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Chemical and Biological Fertilization of *Calendula officinalis* Plant Grown in Sandy Soil

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Abstract: The present study was carried out at the Nursery of Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, Giza, Egypt during the two successive seasons 2014/2015 and 2015/2016. *Calendula officinalis* grown in 30 cm diameter plastic pots filled with sandy soil received ten treatments including (1) NPK conventional fertilization (control); (2) ½NPK + compost (C); (3) ½ NPK + sheep manure (SM); (4) ½ NPK + compost tea (CT); (5) ½ NPK + sheep manure tea (SMT); (6) ½ NPK + CT + SMT; (7) ½ NPK + C + biofertilizer (B), (8) ½ NPK + SM + B, (9) ½ NPK + CT+ B and (10) ½ NPK + SMT+ B. NPK chemical fertilization (control) was added by using ammonium sulphate (20.5%N) at the rate of 400 kg/feddan (7.0 g/ pot), calcium superphosphate (15.5% P_2O_5) at the rate of 400 kg/fed (7.0 g / pot) and potassium sulphate (48% K_2O) at the rate of 150 kg/fed (2.7 g/ pot). ½ NPK was containing the half dose of NPK. The compost was added at 4 ton /fed (71 g/pot) while sheep manure was added at 20 m^3/ fed (353 cm^3/ pot). Both compost tea or sheep manure tea were diluted four times with water and were applied as foliar application (10 ml/plant) in the early morning regularly every 15 days during the vegetative stage starting a week after transplanting and stopped at flowering stage. A commercial biofertilizer Microbin contained N-free living bacteria (*Azotobacter chroococcum* and *Azospirillum brasilense*) and a phosphate dissolving bacteria (*Bacillus megaterium*) was also applied in treatments containing biofertilizer application before sowing and during transplanting. Results showed that ½ NPK + CT+ SMT was the best treatment examined for improving vegetative, flowering and yield parameters as well as chemical composition in most cases.

Key words: *Calendula officinalis* · NPK · Compost tea · Manure tea · Microbin, *Azotobacter* · *Azospirillum* · *Bacillus megaterium*

INTRODUCTION

Calendula officinalis (marigold) is an herbaceous plant belonging to family Asteraceae. Marigold is a valuable ornamental plant in gardens and as a cut flower. Marigold has been used as a medicinal plant in phytotherapy and cosmetics industry. It is cultivated in temperate regions all over the world. The flower heads and the leaves are the parts used, fresh petals are edible in salads and used dried as a food additive and a color agent. A yellow tincture is extracted from the flowers for dyeing fabrics; treating mental tension, insomnia and healing wounds, as well as burns and inflamed skin. A variety of active principles have been reported to be present in marigold which exhibits several pharmacological activities used in drugs, cosmetics and pesticides industries such as carotenoid pigment, oleanolic acid, triterpenoids, saponins, flavonoids (calendoflavoside, rutin, isoquercitrin), essential oil (rich in α-cadinene, α-cadinol, t-muurolol, limonene and 1,8-cineol), coumarins (scopoletin, umbelliferone and esculetin), quinones (plastoquinone, phylloquinone, ubiquinone and phylloquinone) and antioxidants (zeaxanthin, lycopene, vitamins C and E). Marigold has great healing abilities in detoxing the body, treating all kinds of skin problems, cardiovascular disease, cancer, strokes, prominent anti-gastric ulcer, abdominal cramps, constipation and preventing eye disease and blindness as well as protection from sun [1-3].

Nowadays, fertilizers have become essential in agriculture to increase crop production and face needs of the growing population. Conventional NPK chemical fertilization is a fast way of providing plants with essential macro- and micro-nutrients, but excess usage have

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hazardous environmental effects like leaching, runoff, emission causing pollution of aquatic ecosystems and salt accumulation in soil. Phosphorous does not dissolve in water and its overuse causes hardening of soil. Adding inorganic NPK fertilizer replenish nitrogen, potassium and phosphorous, but depletes essential soil nutrients naturally found in fertile soil and leads to decreasing soil fertility. So, it is required to avoid the environmental pollution through applying the optimum amounts of NPK needed by the crop and substituting of the used inorganic fertilizers with organic ones.

Biological fertilization includes soil amendments with various forms of organic matter and/ or rhizosphere microorganisms that interact positively with the plant [4]. In terms of sustainability, there is increasing interest in using organic and bio-fertilization as alternatives to chemical fertilization, aiming at growing healthy and natural food. Compost is a decomposed organic matter used to fertilize soil. It improves physical and mechanical characteristics of the soil and water retention. It slow releases mineral nutrients gradually throughout the growth season, so they give enough time for the plant to develop good root growth; strong stems and resistant immune system to pests and diseases. Organic matter stimulates the growth of root system and foliage resulting in an increase in yield. Compost tea is a liquid extract made by fermenting compost in water. It has been utilized as an active source of organic matter which provides benefits to the soil and plants more quickly than compost. It like any organic fertilizer increases the ability of soil to hold nutrients and retain water [5, 6]. It increases the biomass of beneficial microbes and suppresses soil-borne diseases. Soil microbes produce polysaccharides which bind soil particles and hence improve soil structure [7, 8]. Prior investigators reported the promotion effect of compost and compost tea on some aromatic plants like dragonhead [9], borage [10], thyme [11], sweet marjoram [12] and sand plantain [13]. Manures enhance the ability of the soil to provide physical, chemical and biological needs for the growth of plants through improving microbial biomass, increasing organic carbon stock that is utilized by decomposers for energy as well as N, P and sulfur macronutrients and increasing aggregate stability and decreasing soil bulk density [14, 15]. Sheep manure application promoted both vegetative growth and production of marigold [16], amaranthus [17], peppermint [18], *Lavandula angustifolia* [19] and fennel [20].

Plant growth promoting rhizobateria (PGPR) like *Azotobacter*, *Azospirillum* and Bacillus play a key role in organic matter decomposition, nutrient cycling and they produce phytohormones such as auxins, cytokinins and gibberellins [21]. Auxins increase cell elongation, promote root development and increase root surface area, so the plant absorbs more nutrients and that increases its growth and yield [22, 23]. *Azotobacter* and *Azospirillum* have the ability to fix atmospheric nitrogen, make and leak some active and biological material such as vitamin B, nicotinic acid, pentoterik acid, biotin [24]. Phosphate dissolving *Bacillus megaterium* bacterial inoculation has mineral phosphate solubilization ability by secreting mixtures of phosphatases organic acids which convert insoluble form of phosphate into a soluble form, causing a simultaneous increase in P uptake. El-Ghadban *et al*. [25] pointed out that compost with a mixture of nitrogen fixing bacteria increased vegetative growth characteristics as well as nitrogen, phosphorus and potassium contents of marjoram plants. Abdullah *et al.* [26] revealed that compost and *Azotobacter chroococcum*, *Bacillus megaterium* and *Bacillus circulanse* increased growth, yield and chemical composition of *Rosmarinus officinalis*. Prior studies showed that *Azotobacter* participated in enhancing vegetative characteristics of *Adhatoda vasica*, *Stevia rebaudiana* and *Calendula officinalis* [27-29]. *Bacillus megaterium* affected positively vegetal growth of *Ocimum basilicum* and *Catharanthus roseus* [30, 31] and it increased shoot length, root length and fresh weight of plants and chlorophyll in mustard plants [32]. Eid and El-Ghawwas [33] stated that Microbin and Nitrobin increased plant height, number of branches per plant as well as fresh and dry weights of marjoram plants. Previous studies revealed that biofertilization reduced the full recommended dose of chemical NPK fertilizer without affecting growth and yield. The application of Microbin biofertilizer increased the vegetative growth and yield in sunflower (*Helianthus annus* L. cv. Vedock) and reduced the rate of chemical fertilizers [34]. Bacterial mixture of *Azospirillum* + *Bacillus* showed an increase in growth parameters, nutrients content and yield of sunflower cv. Geza 1 when compared to the full dose of NPK chemical fertilization [35]. There was no significant difference between fertilizing roselle with a mixture of biofertilizers *Azospirillum* and *Bacillus* combined with 50% chemical NPK fertilizer in growth characters and sepal yield compared with the full recommended dose of chemical NPK [36]. Sakr *et al*. [37] found that ¼ NPK+ *Azospirillum brasilense* + *Bacillus megatherium* var. *phosphaticum* was the best treatment to induce vegetative characteristics and chemical composition of marjoram plants. Half dose of the recommended chemical fertilizer combined with nitrogen fixing bacteria (*Azospirillum* *lipoferum*) and phosphate dissolving bacteria (*Bacillus polymxa*) were recommended to obtain high quality *Petunia hybrid* cv. Bravo White [38].

The present study aimed to investigate the effects of NPK chemical fertilization alone or with either organic or bio-organic fertilization on growth, yield and chemical composition of *Calendula officinalis* plants grown under sandy soil conditions, as well as the possibility to substitute chemical fertilizer with biological fertilizers to decrease the worldwide dependence on hazardous chemical fertilizers which deteriorate the agro-ecosystems.

MATERIALS AND METHODS

The present study was conducted during the two successive seasons of 2014/2015 and 2015/2016 at the Experimental Nursery of the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, Giza, Egypt. In both seasons, the seeds of local variety of *Calendula officinalis* (orange flowers) were sown on July 22nd in 8-cm plastic pots filled with a 1:1 (v/v) mixture of sand and clay. The seedlings were transplanted on September 1st of the two seasons in 30 cm diameter plastic pots filled with sandy soil obtained from sixth of October desert. The physical and chemical properties of the experimental soil were shown in Table 1. After two weeks, the seedlings were thinned to one plant per pot. Ten treatments were carried out namely: (1) NPK conventional fertilization (control); (2) ½ NPK + compost (C); (3) ½ NPK + sheep manure (SM); (4) ½ NPK + compost tea (CT); (5) ½ NPK + sheep manure tea (SMT); (6) ½ NPK + CT + SMT; (7) ½ NPK + C + biofertilizer (B), (8) ½ NPK + SM + B, (9) ½ NPK + CT+ B and (10) ½ NPK + SMT+ B.

NPK chemical fertilization (control) was added according to Ministry of Agriculture and Land Reclamation, Egypt recommendation [39] including ammonium sulphate (20.5%N) at the rate of 400 kg/feddan (7.0 g/ pot), calcium superphosphate (15.5% P_2O_5) at the rate of 400 kg/fed (7.0 g / pot) and potassium sulphate (48% K_2O) at the rate of 150 kg/fed (2.7 g/ pot). Calcium superphosphate was added at three doses, the first addition (50%) incorporated with the potting medium before transplanting, the second addition (25%) incorporated with the potting medium after fifty days from transplanting with the second addition of ammonium sulphate and potassium sulphate and the third (25%) incorporated with the potting medium after a month from the second addition. Ammonium sulphate and potassium sulphate (NK) were added equally at five doses, the first three additions were applied starting 21 days after transplanting at a month interval. The other 2 additions were applied after the first and the third harvests of inflorescences (1st January and 15th February, respectively). ½ NPK containing the half dose of NPK and applied as previously described.

Four organic fertilizers were used in this study (1) Compost Alahram, produced in Beba, Beni Suef, Egypt was added at 4 ton /fed (71 g/pot) during soil preparation. (2) Sheep manure (obtained from the animal production farm, Faculty of Agriculture, Cairo University) was added at 20 m^3/ fed (353 cm^3/ pot) at two doses, the first (60%) was incorporated into the soil during soil preparation and the rest was top-dressed forty five days after transplanting. (3) Compost tea was a liquid extract of previous compost prepared by soaking 15 kg of compost with100 liters tap water (previously stored to avoid the harmful effect of Cl_2 on microbes of compost) + 100 cm molasses for 8 days in a 150 liters plastic barrel at room temperature and stirred twice daily until completion of the fermentation process and color turns into light brown [40]. (4) Sheep manure tea was prepared by putting sheep manure in a burlap sack in a barrel, then de-chlorinated water (1:2 W/V) was added and stirred twice daily for a week [41]. 120 L/fed from either compost tea or sheep manure tea were diluted four times with water and were applied as foliar application (10 ml/plant) in the early morning regularly every 15 days during the vegetative stage starting a week after transplanting and stopped at flowering stage to avoid falling of the flowers. The analysis of the used compost; sheep manure; compost tea and sheep manure tea are tabulated in Tables 2 and 3.

Table 1: Physical and chemical properties of the soil used in the study (average of both seasons)

Physical analysis

Clay%	Silt%	Fine sand%	Coarse sand%	Texture class
2.9	1.1	39.6	56.4	Sandy

Chemical analysis

			Soluble cations and anions (meq/L)							Available elements (ppm)		
pH	EC (dS/m)	O.M.%	HCO_3^-	Cl^-	SO_4^{--}	Ca^{++}	Mg^{++}	Na^+	K^+	N	P	K
7.8	1.1	0.34	1.6	5.2	1.5	3.7	2.1	2.3	0.2	10	6	200

Table 2: Physical and chemical analysis of the used compost and sheep manure (average of both seasons)

Parameters	Compost	Sheep manure
Humidity (%)	4.75	11.38
Organic carbon (%)	36.02	63.57
Total N (%)	0.89	1.58
Total P (mg/kg)	248.69	545.67
Total K (%)	1.41	2.13
pH (1:1)	7.60	8.07
EC (dS/m)	4.33	9.07
HCO_3^-(meq/L)	10.00	30.00
Cl^-(meq/L)	20.00	47.00
SO_4^{--}(meq/L)	33.84	57.87
Ca^{++} (meq/L)	3.90	8.58
Mg^{++}(meq/L)	2.73	9.36
K^+(meq/L)	26.82	80.46
Na^+(meq/L)	30.39	36.47

Table 3: Physical and chemical analysis of the used compost tea and sheep manure tea (average of both seasons)

Parameters	Compost tea	Sheep manure tea
Humidity (%)	99.44	98.98
Organic carbon (%)	29.93	40.08
Total N (mg/L)	73.50	257.25
Total P (mg/L)	11.24	15.52
Total K (%)	0.22	0.27
pH	7.87	7.64
EC (dS/m)	6.93	9.85
HCO_3^-(meq/L)	40.00	40.00
Cl^-(meq/L)	34.00	48.00
SO_4^{--}(meq/L)	49.20	53.56
Ca^{++} (meq/L)	7.41	14.82
Mg^{++}(meq/L)	5.85	13.65
K^+(meq/L)	38.31	76.63
Na^+(meq/L)	48.62	36.47

Before sowing the seeds directly, 40% of used seeds were inoculated with a commercial biofertilizer Microbin, contained N-free living bacteria (*Azotobacter chroococcum* and *Azospirillum brasilense*) and a phosphate dissolving bacteria (*Bacillus megaterium*), produced by General Organization for Agricultural Equalization Fund, Agriculture Research Center, Giza, Egypt. The inoculation was carried out by preparing solution of Arabic gum by dissolving 0.25 of the small packet of it in 250 ml of warm water. After that the seeds were spread on polyethylene sheet and seeds were wetted with the previous solution and kept away from sunlight for an hour. Then the Microbin biofertilizer was spread on seeds and mixed with them. The biofertilizer Microbin was used again through the four treatments including compost, sheep manure, compost tea or sheep manure tea. 1 g of Microbin was added around seedlings roots during transplanting

The treatments were arranged in three replicates using complete randomized block design. Each block contained ten fertilization treatments that allocated randomly. Each replicate contained four pots / treatment and each pot contained one plant. All common agricultural practices concern controlling weeds and managing the water supply were followed as needed. Starting from first of January till end of April, all fully opened inflorescences (capitula) were plucked weekly by hand and the number of inflorescences/plant and inflorescence diameter were recorded. The ray flowers were separated, counted and fresh and air dry weights of ray flowers/plant were recorded. Also, at the end of the growing season, the following data were recorded: plant height (cm), number of branches/plant, number of leaves/plant, leaf area (cm^2) of median leaf on main stem, fresh and dry (oven dried at 70 °C) weights of leaves, stems (leafless shoot of main stem and lateral branches) and roots. Total chlorophylls and carotenoids contents were determined in fresh leaves according to Moran [42]. Total carbohydrates, nitrogen, phosphorus and potassium percentages in the dry leaves were determined following methods of Dubois *et al*. [43], A.O.A.C [44], Jackson [45] and Chapman and Pratt [46], respectively. Carotenoids content was determined in fresh ray flowers according to Pineta *et al.* [47]. The resulted data were arranged and subjected to a proper analysis of variance. The means were compared using the Least Significant Difference (LSD) test at 5% level according to the method described by Gomez and Gomez [48].

RESULTS AND DISCUSSION

Vegetative Growth Characteristics: Data presented in Tables 4 and 5 revealed that all fertilization treatments significantly increased vegetative growth parameters (plant height, number of branches/plant, number of leaves/plant, leaf area of median leaf on main stem, as well as fresh and dry weights of leaves, stems and roots/plant) as compared to the control (NPK treatment) in both seasons, in most cases. Few exceptions to this general trend were recorded with the treatment of ½ NPK + compost which recorded insignificantly higher values than the values of control for plant height as well as fresh weights of leaves, stems and roots in both seasons.

Data shown in Tables 4 and 5 revealed that, in most cases, ½ NPK + compost tea +sheep manure tea treatment resulted in the significantly higher values as compared to values recorded with other treatments in most cases for plant height in the first season as well as in both seasons for number of branches/plant, number of leaves/plant, leaf area, dry weight of leaves/plant, fresh and dry weights of stems/plant and fresh weight of roots/plant. ½ NPK +

Table 4: Effect of chemical, organic and bio-organic fertilization on plant height, number of branches and number of leaves/plant as well as leaf area of *Calendula officinalis* plants during the two growing seasons of 2014/2015 and 2015/2016

	plant height (cm)		number of branches / plant		Number of leaves/ plant		Leaf area (cm²)	
Treatments	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control (NPK)	28.37	34.50	9.96	10.95	65.21	71.58	66.00	74.00
½ NPK + C	30.52	36.19	12.63	13.11	87.68	99.96	75.24	86.90
½ NPK + SM	41.42	40.42	13.80	14.46	105.56	110.60	78.00	84.61
½ NPK + CT	36.34	43.33	15.51	15.66	94.29	102.97	79.00	83.80
½ NPK + SMT	40.95	38.77	15.81	15.93	96.04	111.20	82.00	84.00
½ NPK + CT+ SMT	47.53	46.16	17.37	17.64	136.50	147.53	109.31	112.62
½ NPK + C +B	40.92	42.59	15.42	16.92	101.47	114.98	91.18	101.62
½ NPK + SM+B	42.90	45.13	16.95	17.46	97.34	105.60	106.00	90.32
½ NPK + CT+B	43.39	46.61	15.96	16.62	121.91	122.85	96.32	98.01
½ NPK + SMT+B	45.94	43.63	15.93	16.83	132.86	137.55	100.61	104.30
LSD $_{5\%}$	4.32	4.09	1.26	1.32	9.59	10.01	9.23	9.37

Table 5: Effect of chemical, organic and bio-organic fertilization on fresh and dry weights of leaves, stems and roots of *Calendula officinalis* plants during the two growing seasons of 2014/2015 and 2015/2016

	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	2nd season	1st season	2nd season
Control (NPK)	228.80	229.44	42.22	42.28	26.91	26.94
½ NPK + C	245.44	246.56	46.38	46.42	29.93	29.99
½ NPK + SM	329.44	336.80	64.54	64.68	31.50	37.21
½ NPK + CT	326.40	328.96	62.76	63.28	42.87	42.94
½ NPK + SMT	409.92	420.16	68.96	71.60	44.88	44.91
½ NPK + CT+ SMT	414.40	417.28	86.22	87.22	53.35	56.21
½ NPK + C +B	328.96	329.84	66.02	66.24	43.81	44.52
½ NPK + SM+B	410.08	413.44	76.24	76.64	51.44	53.26
½ NPK + CT+B	409.36	410.88	62.84	67.04	40.17	44.55
½ NPK + SMT+B	414.64	417.12	81.00	86.36	44.91	44.94
LSD 5%	39.44	41.04	6.28	6.46	3.93	4.38
	Dry weight of leaves (g/plant)		Dry weight of stems (g/plant)		Dry weight of roots (g/plant)	
Control (NPK)	17.12	24.72	5.14	6.34	2.98	3.33
½ NPK + C	30.56	35.60	8.26	8.46	5.08	5.68
½ NPK + SM	41.04	41.84	12.28	12.62	6.16	6.69
½ NPK + CT	32.00	34.24	10.00	10.24	4.98	5.92
½ NPK + SMT	42.48	44.32	13.06	13.44	7.18	7.64
½ NPK + CT+ SMT	57.92	60.96	16.18	17.04	9.04	8.86
½ NPK + C +B	32.96	33.12	10.28	11.50	5.71	5.91
½ NPK + SM+B	46.96	49.60	12.80	12.92	7.25	7.78
½ NPK + CT+B	49.84	55.44	12.60	12.84	8.85	8.93
½ NPK + SMT+B	57.76	60.64	14.00	15.64	9.44	9.70
LSD 5%	3.44	4.24	1.30	1.46	0.81	0.85

sheep manure tea + biofertilizer treatment resulted in the insignificantly higher value for fresh weight of leaves/plant in the first season and dry weight of roots/plant in both seasons as compared to values recorded with ½ NPK + compost tea +sheep manure tea treatment. Also, this treatment (½ NPK + sheep manure tea + biofertilizer) gave insignificantly different values than the values recorded with ½ NPK + compost tea + sheep manure tea treatment for plant height, number of leaves/plant, leaf area, fresh weights of leaves and stems/plant, dry weights of leaves and roots/plant in both seasons as well as number of branches/plant and dry weight of stems/plant in the second season.

From the above mentioned results, it can be concluded that ½ NPK + compost tea + sheep manure tea treatment was the superior in improving vegetative growth parameters followed by ½ NPK + sheep manure tea + Microbin biofertilizer with no significant difference among them in most cases.

Using ½ NPK + compost tea or sheep manure tea treatments increased the values of most of vegetative growth parameters as compared to the values recorded with ½ NPK + compost or sheep manure treatments, respectively. It is worth to mention that, in both seasons, ½ NPK + compost tea treatment resulted in significantly higher plant height, number of branches/plant, fresh weights of leaves, stems and roots as well as dry weight of stems/plant as compared to values recorded with ½ NPK + compost treatment. Results obtained are in agreement with prior studies using compost and compost tea [9-13] and in harmony with previous studies applied sheep manure [16-20]. The superior effect of compost tea as compared to compost may be attributed to that it is a brewing of compost in which humic acid and nutrients are in the most suitable form for plant uptake as well as it has the ability to improve soil porosity, density. In addition, microorganisms during fermentation enable accumulation of antibiotics that help in disease suppression [49]. Manure might increase nutrients content of the soil and thereby well feed plants for more vigor growth like the observation of Wong *et al*. [50] who revealed that cattle manure compost increased K^+ content in the layer 10-20 cm deep in the sandy soil.

Also, ½ NPK + sheep manure tea treatment resulted in significantly higher number of branches/plant, fresh weights of leaves/plant as well as fresh and dry weights of roots/plant in both seasons as compared to values recorded with ½ NPK + sheep manure treatment.

Using ½ NPK + compost + biofertilizer or ½ NPK + sheep manure + biofertilizer treatments resulted in significantly higher values for most of vegetative growth parameters as compared to the values recorded with ½ NPK + compost or sheep manure treatments, respectively. Also, ½ NPK + compost tea + biofertilizer and ½NPK + sheep manure tea + biofertilizer treatments gave significantly higher values for most of vegetative growth characteristics as compared to values recorded with ½NPK + compost tea or sheep manure tea, respectively. Results obtained are in agreement with previous studies [27-33]. The superior effect resulted from the adding biofertilizer on increasing plants' vegetative growth may be attributed to that the beneficial microorganisms in the biofertilizer break down and release minerals from organic matter to enable easier nutrient uptake in plants. In addition they compete with pathogenic bacteria or fungi for food source and parasitizing them causing inhibition of their growth and activity [51, 52]. Sakr [53] found that available N and P increased in sandy soil after harvesting senna plants fertilized with either cattle or poultry manure alone or accompanied with a biofertilizer of *Azospirillum*, *Bacillus*, *Azotobacter*, *Klebsiella* and *Pseudomonas*.

Flowering and Yield Characteristics: Data presented in Table 6 revealed that all fertilization treatments significantly increased flowering and yield parameters including number of inflorescences (capitula)/plant, inflorescence (capitulum) diameter, number of ray flowers/inflorescence as well as fresh and dry weights of ray flowers/plant as compared to the control (NPK treatment) in both seasons. Only one exception to this general trend was recorded with the treatment of ½ NPK + compost which recorded insignificantly higher value than the value of the control for inflorescence diameter in the second season.

Data shown in Table 6 revealed that ½ NPK + compost tea + sheep manure tea treatment resulted in the significantly higher values as compared to values recorded with other treatments for number of inflorescences/plant, inflorescence diameter, number of ray flowers/inflorescence as well as fresh and dry weights of ray flowers/plant in both seasons. ½ NPK + sheep manure tea + biofertilizer treatment gave insignificantly different values than the values recorded with ½ NPK + compost tea + sheep manure tea treatment for number of inflorescences/plant, inflorescence diameter and number of ray flowers/inflorescence in both seasons as well as fresh weight of ray flowers/plant in the first season.

From the above mentioned results, it can be concluded that ½ NPK+ compost tea + sheep manure tea treatment was the superior in improving flowering and yield parameters. Using ½ NPK + compost tea or sheep manure tea treatments increased the values of most of flowering and yield parameters as compared to the values recorded with ½ NPK + compost or sheep manure treatments, respectively. These results are in agreement with the results reported by previous studies [9, 10, 13, 16].

Using ½ NPK + compost + biofertilizer or ½ NPK + sheep manure + biofertilizer treatments resulted in higher values for all flowering and yield parameters as compared to the values recorded with ½ NPK + compost or sheep manure treatments, respectively. Also, ½ NPK + compost tea + biofertilizer and ½ NPK + sheep manure tea + biofertilizer treatments gave higher values for all flowering and yield characteristics as compared to values recorded with ½NPK + compost tea or sheep manure tea, respectively.

Table 6: Effect of chemical, organic and bio-organic fertilization on number of inflorescences/plant, inflorescence diameter, number of ray flowers/inflorescenc as well as fresh and dry weights of ray flowers/ plant of *Calendula officinalis* plants during the two growing seasons of 2014/2015 and 2015/2016

	Number of inflorescences/plant		Inflorescence diameter (cm)		Number of ray flowers/ inflorescence		Fresh weight of ray flowers/ plant		Dry weight of ray flowers/ plant	
Treatments	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control (NPK)	30.18	36.00	3.02	3.06	34.75	35.02	35.32	50.54	3.55	4.04
½ NPK + C	37.56	41.46	3.46	3.52	42.23	47.02	47.89	63.44	4.70	5.46
½ NPK + SM	36.60	42.30	4.27	4.32	53.45	57.19	44.47	61.67	4.44	5.12
½ NPK + CT	39.00	43.74	4.28	4.34	48.99	57.12	50.31	67.23	5.48	5.96
½ NPK + SMT	49.98	51.00	4.27	4.31	57.56	58.00	71.23	87.21	7.54	8.04
½ NPK + CT+ SMT	52.26	54.96	5.60	5.89	61.57	67.01	75.25	94.98	8.83	9.40
½ NPK + C +B	39.48	42.18	4.27	4.49	50.59	59.70	52.12	66.81	5.55	5.95
½ NPK + SM+B	45.60	48.60	5.03	5.24	55.56	62.97	62.93	80.49	7.00	7.48
½ NPK + CT+B	41.16	47.58	4.63	4.82	55.22	61.23	54.96	76.23	6.48	7.01
½ NPK + SMT+B	50.14	53.06	5.28	5.40	58.99	64.74	71.55	87.47	7.85	8.28
LSD 5%	3.72	4.02	0.36	0.58	4.86	5.30	5.72	6.88	0.56	0.74

Table 7: Effect of chemical, organic and bio-organic fertilization on total chlorophylls, carotenoids and total carbohydrates in leaves as well as carotenoids in ray flowers of *Calendula officinalis* plants during the two growing seasons of 2014/2015 and 2015/2016.

	Total chlorophylls (mg/g fresh matter of leaves)		Carotenoids (mg/g fresh matter of leaves)		Total carbohydrates (% DW of leaves)		Carotenoids (mg/100 g fresh matter of ray flowers)	
Treatments	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control (NPK)	1.43	1.54	0.48	0.55	53.85	54.90	107.1	111.5
½ NPK + C	1.44	1.50	0.57	0.74	58.21	60.25	106.0	113.3
½ NPK + SM	1.51	1.65	0.66	0.71	58.22	58.81	109.4	112.8
½ NPK + CT	1.70	1.73	0.69	0.83	55.47	59.90	108.9	116.2
½ NPK + SMT	1.65	1.71	0.74	0.77	58.36	62.37	111.5	117.5
½ NPK + CT+ SMT	1.88	1.96	0.77	0.83	64.80	66.35	122.4	131.8
½ NPK + C +B	1.63	1.65	0.48	0.60	53.71	62.82	108.7	116.9
½ NPK + SM+B	1.59	1.76	0.69	0.76	59.42	62.51	110.3	115.2
½ NPK + CT+B	1.74	1.82	0.71	0.87	60.36	61.80	111.6	120.1
½ NPK + SMT+B	1.78	1.90	0.76	0.78	58.53	63.05	113.5	118.6
LSD 5%	0.09	0.12	0.05	0.06	4.11	4.35	9.4	10.1

Chemical Characteristics:

Leaf Pigments Content (Total Chlorophylls and Carotenoids): Data presented in Table 7 revealed that in both seasons, all fertilization treatments significantly increased leaf pigments content (total chlorophylls and carotenoids) as compared to control (NPK) treatment, in most cases. Few exceptions to this general trend were recorded with ½ NPK+ compost and ½ NPK + sheep manure treatments in both seasons, in addition to ½ NPK+ compost + biofertilizer treatment in the second season which recorded insignificantly different values of total chlorophylls than the values of control. Also, in both seasons, ½ NPK + compost + biofertilizer treatment recorded insignificantly different values of carotenoids than that recorded in control plants.

In both seasons, ½ NPK+ compost tea + sheep manure tea treatment resulted in the significantly higher values of total chlorophylls and carotenoids in leaves as compared to values recorded with other treatments in most cases.

Using ½ NPK+ compost tea or sheep manure tea treatments resulted in significantly higher values of total chlorophylls and carotenoids in leaves as compared to the values recorded with ½ NPK+ compost or sheep manure treatments, respectively in most cases. These results are in agreement with the results reported by previous studies [9, 16, 19].

In both seasons, in most cases, using ½ NPK+ compost + biofertilizer or ½ NPK+ sheep manure + biofertilizer treatments increased values of total chlorophylls and carotenoids in leaves as compared to the values recorded with ½ NPK+ compost or sheep manure treatments, respectively. Also, ½ NPK + compost tea + biofertilizer and ½ NPK+ sheep manure tea + biofertilizer treatments increased values of total chlorophylls and carotenoids in leaves as compared to values recorded with ½ NPK+ compost tea or sheep manure tea, respectively. These results are in harmony with the result reported by previous studies [29, 32].

Table 8: Effect of chemical, organic and bio-organic fertilization on N, P and K% in leaves of *Calendula officinalis* plants during the two growing seasons of 2014/2015 and 2015/2016

	N (% DW)		P (% DW)		K (% DW)	
Treatments	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control (NPK)	1.51	1.53	0.15	0.17	1.70	1.85
½ NPK + C	1.68	1.81	0.22	0.21	1.86	2.11
½ NPK + SM	1.85	1.80	0.28	0.22	1.76	2.02
½ NPK + CT	1.77	1.78	0.29	0.24	1.87	2.66
½ NPK + SMT	2.02	1.90	0.28	0.23	1.92	2.31
½ NPK + CT+ SMT	2.34	2.51	0.31	0.29	1.93	2.78
½ NPK + C +B	1.78	1.99	0.23	0.22	1.88	2.18
½ NPK + SM+B	1.97	1.89	0.29	0.24	1.85	2.24
½ NPK + CT+B	1.76	1.85	0.29	0.26	1.92	2.74
½ NPK + SMT+B	2.11	2.10	0.29	0.27	2.01	2.46
LSD 5%	0.18	0.21	0.02	0.02	0.14	0.21

Total Carbohydrates in Leaves: Data presented in Table 7 revealed that, in most cases, all fertilization treatments significantly increased total carbohydrates in leaves as compared to values recorded with control (NPK) treatment in both seasons. In both seasons, ½ NPK+ compost tea + sheep manure tea treatment resulted in the significantly higher values of total carbohydrates in leaves as compared to values recorded with other treatments in most cases.

Using ½ NPK + compost tea or sheep manure tea treatments resulted in insignificantly different values of total carbohydrates in leaves as compared to the values recorded with ½ NPK + compost or sheep manure treatments, respectively. These results are in agreement with the results reported by previous studies [9, 19, 20].

In most cases, using ½ NPK + compost + biofertilizer or ½ NPK + sheep manure + biofertilizer treatments insignificantly improved values of total carbohydrates in leaves as compared to the values recorded with ½ NPK + compost or sheep manure treatments, respectively. Also, ½ NPK + compost tea + biofertilizer and ½ NPK + sheep manure tea + biofertilizer treatments insignificantly improved values of total carbohydrates in leaves as compared to values recorded with ½ NPK + compost tea or sheep manure tea treatments, respectively.

Carotenoids Content in Ray Flowers: Data presented in Table 7 revealed that, in both seasons, all fertilization treatments increased carotenoids content in ray flowers as compared to the control (NPK) treatment in most cases. Only one exception to this general trend was recorded in the first season with the treatment of ½ NPK + compost which recorded insignificantly lower value of carotenoids in ray flowers than the value recorded with the control treatment.

In both seasons, ½ NPK+ compost tea + sheep manure tea treatment resulted in higher values of carotenoids in ray flowers as compared to values recorded with other treatments. Using ½ NPK+ compost tea or sheep manure tea treatments increased carotenoids in ray flowers as compared to the values recorded with ½ NPK+ compost or sheep manure treatments, respectively. Using ½ NPK+ compost + biofertilizer or ½ NPK+ sheep manure+ biofertilizer treatments increased carotenoids in ray flowers as compared to the values recorded with ½ NPK+ compost or sheep manure treatments, respectively. Also, ½ NPK+ compost tea+ biofertilizer and ½ NPK + sheep manure tea + biofertilizer treatments gave higher values for carotenoids in ray flowers as compared to values recorded with ½ NPK+ compost tea or sheep manure tea treatments, respectively.

N, P and K (% DW): Data presented in Table 8 revealed that in both seasons, all fertilization treatments significantly increased N, P and K% in leaves as compared to control (NPK) treatment in most cases. Two exceptions to this general trend were recorded in the first season with the treatment of ½ NPK+ compost and in both seasons with the treatment of ½ NPK + sheep manure which recorded insignificantly higher values of N% and K%, respectively than the values recorded with the control treatment.

In both seasons, ½ NPK+ compost tea + sheep manure tea treatment resulted in the significantly higher values of N, P and K% in leaves as compared to values recorded with other treatments in most cases. Only one exception to this trend was recorded in the first season with ½ NPK+ SMT+B treatment which resulted in the highest K% in leaves followed by ½ NPK+ CT+SMT, ½ NPK+ SMT, ½ NPK+ CT+B and ½ NPK+ C+B treatments with no significant difference among them.

In most cases, using ½ NPK+ compost tea or sheep manure tea treatments increased the N, P and K% in leaves as compared to the values recorded with ½NPK+ compost or sheep manure treatments, respectively. Results obtained are in agreement with previous studies [17, 19, 20].

Using ½ NPK+ compost + biofertilizer or ½ NPK+ sheep manure+ biofertilizer treatments increased N, P and K% in leaves as compared to the values recorded with ½ NPK+ compost or sheep manure treatments, respectively. Also, ½NPK+compost tea+ biofertilizer and ½ NPK+ sheep manure tea + biofertilizer treatments gave higher values for N, P and K% in leaves as compared to values recorded with ½ NPK + compost tea or sheep manure tea treatments, respectively. Results obtained are in agreement with previous studies [27-31, 33].

Conclusion and Recommendation: From the above results, it can be concluded that although there was no significant difference between ½ NPK+ compost tea + sheep manure tea treatment and ½ NPK + sheep manure tea + Microbin biofertilizer in improving vegetative growth parameters and most of flowering characteristics of *Calendula officinalis* plants, but ½ NPK+ compost tea + sheep manure tea treatment is recommended because of increasing fresh and dry yield of ray flowers and chemical composition especially carotenoids content in ray flowers significantly than the other treatments.

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