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PARTIAL SUBSTITUTION OF CHEMICAL FERTILIZATION OF CHIA PLANT (*Salvia hispanica* L.) BY ORGANIC FERTILIZATION IN PRESENCE OF BIO FERTILIZATION

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ABSTRACT: Two field experiments were carried out at the Experimental Farm and in the Laboratory of Horticulture Department Faculty of Agriculture at Moshtohor, Benha Univ., during 2018/2019 and 2019/2020 seasons to study the effect of some fertilizer treatments (chemical, organic and bio-fertilizers) on vegetative growth, essential oil productivity and some chemical constituents of chia (*Salvia hispanica* L.) plants. Obtained results showed that: The tallest plant was gained by T_2 (100 % R.D. chemical NPK) followed in descending order by T_3 (75 % R.D. chemical NPK + 25% organic + bio) in the two seasons. The greatest number of branches and the heaviest fresh and dry weight of herb per plant of chia were gained by T_3 treatment, followed by T_2 treatment. The heaviest fresh and dry weights of inflorescence per plant, the heaviest 1000 seeds and seeds weight / plant of chia plants were scored by T3 treatment, followed by T2 treatment in the two seasons. The greatest herb oil percentage of chia was scored by T4 (50 % R.D. chemical NPK + 50% organic + bio) treatment, followed by T3 treatment. The greatest fixed oil yield per plant and per fed. of chia were scored by T3 treatment, followed by T2 treatment in the two seasons. The highest herb chemical constituents of chia plants were recorded by T2 and T3 treatments in the two seasons.

Key words: Chia, fertilization, growth and productivity.

INTRODUCTION

Chia (*Salvia hispanica* L.) is an annual plant belonging to family Lamiaceae (Labiate) native to Mexico and Guatemala (**Ixtaina *et al.*, 2008**). In pre-Columbian times, chia is one of the basic foods of Central American civilizations (**Ayerza and Coates, 2005**). Owing to the fact that it can grow in arid environments, it has been highly recommended as an alternative crop for the field crop industry **(Peiretti and Gai, 2009).** The cultivation of chia is gaining popularity in Africa because it is considered as a healthy food and good nutrition (**Ayerza and Coates, 2000**).

Chia can grow up to 1 m tall and has opposite arranged leaves. Chia flowers are small flower (3 - 4 mm) with small corollas and fused flower parts that contribute to a high self-pollination rate. The seed color varies from black, gray, and black spotted to white, and the shape is oval with size ranging from 1 to 2 mm. Chia seeds are rich in dietary fibers, protein, oil and mucilage. It contains from 25% to 40% oil with 60-68% of its comprising (omega) ω-3 α-linolenic acid (ALA) and 20% of (omega) ω-6 linoleic acid **(Ayerza, 2013).** Chia seeds are composed of protein (15-25%), fats (30-33%), carbohydrate (26-41%), high dietary fiber (18-30%), ash (4-5%), minerals, vitamins, and dry matter (90-93%). It also contains a high amount of antioxidants **(Ixtaina *et al.*, 2008).** Clearly, any reliable source of omega 3 fatty acids that can be found which is safe for consumption would be attractive. Chia contains omega 3 fatty acids and its oil provides the richest plant alpha linolenic fatty acid known **(Ayerza and Costa, 2005)**. Another key feature of the chia seed is that it does not contain gluten **(Bueno *et al.*, 2010)**.

Unconventional efforts are used to minimize the amounts of chemical fertilizers which applied to medicinal and aromatic plants in order to reduce production cost and environmental pollution without reduction of yield. Therefore, the trend now is using the bio and organic fertilizers. Bio-fertilizers are reasonably safer to the environment than chemical fertilizers and play an important role in decreasing the use of chemical fertilizers. Consequently, it causes a reduction in environmental pollution. Bio fertilizers are microbial inoculants consisting of living cells of micro-organism like bacteria, algae and fungi alone or combination which may help in increasing crop productivity. Bio fertilizers can influence plant growth directly through the production of phytohormones such as gibberellins, cytokinins and IAA that act as growth regulators and indirectly through nitrogen fixation and production of bio-control agents against soil-borne phytopathogens and consequently increase formation of metabolites which encourage the plant vegetative growth and enhance the meristematic activity of tissues to produce more growth (**Glick, 2003; Ahmed and Kibret, 2014**).

Organic fertilizers are obtained from animal sources such as animal manure or plant sources like green manure. Continuous usage of inorganic fertilizer affects soil structure. Hence, organic manures can serve as alternative to mineral fertilizers for improving soil structure **(Shahram and Ordookhani, 2011)** and microbial biomass (**Suresh *et al.*, 2004).** The addition of organic fertilizers to agricultural soils has beneficial effects on crop development and yields by improving soil physical and biological properties (**Zheljazkov and Warman, 2004**). Organic and bio fertilizers in comparison of the chemical fertilizers have lower nutrient content and are slow release but they are as effective as chemical fertilizers over longer periods of use (**Naguib, 2011 and Mohamed *et al*., 2012**).

The target of this work was to evaluate the benefits of supplementing organic fertilizers with chemical fertilizer in presence of bio fertilizer on growth and fixed oil productivity of chia plants and to minimize consuming of chemical fertilizers.

MATERIALS AND METHODS

Two field experiments were carried out at the Experimental Farm and in the Laboratory of Horticulture Department Faculty of Agriculture at Moshtohor, Benha Univ., during 2018/2019 and 2019/2020 seasons to study the effect of some fertilizer treatments (chemical, organic and bio-fertilizers) on vegetative growth, essential oil productivity and some chemical constituents of chia (*Salvia hispanica* L.) plants. Chia seeds were obtained from Floriculture Farm, Horticulture Department, Faculty of Agriculture, Benha Univ. Seeds were sown in clay loam soils on 19th October in both seasons in plots (1x1 m) containing two rows (50 cm. in between) every row contains two hills (50 cm. apart) and 45 days later, the plants were thinned, leaving only one seedling/hill.

Physical and chemical characters of the used soil are shown in Tables (a) and (b), Physical analysis was estimated according to **Jackson (1973)** whereas, chemical analysis was determined according to **Black *et al*. (1982).**

Table (a). Physical analysis of the experimental soil

Parameters	Unit	Seasons	
		2018/2019	2019/2020
Coarse sand	%	5.08	4.84
Fine sand	%	15.98	16.41
Silt	%	26.47	27.33
Clay	%	52.47	51.42
Textural class	---	Clay loam	Clay loam

Table (b). Chemical analysis of the experimental soil

Parameters	Unit	Seasons	
		2018/2019	2019/2020
$CaCo_3$	%	1.06	1.17
Organic matter	%	2.18	2.21
Available nitrogen	%	0.86	0.88
Available phosphorus	%	0.36	0.39
Available potassium	%	0.71	0.74
E.C	$dS.m^{-1}$	0.83	0.81
pH	---	7.67	7.69

Bio-fertilizer treatment

Chia seeds were inoculated with a mixture of nitrobein + phosphorein contained efficient strains of nitrogen fixing bacteria namely, *Azotobacter chroococcum* + phosphate dissolving bacteria (*Bacillus megaterium* var phosphaticum) which supplied by the Department of Microbiology, Agric. Res. Center, Giza was used in this study as biological activators. The strains were characterized by a good ability to infect its specific host plant and by its high efficiency in N-fixation and phosphate solublizing. Seeds of chia plants were washed with water and air-dried, thereafter the seeds were soaked in cell suspension of the mixture of nitrobein and phosphorein (1ml contains 10^9 viable cell) for 30 min. Gum arabic (16 %) was added as an adhesive agent prior to soaking. The inoculated seeds were air dried at room temperature for one hour before sowing. Another two applications were applied (2kg/fed.) as an aqueous solution, the first one was applied just before irrigation after 30 days from sowing date, whereas the second one was done after 60 days from sowing date to increase the power ability of bacteria.

Organic fertilizer treatment

Organic fertilizer i.e. compost containing plant sources and cattle manure at the rate of 8.33, 6.25,4.17 and 2.1 ton/fed., was thoroughly mixed with the soil before planting, the chemical properties of the tested compost are presented in Table (c).

Table (c). Chemical properties of the used compost

Parameters / Determinations	Ec dS.m^{-1} (1:5)	pH (1:5)	Total C %	Total N %	Total P %	Total K %	Total Fe (ppm)	Total Zn (ppm)	C:N ratio
Reading	2.01	6.84	22.29	1.20	0.79	1.52	1484	378	18.6:1

Chemical fertilizer treatment

The plants were fertilized with full chemical fertilizer dose as a recommended dose **(Ismail, 2004)**; where ammonium nitrate (33.5% N) was added at the rate of 100Kg N/fed., calcium superphosphate (15.5 % P_2O_5) was added at the rate of 200 Kg and potassium sulphate (48.5 % K_2O) at the rate of 75 Kg /fed. The amount of N and K fertilizers were divided into four equal portions as side dressing at 50, 65, 80 and 95 days after sowing date of both seasons. However, the amount of P-fertilizer was added to the soil before seed sowing during soil preparation.

The studied treatments were as follow:

Sampling and collecting data

At the flowering stage (2th March), vegetative growth parameters, flowering growth parameters and chemical composition were estimated as following:

Vegetative growth parameters

1- Plant height (cm), 2- Number of branches/ plant, 3- Fresh weight of herb / plant (g), 4- Dry weight of herb / plant (g)

Flowering growth parameters

1- Inflorescence fresh weight / plant, 2- Inflorescence dry weight / plant,

Fruiting yield parameters

1- Weight of 1000 seeds, 2- Seeds yield / plant, and 3- Seeds yield / fed.

Fixed oil yield parameters

1- Herb fixed oil percentage, 2- Fixed oil yield / plant (cm^3), and 3- Fixed oil yield / fed. (L).

Chemical composition determination

N, P, K and total carbohydrates percentages

- Nitrogen (%) was determined according to the modified Microkjeldahle method as described by **A.O.A.C. (1990)**.

- Phosphorus (%) was determined colorimetrically by the spectrophotometer at wavelength of 650 µm according to the method of **Murphy and Riley (1962).**

- Potassium (%) was determined using flame-photometry method according to **Cottenie *et al.* (1982)**.

- Total carbohydrates (%) were determined colorimetrically by the spectrophotometer according to the method of **Herbert *et al.* (1971)**.

Fixed oil percentage and productivity

The clean air-dried seeds of chia were separately crushed in a willey mill, then extracted in Soxhlet apparatus, samples of 10 g of seeds were moved into soxhlet apparatus in 100 ml of N-hexane and the extraction period extended to three hours (30-36syphon cycle approx.). The N-hexane extract was dried over anhydrous sodium sulfate, then filtered and the oil was obtained by distillation under vacuum. The percent of fixed oil was calculated as weight/weight using the following equation:

$$\text{Fixed oil percentage} = \frac{\text{Extracted fixed oil weight}}{\text{Seed sample weight}} \times 100$$

The fixed oil percentage was used to calculate fixed oil yield/plant as well as fixed oil yield/fed using the following equations:

$$\text{Fixed oil yield/ plant (g)} = \frac{\text{Fixed oil percentage} \times \text{Seed dry weight/plant}}{100}$$

$$\text{Fixed oil yield/ fed. (kg)} = \frac{\text{Fixed oil yield/ plant} \times \text{Number of plants/ fed.}}{1000}$$

Statistical analysis

The obtained data in both seasons of study were subjected to analysis of variance as a simple experiment in RCBD, L.S.D. method was used to differentiate between means according to **Snedecor and Cochran (1989).**

RESULTS AND DISCUSSION

Vegetative growth parameters

1-Plant height (cm)

Data tabulated in Table ,1. revealed that, all tested fertilization treatments succeeded in increasing plant height of chia (*Salvia hispanica, L)* plants with significant differences, in all cases when compared with un-fertilized plants in the two seasons. In this regard, the tallest plant was gained by T_2 (100 % R.D. chemical NPK) as it recorded 121.7 and 126.4 cm followed in descending order by T_3 (75 % R.D. chemical NPK+25% organic+bio) which recorded 118.2 and 121.3 cm in the first and second seasons, respectively. The differences between the abovementioned two treatments were so small to reach the level of significance in both seasons.

Table 1. Effect of some fertilization treatments on plant height and branches number / plant of chia plants during the two successive seasons of 2019 and 2020

Characters	Plant height (cm)		Branches Number/plant	
Seasons / Treatments	1st season	2nd season	1st season	2nd season
Control (T_1)	86.4	91.7	16.8	19.6
100% R.D. of mineral NPK (T_2)	121.7	126.4	29.7	26.8
75% R.D. +25% organic+bio (T_3)	118.2	121.3	31.2	28.0
50% R.D. +50% organic+bio (T_4)	112.8	114.2	24.3	21.9
25% R.D. +75% organic+bio (T_5)	104.3	108.5	21.8	19.3
0.0% R.D. +100% organic+bio(T_6)	102.9	106.8	19.4	18.4
LSD at 0.05	4.26	6.19	1.84	1.04

2- Number of branches / plant

Data presented in Table 1, show that, the all studied fertilization treatments significantly increased the number of branches per plant in the two seasons. In this respect, the greatest number of branches of chia (*Salvia hispanica, L),* was gained by T_3 treatment as it scored 31.2 and 28.9 branches per plant, followed by T_2 treatment which gave 29.7 and 26.8 branches per plant, without significant differences between them in the first and second seasons, respectively. Besides, T_4 and T_5 gave high increments in this concern in the two seasons.

3- Herb fresh and dry weights / plant (g)

Data in Tables, 2 and 3 revealed that fresh and dry weights of herb per plant were positively increased by all fertilization in the two seasons. In this concern, the heaviest fresh and dry weights of herb per plant of chia plants was recorded by T2, followed by T3, with non-significant differences between them in the two seasons. Irrespective un-fertilized plants the lowest value of this parameter was scored by T6, followed in ascending order by T5 in the two seasons.

Table 2. Effect of some fertilization treatments on herb fresh and dry weights / plant of chia plants during the two successive seasons of 2019 and 2020

Characters	Herb fresh weight (g)		Herb dry weight(g)	
Seasons / Treatments	1st season	2nd season	1st season	2nd season
Control (T_1)	84.3	89.6	13.4	15.1
100% R.D. of mineral NPK (T_2)	134.2	138.7	21.6	23.8
75% R.D. +25% organic+bio (T_3)	128.9	131.2	20.2	22.1
50% R.D. +50% organic+bio (T_4)	118.4	122.4	18.6	20.9
25% R.D. +75% organic+bio (T_5)	107.2	113.4	17.2	19.4
0.0% R.D. +100% organic+bio(T_6)	104.7	108.0	16.8	18.6
LSD at 0.05	8.17	7.64	1.94	1.87

The aforementioned results of fertilization concerning vegetative growth are in parallel with those obtained by **Abd-Allah (2012**) on fennel, **Abo-Baker and Mostafa (2011)** on *Hibiscus sabdariffa*, **Abd El-Wahab (2013)** on *Tanacetum vulgare*, **Abdel-Aziez *et al.* (2014)** on black cumin, **Said-Al Ahl *et al.* (2015)** on *Anethum graveolens* L., **Mahmoud *et al.* (2017)** on caraway, **Gomaa *et al.* (2018)** on roselle, **Youssef *et al.* (2020)** on Dutch fennel and **Youssef *et al.* (2020)** on caraway plant**.**

Flowering growth parameters

Data outlined in Table,3 pointed out that fresh and dry weights of inflorescence per plant was statistically increased by all fertilization treatments in the two seasons. In this respect, the heaviest fresh and dry weights of inflorescence per plant of chia plants was scored by T3 treatment, followed by T2 treatment, with nonsignificant differences between them in the two seasons. Irrespective un-fertilized plants the lowest value of this parameter was scored by T6, followed in ascending order by T5 in the two seasons. The rest treatments occupied an intermediate position between the abovementioned treatments in the two seasons.

These results are in close agreement with those reported by **Talaei *et al*. (2014)** on cumin, **Badran *et al.* (2017)** on fennel, **Sakr (2017)** on *Calendula officinalis*, **Hassanain *et al*. (2018)** on *Tagetes erecta*, **Gomaa *et al.* (2018)** on roselle**, Youssef, et al., (2020)** on dutch fennel and **Youssef *et al.* (2020)** on caraway plant.

Table 3. Effect of some fertilization treatments on inflorescences fresh and dry weights / plant of chia plants during the two successive seasons of 2019 and 2020

Characters	Inflorescences F.W/plant (g)		Inflorescences D.W/plant (g)	
Seasons / Treatments	1st season	2nd season	1st season	2nd season
Control (T_1)	8.36	9.18	1.08	1.27
100% R.D. of mineral NPK (T_2)	13.17	14.92	1.71	2.09
75% R.D. +25% organic+bio (T_3)	14.82	15.60	1.92	2.18
50% R.D. +50% organic+bio (T_4)	11.86	13.84	1.51	1.93
25% R.D. +75% organic+bio (T_5)	10.30	12.19	1.34	1.70
0.0% R.D. +100% organic+bio(T_6)	9.26	10.80	1.20	1.54
LSD at 0.05	0.64	0.78	0.10	0.12

Fruiting growth parameters

1- Weight of 1000 seeds (g)

Data in Table,4 revealed that Weight of 1000 seeds (g) was positively increased by all fertilization in comparison with un-fertilized plants in the two seasons. In this concern, the heaviest 1000 seeds (g) of chia plants was recorded by T3 as it scored 1.43.2 and 1.42 g, followed by T4 which recorded 1.41 and 1.39 g, with nonsignificant differences between them in the two seasons. Also, T2 achieved high increment in this parameter in the two seasons.

Irrespective un-fertilized plants the lowest value of this parameter was scored by T6, followed in ascending order by T5 in the two seasons.

2- Seeds weight / plant (g)

Data in Table,4 showed that all fertilization treatments statistically affected seeds weight / plant as compared with un-fertilized plants in the two seasons. In this sphere, the heaviest seeds / plant of chia plants was scored by T3 treatment as it scored 8.19 and 7.82 g, followed by T2 treatment which gave 7.96, with nonsignificant differences between them in the two seasons.

3- Seeds weight / fed. (kg)

Data in Table,5 pointed out that all fertilization treatments significantly increased seeds weight / fed as compared with un-fertilized plants in the two seasons. In this respect, the highest seeds weight / fed. of chia plants was scored by T3 treatment as it registered 137.6 and 131.4 kg, followed by T2 treatment which recorded 129.2 and 120.0 kg, with significant differences between them in the two seasons.

These results are in close agreement with those reported by **Abdel Wahab *et al*. (2016)** on fennel, **Nabizadeh *et al*. (2012)** on anise, **Tajpoor *et al.* (2013)** on dill, **Talaei *et al*. (2014)** on cumin, **Patidar *et al*. (2016)** on coriander, **Hassanain *et al*. (2018)** on *Tagetes erecta***, Gomaa *et al.* (2018)** on roselle **Youssef *et al.* (2020)** on dutch fennel and **Youssef *et al.* (2020)** on caraway plant.

Table 4. Effect of some fertilization treatments on weight of 1000 seeds and seeds weight / plant of chia plants during the two successive seasons of 2019 and 2020

Characters	Weight of 1000 seeds (g)		Seeds weight / plant (g)	
Seasons / Treatments	1st season	2nd season	1st season	2nd season
Control (T_1)	1.24	1.21	3.17	3.28
100% R.D. of mineral NPK (T_2)	1.39	1.40	7.96	7.40
75% R.D. +25% organic+bio (T_3)	1.43	1.42	8.19	7.82
50% R.D. +50% organic+bio (T_4)	1.41	1.39	6.17	6.04
25% R.D. +75% organic+bio (T_5)	1.32	1.34	5.26	5.10
0.0% R.D. +100% organic+bio(T_6)	1.29	1.28	4.08	3.96
LSD at 0.05	0.11	0.12	0.81	0.62

Fixed oil yield parameters

1- Herb fixed oil percentage

Data presented in Table,5 revealed that, the all studied fertilization treatments increased herb fixed oil percentage per plant in the two seasons. In this respect, the greatest herb oil percentage of chia was scored by T4 treatment as it scored 31.2 and 32.8 %, followed by T3 treatment which gave 29.4 and 31.2 % in the first and second seasons, respectively. Also, T2 gave high significant increases in this concern in the two seasons. The lowest value of this parameter was recorded by un-fertilized plants (T1) as it recorded 23.8 and 25.2 %, followed in ascending order by T6 which scored 25.7 and 27.6 % in the first and second seasons, respectively.

Table 5. Effect of some fertilization treatments on seeds yield / fed. and fixed oil % of chia plants during the two successive seasons of 2019 and 2020

Characters	Seeds yield /fed (kg)		Fixed oil %	
Seasons / Treatments	1st season	2nd season	1st season	2nd season
Control (T_1)	53.3	55.1	23.8	25.2
100% R.D. of mineral NPK (T_2)	129.2	120.0	28.1	29.4
75% R.D. +25% organic+bio (T_3)	137.6	131.4	29.4	31.2
50% R.D. +50% organic+bio (T_4)	103.7	101.5	31.2	32.8
25% R.D. +75% organic+bio (T_5)	88.4	85.7	26.9	28.5
0.0% R.D. +100% organic+bio(T_6)	68.5	66.5	25.7	27.6
LSD at 0.05	6.91	5.84	1.46	1.65

2- Fixed oil yield / plant (ml) and fed. (L)

It is clear from data presented in Table,6 that fixed oil yield per plant and per fed. were increased by using the all studied fertilization treatments when compared with un-fertilized plants in the two seasons. In this concern, the greatest fixed oil yield per plant and per fed. of chia were scored by T3 treatment, followed by T2 treatment without significant differences between them in the first and second seasons, respectively. The lowest value of this parameter was recorded by un-fertilized plants (T1), followed in ascending order by T6 in the two seasons.

The results of oil parameters go on line with those obtained by **Toaima (2005)** on *Achillea millefolium* L., **Saker *et al.* (2012)** on marjoram, **Amran (2013)** on *Pelargonium graveolens*, **Sakr *et al*. (2014)** on sweet basil, **Talaei *et al*. (2014)** on cumin, **Sakr (2017)** on *Calendula officinalis,* **Hassanain *et al*. (2018)** on *Tagetes erecta*, **Gomaa *et al.* (2018)** on roselle, **Youssef *et al.* (2020)** on Dutch fennel and **Youssef *et al.* (2020)** on caraway plant**.**

Table 6. Effect of some fertilization treatments on fixed oil yield / plant and fed. of chia plants during the two successive seasons of 2019 and 2020

Characters	Oil yield /plant (ml)		Oil yield/fed. (L)	
Seasons / Treatments	1st season	2nd season	1st season-	2nd season
Control (T_1)	0.75	0.83	12.6	13.9
100% R.D. of mineral NPK (T_2)	2.16	2.10	36.3	35.3
75% R.D. +25% organic+bio (T_3)	2.14	2.44	40.5	41.0
50% R.D. +50% organic+bio (T_4)	1.93	1.98	32.4	33.3
25% R.D. +75% organic+bio (T_5)	1.41	1.45	23.7	24.4
0.0% R.D. +100% organic+bio(T_6)	1.05	1.09	17.6	18.3
LSD at 0.05	0.21	0.24	2.40	2.81

Chemical constituents' parameters:

1- Leaves nitrogen percentage

Data outlined in Table,7 emphasized that leaves nitrogen percentage was positively affected by all fertilization treatments in the two seasons. In this regard, the highest leaves nitrogen percentage of chia plants was recorded by T2 treatment, followed by T3 treatments, with nonsignificant differences between them in the two seasons.

2- Leaves phosphorus percentage

It obvious from data in Table 7 that all applied fertilization treatments increased the leaves phosphorus percentage in the two seasons. In this concern, the highest leaf phosphorus percentage of chia was scored by T2 treatment as it scored 0.286 and 0.292 %, followed by T3 treatment which recorded 0.281 and 0.287 in the first and second seasons, respectively.

Table 7. Effect of some fertilization treatments on leaves N and P % of chia plants during the two successive seasons of 2019 and 2020

Characters	N%		P%	
Seasons / Treatments	1st season	2nd season	1st season-	2nd season
Control (T_1)	1.39	1.32	0.219	0.213
100% R.D. of mineral NPK (T_2)	1.94	1.91	0.286	0.292
75% R.D. +25% organic+bio (T_3)	1.89	1.84	0.281	0.287
50% R.D. +50% organic+bio (T_4)	1.63	1.79	0.267	0.273
25% R.D. +75% organic+bio (T_5)	1.54	1.62	0.260	0.271
0.0% R.D. +100% organic+bio(T_6)	1.48	1.57	0.251	0.259
LSD at 0.05	0.12	0.14	0.024	0.026

3- Leaves potassium percentage

Data presented in Table,8 pointed out that leaves potassium percentage was increased by using the all studied fertilization treatments in comparison with un-fertilized plants in the two seasons. In this concern, the highest leaf potassium percentage of chia was recorded by T3 treatment as it scored 1.64 and 1.69 %, followed by T2 treatment which gave 1.56 and 1.60 % without significant differences between them in the first and second seasons, respectively.

4- Leaves total carbohydrates percentage

Data in Table, 8 showed that all studied fertilization treatments increased leaves total carbohydrates percentage of chia plants in comparison with un-fertilized plants in the two seasons. Anyhow, the highest leaves total carbohydrates percentage was detected by T3 treatment as it recorded 17.94 and 18.70 %, followed by T2 treatment which gave 16.27 and 17.05 % with significant differences between them in the first and second seasons, respectively.

Table 8. Effect of some fertilization treatments on leaves K and total carbohydrates % of chia plants during the two successive seasons of 2019 and 2020

Characters	K%		Total carbohydrates %	
Seasons / Treatments	1st season	2nd season	1st season-	2nd season
Control (T_1)	1.14	1.19	12.14	11.82
100% R.D. of mineral NPK (T_2)	1.56	1.60	16.27	17.05
75% R.D. +25% organic+bio (T_3)	1.64	1.69	17.94	18.70
50% R.D. +50% organic+bio (T_4)	1.51	1.48	15.14	16.25
25% R.D. +75% organic+bio (T_5)	1.43	1.14	14.62	15.82
0.0% R.D. +100% organic+bio(T_6)	1.36	1.38	14.20	15.05
LSD at 0.05	0.18	0.14	1.06	1.29

The aforementioned results of fertilization concerning chemical constituents are in parallel with those obtained by **Amran (2013)** on *Pelargonium graveolens*, **Mady and Youssef (2014)** on dragonhead, **Ghatas and Abdallah (2016)** on *Echinacea purpurea*, **Mohamed and Ghatas (2016)** on violet, **Badran *et al.* (2017)** on fennel, **Sakr (2017)** on *Calendula officinalis*, **Gomaa *et al*. (2018)** on roselle, **Hassanain *et al*. (2018)** on *Tagetes erecta*, **Youssef *et al.* (2020)** on Dutch fennel and **Youssef *et al.* (2020)** on caraway plant.

The obtained results of this study may be due to the role of fertilization in growth and development of the plants; where the use of N-fixing bacteria (nitrobein) as a bio-fertilizer product containing nitrogen fixing bacteria, e.g. Azotobacter and Azospirillum was found to have not only the ability to fix nitrogen but also to release certain phytohormones of cytokinins, gibberellins and auxins which could enhance plant growth through absorption of nutrients and so on enhancing photosynthesis process **Hegde *et al.* (1999).** Microorganisms used as bio-fertilizers may affect the integrity of growing plants by one mechanism or more such as nitrogen fixation production of growth promoting substances or organic acids, enhancing nutrients uptake or protection against plant pathogens (**Hawaka, 2000).** Also, N-fixers synthesize stimulatory compounds such as, gibberellins, cytokinins and IAA. They act as growth regulators, which increased the surface area per unit of root length and were responsible for root hair branching with an eventual increase in the uptake of nutrients from the soil (**Sperenat, 1990 and Dadarwal *et al.*, 1997).** Besides, the use of Phosphate dissolving bacteria (phosophorein) as a bio-fertilizer product containing very active phoshphate dissolving bactcria has proved its efficiency in enhancing different aspects of growth and development of many plant species including medicinal and aromatic ones. Establishment of a strong root system is related to the level of available phosphate in the soil. Phosphate dissolvers or vesicular arbuscular mycorrhizae and silica bacteria are capable of converting tricalcium phosphate to monocalcium phosphate ready for plant nutrition. Phosphate also increased mineral uptake and water use efficiency (**Hawaka, 2000).** The use of microbial inoculants as biofertilizers is a sustainable agriculture for providing alternative tool of chemical fertilizer by using these farmers can mobilize the potassium present in their own field soil and save some percentage of their potassium fertilizer requirement. Several reports have examined the ability of different bacterial species to solubilize insoluble inorganic phosphate compounds, such as tri-calcium phosphate, di-calcium phosphate, hydroxyl-apatite, and rock phosphate. Among the bacterial genera with this capacity are pseudomonas, Bacillus, Rhizobium, Burkholderia, Achromobacter, Agrobacterium, Microccocus, Aereobacter, Flavobacterium and Erwinia32, 33. There are considerable populations of phosphate solubilizing bacteria in soil and in plant rhizosphere. These include both aerobic and anaerobic strains, with a prevalence of aerobic strains in submerged soils. A considerably higher concentration of phosphate solubilizing bacteria is commonly found in the rhizosphere in comparison with non-rhizosphere soil.

Therefore, the inoculations with KSB and other useful microbial inoculants in the soil become mandatory to restore and maintain the effective microbial populations for solubilization of chemically fixed potassium and availability of other macro and micronutrients to harvest good sustainable yield of various crops. Moreover, when organic manures (compost) added as fertilizer, it led to decrease soil pH which in turn increasing solubility of nutrients for plant uptake, in some cases organic materials may act as low release fertilizer. Recently, on the way of sustainable agriculture with minimum effects, the use of organic manures (compost or chicken manure, ...etc) as natural soil amendments is recommended to replace the soluble chemical fertilizers. They improve the structure of weak-structured sandy soils and increase their water holding capacity. Also, they improve soil fertility, and stimulate root development, induce active biological conditions and enhancing activities of micro-organisms especially those involved in mineralization (**Suresh *et al.*, 2004)**. Furthermore, to interpret and evaluate the effect of chemical fertilization concerned in this study, on augmenting the different tested vegetative growth parameters, yield component parameters and chemical constituents of dragonhead plants it is important to refer to the physiological roles of nitrogen, phosphorus and potassium in plant growth and development. Such three macronutrient elements are the common elements usually included in fertilizers. Plant supplement with these macronutrients in form of fertilizers is necessary because the soil is usually in deficient of them due to plant removal leaching or they are not readily available for plants. Therefore, such addition of well-balanced NPK fertilization quantities insured production of high productivity and chemical constituents of dragonhead plants.

The role of NPK fertilization in promoting vegetative growth characters, enhancing yield component parameters and increasing growth, as well as stimulating the chemical constituent's content of dragonhead plants could be explained by recognizing their fundamental involvement in the very large number of enzymatic reactions that depend on NPK fertilization. NPK reflected directly on increasing the content of total carbohydrates, total sugars and total free amino acids as well as NPK % in the leaves were indirectly the cause for enhancing the augmenting of all other vegetative growth traits and chemical constituents of chia plants (**Cooke, 1982).**

Consequently, it is preferable to treat chia plants with the combined treatment of 75% recommended dose of chemical fertilizer + 25% organic + bio or for enhancing growth and productivity of this plant.

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