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Response of caraway (*Carum carvi* L.) plant to bio-fertilizers in substitution of chemical fertilization

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Abstract
The present work was performed on caraway plants during the two successive seasons of 2017/2018 and 2018/2019 at Baloza Research Station, North Sinai Governorate, Egypt to evaluate the effect of bio-fertilizer applications as partial replacement for chemical fertilization on vegetative growth, seeds yield, essential oil productivity and its main components as well as chemical constituents of caraway under Sinai conditions. Treatments were the combination of five fertilization levels (full recommended dose of NPK only, 75, 50, 25% of the recommended NPK plus bio-fertilizers and bio-fertilizers only). The results showed that application of T_2 (75% of the recommended NPK plus bio-fertilizers) increased vegetative growth, yield, maximized essential oil percentage and yield compared to other treatments. However, the minimum values of vegetative growth, yield and essential oil productivity were obtained with plants treated by T_5 (bio-fertilizers only). Finally, carvone and limonene were the main chemical constituents of essential oil for caraway.

Keywords: *Carum carvi*, bio-fertilizers, growth, yield and essential oil

Introduction
Caraway (*Carum carvi* L.), is an annual or perennial herb. It is originally from Europe and West Asia [1]. It is an important plant containing volatile oil in the fruits. Seeds of caraway contain essential oil (2-8%), with carvone and limonene as the main constituents, lipids, nitrogen compounds, fiber and water [2]. The quality and effect of essential oil of caraway seeds depends mainly on carvone and limonene content [3]. Caraway has carminative, anti-diabetic, antioxidant, anti-cancer and diuretic effects. Also, it promotes digestion and encourages the production of breast milk and lactation [4, 5].
Many factors influence oil content in umbelliferous crops i.e. fertilization, salinity and irrigation [6]. Also, balanced mineral fertilization of aromatic plants is an important cultivation factor determining essential oil quantity and quality [7]. Chemical fertilization is used for increasing the productivity of medicinal and aromatic plants. However, excessive use of manufactured fertilizers raised production cost, caused environmental pollution and decreased the acceptance of the crops for export as well as affected the soil fertility [8]. For these purposes it is recommended that mineral fertilization (NPK) be completely or partially substituted by the use of healthy and economical biofertilizers for farmers.
Bio-fertilizers are substances contain living microorganisms when added to seeds, plants, or soil colonize the rhizosphere and promote growth by increasing the supply or availability of primary nutrients to the host plant [9]. Recently, bio-fertilizers have been increasingly used in modern agriculture due to the wide knowledge in rhizospheric biology and the discovery of the promotive microorganism. Bio-fertilizers are environmentally friendly, economical and their continuous use improves soil fertility [10] by fixing the atmospheric nitrogen and solubilizing insoluble phosphate and produce plant growth-promoting substances in the soil [11]. Also, [12] reported that the use of bio-fertilizers improved crop yield by increasing contents of vitamins, essential amino acids, proteins and nitrogen fixation. *Azotobacter chroococcum* (nitrogen fixing bacteria), *Bacillus megaterium* var. *phosphaticum* (phosphate solubilizing bacteria) are known as plant growth promoting rhizobacteria [13]. *Bacillus megaterium* dissolves phosphate in the soil into a soluble form by producing organic acids which lower the pH and cause the dissolution of bound forms of phosphate. Active dry yeasts (*Saccharomyces cerevisiae*) are

natural safety bio-fertilizers cause various promotive effect on plants. They are natural source of cytokinin which stimulates cell division and enlargement as well as the synthesis of protein, nucleic acid and vitamin B. Also, they release carbone dioxide which improves net photosynthesis [14, 15].

The Egyptian government promotes the cultivation of medicinal and aromatic plants in Sinai, since the climate conditions are ideal for growing these plants in order to increase exports. The goal of the present research was thus to evaluate the effect of chemical and biofertilization on vegetative growth, seed yield, oil production and the main components of caraway plants under sandy soil conditions at Baloza Station, North Sinai, Egypt.

Materials and Methods

During the two successive seasons of 2017/2018 and 2018/2019, a two-year experiment was performed at the Desert Research Center Agricultural Experimental Station in Baloza Village, North Sinai Governorate, Egypt. Seeds of caraway (*Carum carvi* L.) were imported from the Department of Medicinal and Aromatic plants, Horticulture Research Institute (HRI), Dokki, Giza to be used in this work.

Soil

A soil sample was randomly collected from the depth of 0 to 30 cm to determine the chemical and physical properties of the soil. The texture of experimental farm soil in Baloza Station was of sandy nature. This was demonstrated clearly from the mechanical and chemical analysis of the used soil before cultivation as shown in Tables (A, B).

Water irrigation

Water sample was taken from the irrigation system of Baloza Experimental Station for analysis. The water analysis of the used irrigation water is shown in Table (C).

Compost

Compost manure was added at 10 m^3/feddan throughout soil preparation before planting in each season. The chemical analysis of the used compost manure is shown in Table (D).

Table A. Mechanical analysis of the experimental soil area.

Depth (cm)	Sand (%)	Silt (%)	Clay (%)	Soil texture
0-30	90	5	5	Sandy

Table B. Chemical analysis of the experimental soil area.

pH		E.C. (ds/m)	O.M. (%)	Soluble anions (meq/l)				Soluble cations (meq/l)			
				CO_3^{--}	HCO_3^-	Cl^-	SO_4^{--}	Ca^{++}	Mg^{++}	Na^+	K^+
8.17		5.99	0.17	-	2.46	40.71	17.17	19.53	13.25	26.91	0.65

Table C. The chemical analysis of irrigation water.

pH	E.C. (ppm)	Soluble anions (meq/l)				Soluble cations (meq/l)			
		CO_3^{--}	HCO_3^-	Cl^-	SO_4^{--}	Ca^{++}	Mg^{++}	Na^+	K^+
7.76	2491	0.52	1.96	28.86	7.59	4.51	11.75	22.08	0.59

Table D: Chemical analysis of the used compost manure

pH	EC (ds/m)	O.M. (%)	C/N Ratio (%)	N (%)	P (%)	K (%)	Fe (%)	Mn (mg/kg)	Zn (mg/kg)	Cu (mg/kg)
8.8	4.6	20.5	18.85	1.03	0.22	2.04	3.43	606.8	85.65	43.60

Seed Sowing

Seeds were sown into hills at 6th October in the first season and 8th October in the second season within the drippers of irrigation lines and irrigation was done immediately after sowing. The distance between rows was 75 cm and the distance between plants was 30 cm, drip irrigation lines with 4 liter/hour were used. Thinning was conducted one month after sowing leaving two plants per hill.

Chemical fertilizer sources

The sources of NPK chemical fertilizers were ammonium sulphate (20.5% N), calcium superphosphate (15.5% P_2O_5) and potassium sulphate (48% K_2O).

Bio-fertilization Treatments

Bio-fertilizers used were *Azotobacter chroococcum*, *Bacillus megaterium* var. phosphaticum, and Active dry yeast (*Saccharomyces cerevisiae*). A liquid bio-fertilizer from *Azotobacter chroococcum* and *Bacillus megaterium* var. phosphaticum was obtained from soil fertility and Microbiology Department, Desert Research Center (DRC), Egypt. The liquid bio-fertilizer was added to the soil as a soil drench. Active dry yeast (*Saccharomyces cerevisiae*) was dissolved in water followed by adding sugar at the ratio of 1:1 (6g/l from active dry yeast and 6 g/l from sugar) and kept overnight for activation and reproduction of yeast [16]. Active dry yeast was added as a foliar spray.

All horticultural practices including irrigation and pest and diseases control were done as used recommendation in this respect.

Treatments

Basically, the experiment consisted of five fertilization levels, as follow:

1. Application of full recommended dose of NPK (300 kg ammonium sulphate, 300 kg calcium superphosphate and 80 kg potassium sulphate per feddan).
2. Application of 75% from full recommended dose of NPK (225 kg ammonium sulphate, 225 kg calcium superphosphate and 60 kg potassium sulphate per feddan) + bio-fertilizers.
3. Application of 50% from full recommended dose of NPK (150 kg ammonium sulphate, 150 kg calcium superphosphate and 40 kg potassium sulphate per feddan) + bio-fertilizers.
4. Application of 25% from full recommended dose of NPK (75.5 kg ammonium sulphate, 75.5 kg calcium superphosphate and 20 kg potassium sulphate per feddan) + bio-fertilizers.
5. Application of bio-fertilizers only.

Application time of chemical fertilizers and bio-fertilizers

Chemical fertilization with calcium superphosphate was conducted immediately before sowing in each season in only one dose during the preparation of the soil for cultivation. Nitrogen and potassium fertilizers were applied in three equal

doses at 60, 90 and 120 days after seed sowing in both seasons. Bio-fertilizers were added at three times per season. The first one was after 60 days after seed sowing, followed by second and third one after 90 and 120 days from seed sowing in both seasons, respectively.

Growth evaluation

Harvesting was carried out at 15th May in the first season and 19th May in the second season. Plant height (cm) and number of branches/plant were recorded before harvest. After harvest, data recorded were herb fresh and dry weights/plant (g), number of umbels/plant, weight of 1000 seeds (g), fruit yield/plant (g) and fruit yield/feddan (kg). Essential oil percentage of caraway seeds was determined by hydro distillation for 3 h using the method of [17], the oil percentage was used to calculate essential oil yield/plant (ml), and per feddan (l). The extracted volatile oil was dehydrated over anhydrous sodium sulphate and stored in a refrigerator until GC/MS analysis.

Chemical analysis

Chlorophyll a, chlorophyll b, total chlorophyll and carotenoids (mg/g f.w.) were determined in leaf fresh samples in both seasons as described by [18]. N, P and K percentages as well as total carbohydrates in the dry herb were estimated at flowering time. Nitrogen was determined by modified micro Kjeldahle method as described by [19]. Phosphorus was colorimetrically determined using the method described by [20] using spectrophotometer at 882 µv. Potassium percentage was estimated using flame photometry according to [21]. Total carbohydrates in the dry leaves were determined by using a colorimetric method of [22].

Essential oil GC/MS analysis

The GC-MS analysis of the essential oil samples was carried out using gas chromatography-mass spectrometry instrument stands at the Laboratory of Medicinal and Aromatic Plants Research, National Research Centre with the following specifications. Instrument: a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with: a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GCMS system was equipped with a TR5 MS column (30 m x 0.32 mm i.d., 0.25 µm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.0 mL/min at a split ratio of 1:10 and the following temperature program: 60°C for 1 min; rising at 4.0 C/min to 240°C and held for 1 min. The injector and detector were held at 200 and 240°C, respectively. Diluted samples (1:10 hexane, v/v) of 1 µL of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450. Most of the compounds were identified using two different analytical methods: mass spectra (authentic chemicals, Wiley spectral library collection and NSIT library).

Experiment layout and statistical analysis

This experiment was arranged in a complete randomized block design system, each treatment contained three replicates of 10 plants for each replicate. The obtained results were statistically analyzed by using MSTATC program [23]. Analysis of variance was performed to determine significant differences. Means were compared using LSD test at 0.05 level according to [24].

Results and Discussion

Vegetative growth measurements

1. Plant height: It is observed from Data in Table (1) that, the different fertilization treatments had a noticeable effect on the average plant height of caraway plants during the two successive seasons. The results indicate that, fertilization with T_2 (75% NPK + bio-fertilizers) gave the highest values of plant height (90.67 and 91.12 cm) compared to other fertilization treatments in the first and second seasons, respectively, and came in the second place the plants which were treated by T_1 (full NPK dose only) as its values were (87.00 and 88.68 cm) in the first and second seasons, respectively with significant differences between them in the first season but there were not significant differences between them in the second season. Meanwhile, T_5 (bio-fertilizers only) produced the shortest plants (76.27 and 77.27 cm) in the first and second seasons, respectively and followed in ascending order by those received T_4 (25% NPK + bio-fertilizers) as it scored 76.93 and 78.27 cm in the first and second seasons, respectively.

2. Number of branches per plant: Data recorded on number of branches per plant as affected by different fertilization treatments shown in Table (1). Data reveal that, growth behavior showed logical relationship to different fertilization levels. It is obvious that application of T_2 (75% NPK + bio-fertilizers) gave the best plant height resulted in the highest number of branches/plant as it recorded 11.47 and 11.73 branches/plant in the first and second seasons, respectively. Whereas T_1 (full NPK dose only) produced the next lower values in this parameter as it recorded 10.73 and 11.00 branches/plant in the first and second seasons, respectively. The differences between the above-mentioned treatments were non-significant in the two seasons. While, the lowest number of branches per plant (5.33 and 5.40 branches/plant) were recorded with plants treated with T_5 (bio-fertilizers only) in the first and second seasons, respectively, followed in ascending order by T_4 and T_3 in the two seasons.

Table 1: Effect of chemical fertilizers and bio-fertilizers on some vegetative growth parameters of caraway plants during the two seasons of 2017/2018 and 2018/2019.

Treatment	Plant height (cm)	Number of branches/plant	Fresh weight of herb/plant (g)	Dry weight of herb/plant (g)
First season				
T1	87.00	10.73	202.21	78.83
T2	90.67	11.47	233.68	91.53
T3	85.00	10.33	190.48	74.30
T4	76.93	6.73	104.00	49.65
T5	76.27	5.33	75.54	30.81
LSD at $p<0.05$	2.96	1.44	36.05	13.81
		Second season		
T1	88.68	11.00	231.12	79.43
T2	91.12	11.73	249.06	95.55
T3	86.20	10.53	209.55	79.35

T4	78.27	7.13	129.46	54.02
T5	77.27	5.40	78.57	35.03
LSD at $p<0.05$	3.55	2.15	43.41	7.87

T_1 (full recommended dose of NPK), T_2 (75% NPK recommended dose plus bio-fertilizers), T_3 (50% NPK recommended dose plus bio-fertilizers), T_4 (25% NPK recommended dose plus bio-fertilizers), T_5 (bio-fertilizers only).

3. Fresh weight of herb/plant (g): Results in Table (1) indicate that, plants treated with T_2 (75% NPK + bio-fertilizers) gave the highest fresh weight of herb (233.68 and 249.06 g/plant) in the first and second seasons, respectively compared to other treatments and came in the second place T_1 (full recommended dose) {202.21 and 231.12 g/plant} in the first and second seasons, respectively with non-significant differences between them in both seasons. Meanwhile, T_3 (50% NPK + bio-fertilizers) scored the third place in both seasons. Furthermore, the lowest values resulted from T_5 (bio-fertilizers only) as (75.54 and 78.57 g/plant) in the both seasons, respectively.

4. Dry weight of herb/plant (g): Table (1) show that, the heaviest dry weight of herb (91.53 and 95.55 g/plant) in the first and second seasons, respectively, was obtained with T_2 (75% NPK + bio-fertilizers), followed in descending order by those treated with T_1 (full recommended dose) as its values were 78.83 and 79.43 g/plant, T_3 (50% NPK + bio-fertilizers) and T_4 (50% NPK + bio-fertilizers) respectively. The differences between T_2 and T_1 were non-significant in the first season and significant in the second one. But, the lowest plant dry weight of caraway was obtained by T_5 (bio-fertilizers only) as it recorded 30.81 and 35.03 g/plant in both seasons, respectively, compared to other treatments in both seasons.

The aforementioned results of fertilization concerning vegetative growth are in parallel with those obtained by [25-27] on fennel, [28] on *Hibiscus sabdariffa*, [29] on *Tanacetum vulgare*, [30] on black cumin, [31] on *Anethum graveolens* L., [32] on caraway and [33] on roselle.

Yield Characters

1. Number of umbels per plant: Data in Table (2) illustrated that, treated caraway plants by T_2 (75% NPK + bio-fertilizers) produced the highest values of number of umbels per plant as it recorded 67.53 and 70.00 umbels/plant in the first and second seasons, respectively compared to other treatments. The next lower place was occupied by T_1 (full dose of NPK only) as it scored 64.73 and 66.27 umbels/plant in the first and second seasons, respectively with non-significant differences between the above-mentioned treatments in both seasons. Meanwhile T_5 (bio-fertilizers only) gave the lowest values of number of umbels per plant (42.00 and 43.33) in the first and second seasons, respectively and followed in ascending order by T_4 (25% NPK + bio-fertilizers) and T_3 (50% NPK + bio-fertilizers) in the two seasons, respectively.

2. Weight of 1000 seeds (g): It is observed from data in Table (2) that, the highest weight of 1000 seeds of caraway was obtained with T_2 (75% NPK plus bio-fertilizers) as it scored 9.27 and 9.53 g in the first and second seasons, respectively, followed by T_1 (full recommended dose) with non-significant differences in the two seasons. However, the lowest values were recorded with T_5 (bio-fertilizers only) as it scored 5.96 and 6.08 g in first and second seasons, respectively and followed in ascending order by T_4 (25% NPK + bio-fertilizers) and T_3 (50% NPK + bio-fertilizers) in the two seasons, respectively.

Table 2: Effect of chemical fertilizers and bio-fertilizers on yield parameters of caraway plants during the two seasons of 2017/2018 and 2018/2019.

Treatment	Number of umbels/plant	Weight of 1000 seeds (g)	Seed yield/plant (g)	Seed yield/feddan (kg)
		First season		
T1	64.73	9.17	19.05	711.27
T2	67.53	9.27	22.50	840.16
T3	63.87	8.98	18.96	708.03
T4	49.53	8.39	12.11	452.14
T5	42.00	5.96	6.18	230.70
LSD at $p<0.05$	3.91	0.27	7.31	172.76
		Second season		
T1	66.27	9.30	21.19	790.93
T2	70.00	9.53	23.72	885.65
T3	65.33	9.20	20.97	782.99
T4	51.53	8.55	14.15	528.10
T5	43.33	6.08	7.85	292.97
LSD at $p<0.05$	8.44	0.54	4.70	175.56

T_1 (full recommended dose of NPK), T_2 (75% NPK recommended dose plus bio-fertilizers), T_3 (50% NPK recommended dose plus bio-fertilizers), T_4 (25% NPK recommended dose plus bio-fertilizers), T_5 (bio-fertilizers only).

3- Seed yield/plant (g): Table (2) showed that, seed yield per plant takes the similar trend with number of umbels as the highest values were recorded with plants treated with T_2 (75% NPK plus bio-fertilizers) as the values were 22.50 and 23.72 g/plant in the first and second seasons, respectively, followed in descending order by T_1 (full NPK recommended dose) {19.05 and 21.19 g/plant} in the first and second seasons, respectively with non-significant differences between them in the two seasons. Meanwhile, the lowest values were observed in the fertilized plants with T_5 (bio-fertilizers only) {6.18 and 7.85 g/plant} in the first and second seasons, respectively, compared to other treatments in both seasons and the next higher values were obtained from application of T_4 (25% NPK + bio-fertilizers) and T_3 (50% NPK + bio-fertilizers) in the two seasons, respectively.

4. Seed yield/feddan (kg): According to data in Table (2), seed yield/feddan (kg), as well as seed yield/plant (g), gave the highest values with application of T_2 (75% NPK + bio-fertilizers) as it scored 840.16 and 885.65 kg/feddan in first

and second seasons, respectively and application of T_1 (full NPK recommended dose) recorded the next lower values (711.27 and 790.93 kg/feddan) in the first and second seasons, respectively. The differences between the above-mentioned treatments were non-significant. However, the lowest seed yield/feddan of caraway was recorded with T_5 (bio-fertilizers only) as its values were 230.70 and 292.97 kg/feddan in the first and second seasons, respectively, compared to other treatments in the both seasons and followed in ascending order by T_4 (25% NPK + bio-fertilizers) and T_3 (50% NPK + bio-fertilizers) in both seasons, respectively.

These results are in close agreement with those reported by [34-39] on fennel, [40, 41] on caraway, [42] on anise, [43, 44] on dill, [45, 46] on coriander, [47, 48] on cumin.

Oil Characters

1. Essential oil percentage: As indicated in Table (3), the richest essential oil percentage was obtained with T_2 (75% NPK + bio-fertilizers) as it scored 2.76 and 2.92% in the first and second seasons, respectively, followed in descending order by T_1 (full NPK recommended dose) in the first season with non-significant differences between them and T_3 (50% NPK + bio-fertilizers) came in the second place in the second season with non-significant differences between T_3 and T_1. Meanwhile, the lowest values were recorded with T_5 (bio-fertilizers only) as the values were 2.33 and 2.38% in the first and second seasons, respectively and the next higher values were obtained with T_4 (25% NPK + bio-fertilizers) in the two seasons.

2. Oil yield/plant (ml): Concerning oil yield per plant (ml), data in Table (3) show that application of T_2 (75% NPK recommended dose + bio-fertilizers) recorded the highest values as it scored 0.62 and 0.70 ml/plant in the first and second seasons, respectively, followed by T_1 (full NPK recommended dose) as the values were 0.49 and 0.56 ml/plant in the first and second seasons, respectively. While, the lowest values were obtained with plants treated by T_5 (bio-fertilizers only) as it scored 0.14 and 0.19 ml/plant in the first and second seasons, respectively, compared to other treatments in both seasons and followed in ascending order by T_4 (25% NPK + bio-fertilizers) and T_3 (50% NPK + bio-fertilizers) in the two seasons, respectively.

3. Oil yield/feddan (liter): Table (3) show that, oil yield per feddan takes the same line with oil yield per plant as the highest values occurred with T_2 (75% NPK recommended dose + bio-fertilizers) as its values were 23.05 and 26.05 L/feddan in the first and second seasons, respectively. The next lower place was occupied by T_1 (full NPK recommended dose) {18.41 L/feddan} in the first season and T_3 (50% NPK recommended dose + bio-fertilizers) occupied the second place (20.95 L/feddan) in the second season. The differences between the above-mentioned treatments were not significant in both seasons. However, T_5 (bio-fertilizers only) recorded the lowest values (5.35 and 6.97 L/feddan) in the first and second season, respectively and the next higher values were recorded by T_4 (25% NPK recommended dose + bio-fertilizers) in both seasons.

Table 3: Effect of chemical fertilizers and bio-fertilizers on essential oil productivity of caraway plants during the two seasons of 2017/2018 and 2018/2019.

Treatment	Essential oil percentage	Oil yield/plant (ml)	Oil yield/feddan (l)
First season			
T1	2.58	0.49	18.41
T2	2.76	0.62	23.05
T3	2.54	0.48	17.99
T4	2.46	0.30	11.07
T5	2.33	0.14	5.35
LSD at $p<0.05$	0.24	0.18	6.82
Second season			
T1	2.63	0.56	20.84
T2	2.92	0.70	26.05
T3	2.68	0.56	20.95
T4	2.53	0.36	13.41
T5	2.38	0.19	6.97
LSD at $p<0.05$	0.27	0.16	6.00

T_1 (full recommended dose of NPK), T_2 (75% NPK recommended dose plus bio-fertilizers), T_3 (50% NPK recommended dose plus bio-fertilizers), T_4 (25% NPK recommended dose plus bio-fertilizers), T_5 (bio-fertilizers only).

The results of oil parameters go on line with those obtained by [34, 35, 38, 49, 50, 51] on fennel, [52, 53] on coriander, [54] on *Achillea millefolium* L., [55] on *Mentha piperita*, [56 - 58] on dill, [59] on marjoram, [60] on *Pelargonium graveolens*, [61] on sweet basil, [48] on cumin

Photosynthetic Pigments

1. Chlorophyll a: Data in table (4) indicate that, the highest values of chlorophyll a (1.352 and 1.353 mg/g) in the first and second seasons, respectively were obtained from T_2 treatment (75% NPK plus bio-fertilizers) and followed in descending order by T_1 (full NPK recommended dose) as it recorded 1.160 and 1.155 mg/g in the first and second seasons, respectively with significant differences between them. While, the treated plants by T_5 (bio-fertilizers only) gave the lowest values (0.746 and 0.753 mg/g) in the first and second seasons, respectively, compared to other treatments in both seasons and the next higher values were obtained from plants treated with T_4 (25% NPK recommended dose + bio-fertilizers) and T_3 (50% NPK recommended dose + bio-fertilizers) in both seasons, respectively.

2. Chlorophyll b: Data in Table (4) regarding chlorophyll b content in caraway reveal that, the highest values were recorded by T_5 as it scored 0.617 and 0.611 mg/g in the first and second seasons, respectively. The next lower place was occupied by T_2 as the values were 0.602 and 0.600 mg/g in the first and second seasons, respectively with non-significant differences between them. Meanwhile, the lowest values (0.479 and 0.481 mg/g) were recorded by T_4 treatment, in the

first and second seasons, respectively, followed in ascending order by T_3 treatment in both seasons.

3. Total Chlorophyll: It is obvious from data in Table (4) that, total chlorophylls in treated plants by T_2 (75% NPK recommended dose plus bio-fertilizers) gave the highest values (1.954 and 1.953 mg/g) in the first and second seasons, respectively and followed in descending order by T_1 (full NPK recommended dose) as it recorded 1.761 and 1.748 mg/g in the first and second seasons, respectively with significant differences between them. While, the lowest values were recorded by T_4 treatment (25% NPK recommended dose plus bio-fertilizers) as it scored 1.253 and 1.263 mg/g in the first and second seasons, respectively and T_5 occupied the next higher values in both seasons.

4. Carotenoids: Results in Table (4) show that, T_2 (75% NPK recommended dose plus bio-fertilizers) recorded the highest values of carotenoids (0.721 and 0.395 mg/g) in the first and second seasons, respectively and the next lower values (0.613 and 0.328 mg/g) resulted from application of T_1 treatment (full NPK recommended dose). The differences between the above-mentioned treatments were significant in both seasons. On the other hand, the lowest values of carotenoids in the herb of caraway were recorded by T_5 treatment (bio-fertilizers only) {0.370 and 0.196 mg/g} in the first and second seasons, respectively, compared to other treatments.

Table 4: Effect of chemical fertilizers and bio-fertilizers on photosynthetic pigments of caraway plants during the two seasons of 2017/2018 and 2018/2019.

Treatment	Chl. a (mg/g)	Chl. B (mg/g)	Total chl. (mg/g)	Carotenoids (mg/g)
First season				
T1	1.160	0.601	1.761	0.613
T2	1.352	0.602	1.954	0.721
T3	0.839	0.512	1.351	0.429
T4	0.774	0.479	1.253	0.421
T5	0.746	0.617	1.363	0.370
LSD at $p<0.05$	0.114	0.051	0.134	0.048
Second season				
T1	1.155	0.593	1.748	0.328
T2	1.353	0.600	1.953	0.395
T3	0.847	0.516	1.363	0.235
T4	0.782	0.481	1.263	0.242
T5	0.753	0.611	1.364	0.196
LSD at $p<0.05$	0.107	0.055	0.128	0.046

T_1 (full recommended dose of NPK), T_2 (75% NPK recommended dose plus bio-fertilizers), T_3 (50% NPK recommended dose plus bio-fertilizers), T_4 (25% NPK recommended dose plus bio-fertilizers), T_5 (bio-fertilizers only).

Chemical Composition

Nitrogen percentage: Concerning nitrogen percentage, data in Table (5) show that the highest values of nitrogen percentage in the herb of caraway were obtained by T_5 (1.963 and 2.006%) in the first and second seasons, respectively and followed in descending order by T_3 with significant differences between them in both seasons. On the opposite, the lowest values of nitrogen percentage were obtained by treating the plants with T_4 treatment (25% NPK recommended dose plus bio-fertilizers) {1.189%} in the first season while T_1 recorded the lowest values in the second season (1.152%) respectively. However, the rest treatments occupied intermediate place between the aforesaid treatments.

Phosphorus percentage: Data indicated in Table (5) reveal that, T_5 recorded the highest values (0.220 and 0.221%) in the first season and second seasons, respectively followed by T_3 treatment as (0.146%) in the first season with significant differences between them and followed in the second season by T_4 as (0.112%) with significant differences between them. Meanwhile, the lowest values of phosphorus in the herb of caraway were recorded by T_2 as the values were (0.101 and 0.103%) in the first and second seasons, respectively.

Potassium percentage: Data of Table (5) indicate that, the highest values of potassium percentage in the herb of caraway were obtained as a result of using T_5 treatment in the two seasons as it recorded 1.577 and 1.683%, respectively followed by T_3 treatment in the first and second seasons with non-significant differences between them in the first season but the differences were significant in the second season. On the opposite, the lowest percentage of potassium was obtained by treating the plants with T_2 treatment in the first season as the values were 1.340% but in the second seasons T_1 gave the lowest values (1.380%).

Total carbohydrates (%): It is obvious from data in table (5) that, the highest total carbohydrates percentage in the herb of caraway was obtained from the treatment of T_2 (75% NPK recommended dose plus bio-fertilizers) as it recorded 17.90% in the first season only but T_3 (50% NPK recommended dose plus bio-fertilizers) gave the highest value (18.02%) in the second season. While, the lowest values (12.71 and 13.59%) were occurred with T_5 (bio-fertilizers only) in the two seasons.

The aforementioned results of fertilization concerning chemical constituents are in parallel with those obtained by [26, 39, 49 - 51, 62] on fennel, [56] on dill, [60] on *Pelargonium graveolens*, [63] on dragonhead, [64] on *Echinacea purpurea*, [65] on violet, [66] on *Calendula officinalis*, [33] on roselle, [67] on *Tagetes erecta* and [68] on collard.

Table 5: Effect of chemical fertilizers and bio-fertilizers on herb chemical composition of caraway plants during the two seasons of 2017/2018 and 2018/2019.

Treatment	N percentage	P percentage	K percentage	Total Carbohydrates
First season				
T1	1.593	0.142	1.384	17.80
T2	1.296	0.102	1.340	17.90
T3	1.800	0.146	1.529	17.77
T4	1.189	0.109	1.491	17.72
T5	1.963	0.220	1.577	12.71
LSD at $p<0.05$	0.116	0.059	0.092	0.059
Second season				
T1	1.152	0.112	1.380	17.59
T2	1.169	0.103	1.386	17.57
T3	1.712	0.110	1.506	18.02
T4	1.467	0.112	1.447	17.49
T5	2.006	0.221	1.683	13.59
LSD at $p<0.05$	0.146	0.059	0.084	0.059

T_1 (full recommended dose of NPK), T_2 (75% NPK recommended dose plus bio-fertilizers), T_3 (50% NPK recommended dose plus bio-fertilizers), T_4 (25% NPK recommended dose plus bio-fertilizers), T_5 (bio-fertilizers only).

Essential oil GC/MS analysis of caraway plants

The GC/MS analysis of caraway essential oil as indicated in Table (6) showed the presence of 22 compounds with the

presence of Carvone as the main components followed by Limonene. The highest percentage of Carvone (77.33%) was obtained by T_5 (bio-fertilizers only), followed by T_1 (full recommended dose of NPK) {75.83%} and T_4 (25% NPK recommended dose plus bio-fertilizers) {75.07%}, respectively. While, the lowest percentage of Carvone (68.89%) occurred with T3 (50% NPK recommended dose plus bio-fertilizers) compared to other treatments. The highest percentage of Limonene (28.75%) resulted from T_3 (50% NPK recommended dose plus bio-fertilizers) and came in the second place T_2 (75% NPK recommended dose plus bio-fertilizers) {25.10%} and T_4 (25% NPK recommended dose plus bio-fertilizers) {24.25%}, respectively. While, the lowest percentage of Limonene (21.56%) was obtained by T_5 (bio-fertilizers only) compared to other treatments.

Table 6: Essential oil GC/MS analysis of caraway plants treated by chemical fertilizers and bio-fertilizers treatments in the second season 2018/2019.

Compounds		Concentration of compounds (%)				
	R.T.	T1	T2	T3	T4	T5
α-Pinene	4.28	–	–	0.09	0.05	0.03
Sabinene	5.25	0.03	0.03	0.06	0.04	0.03
ß-Pinene	5.39	–	0.04	0.13	0.05	0.03
α-Myrcene	5.76	0.05	0.06	0.08	0.06	0.05
Limonene	6.89	23.45	25.10	28.75	24.25	21.56
Fenchone	9.09	–	–	–	0.04	0.10
Verbenol	10.52	–	0.03	–	–	–
Limonene oxide	10.99	0.03	–	–	–	0.04
Camphor	11.40	–	–	0.07	–	–
Terpinen-4-ol	12.75	–	–	0.08	–	–
α-Terpineol	13.46	0.03	0.04	0.09	0.05	0.07
Dihydrocarvone	13.84	0.15	0.16	0.13	0.14	–
Carveol	14.57	0.03	–	–	0.03	–
Carveol, dihydro-, cis-	14.93	0.10	0.11	1.30	0.12	–
Estragole	14.94	–	–	–	–	0.69
Carvone	15.60	75.83	74.20	68.89	75.07	77.33
Caryophyllene	22.20	0.10	0.05	0.21	0.10	0.04
Germacrene D	24.80	0.07	0.04	–	–	–
α-elemene	25.86	–	0.08	–	–	–
Cubedol	26.37	0.04	–	–	–	–
Caryophyllene oxide	28.91	0.05	0.04	0.05	–	0.03
Veridiflorol	29.46	0.04	–	0.07	–	–
Total		100	99.98	100	100	100

T_1 (full recommended dose of NPK), T_2 (75% NPK recommended dose plus bio-fertilizers), T_3 (50% NPK recommended dose plus bio-fertilizers), T_4 (25% NPK recommended dose plus bio-fertilizers), T_5 (bio-fertilizers only).

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 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 [69]. 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 [70]. 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 [71, 72]. Besides, the use of Phosphate dissolving bacteria as a bio-fertilizer product containing very active phoshphate dissolving bacteria 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 [70].

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 caraway 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 caraway plants.

The role of NPK fertilization on promoting vegetative growth characters, enhancing yield component parameters and oil yield and as well as stimulating the chemical constituents content of caraway plants could be explained by recognizing their fundamental involvement in the very large number of enzymatic reaction 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, oil yield and components of caraway plants [73]. In this context, [53] stated that using full dose of NPK or 75% NPK + Bio. + SA. gave the best growth and chemical constituents parameters of coriander plants.

Conclusion

It is preferable from previous results to treat caraway plants with the treatment of 75 percent of the prescribed NPK plus bio-fertilizers to improve this plant's growth and oil productivity. The present study therefore strongly admits the use of such treatment to provide good and high export characteristics due to its role in human health in terms of protection.

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