Effect of organic and chemical fertilizers on morpho-physiological and biochemical properties of ajowan (*Trachyspermum ammi* L.)

Shahnaz Fathi[1] and Sharareh Najafian[2]*

1. Department of Medicinal and Aromatic Plants, Shahid Bakeri Higher Education Center of Miandoab, Urmia University, Iran
2. Department of Agriculture, Payame Noor University, Tehran, Iran

---

Abstract

Organic fertilizers play a significant role in organic systems and sustainable soil management. In general, organic fertilizers increase soil fertilization and can reduce the negative effects of the excessive use of chemical fertilizers and synthetic man-made fertilizers. This study was carried out in the Medicinal Plants Farm of Shahid Bakeri Higher Education Center of Miandoab during summer 2017. Thus, the main objective of the present investigation was to study the effect of organic and chemical fertilizers on the morpho-physiological features (diameter of plant, plant height, number of branch, 1000-seed weight, and seed yield), biochemical contents (amount of N, P, K, Soluble sugar, Carotenoids, Chlorophyll a, b, total phenol content, and Proline content) and essential oil content of ajowan. Results showed that the fertilizers could significantly enhance all determined factors as compared to control samples. Vermicompost had a better effect than the chemical fertilizer in some factors including total phenol, chlorophylls, carotenoids, total soluble sugar, nitrogen, phosphate, and potassium contents (P<0.05). The vermicompost as a potential source of plant nutrients for sustainable ajowan production can reduce health and environmental problems.

Keywords: Compost, Cow manure, total phenol, *Trachyspermum ammi* (L.), Soluble sugar, Vermi-compost.

Fathi, S. and S. Najafian. 2020. 'manuscript title: Effect of organic and chemical fertilizers on morpho-physiological and biochemical properties of ajowan (*Trachyspermum ammi* L.) '. *Iranian Journal of Plant Physiology* ,11(1), 3405- 3415.

Introduction

Ajowan (*Trachyspemum ammi* L.) is a medicinal plant that belongs to Apiaceae family with valuable secondary metabolites. The most valuable part of the ajowan in the food and pharmaceutical industries is its seeds (Rahimmalek et al., 2017). Ajowan seeds are widely used as spice in the curry powder because of their aromatic odor and spicy taste (Ishikawa et al., 2001). Ajowan seeds have an essential oil that contains about 50% thymol, which has a strong germicide, anti-spasmodic, and fungicidal effect (Noori et al., 2017). Different studies reported different components for essential oil of ajowan (Rasooli et al., 2008).

Chemical fertilizers are applied to soils to compensate for poor nutrients. However, this could lead to several problems such as water, air and, soil pollution.

*Corresponding author
E-mail address: shararehnajafian@yahoo.com
Received: January, 2020
Accepted: September, 2020

Table 1
Physico-chemical characteristics of the study soil

Soil Properties	Value
Soil texture class	Silt/loam
Sand (%)	25
Silt (%)	49
Clay (%)	26
Organic matter, OM (%)	1.36
Total nitrogen, N (%)	0.13
PH of saturated paste	8.3
Electrical conductivity of saturated extract, EC_e (dSm^{-1})	2.1
Olsen (sodium-bicarbonate extractable) phosphorus (mg kg^{-1})	13.24

Organic materials are eco-friendly natural sources. The idea behind applying these products is to overcome the disadvantages of the chemical fertilizers and development of agriculture (Mondal et al., 2017). A category of organic fertilizer, vermicompost treatment increases nutrient content and the main essential oil ingredients of the medicinal plants. Salehi et al. (2018) examined the impact of different chemical and organic fertilizers on German chamomile yield and essential oil composition. This experiment came up with the result which demonstrated that application of fertilizers especially vermicompost is among the main reasons which can limit herbage growth, yield and, quality. Based on this report, biochemical and physiological parameters improve under the influence of growth regulating agents which in turn increases the quantity and quality of essential oils in the herbage industry (Bano et al., 2016).

One of the best methods that have been identified today for sustainable agriculture, environmental protection against contamination, and reduced use of chemical fertilizers is organic farming. The purpose of application of organic fertilizer to the soil is to increase organic matter and soil fertility in the long run and plant production (Abadi et al., 2011).

This survey is done to specify the efficacy of fertilizers with different sources known as organic and chemical, on growth, flowering, and fruiting of ajwain plant (*Trachyspermum ammi*) in a soil with high pH to obtain a comprehensive view about this subject and to get new insights on the shifting from conventional nutrient management system to integrated nutrient management system.

Materials and Methods

Soil characteristics

This study was carried out on a silt loam soil (Table 1) during summer 2017. Hydrometer method was used for determination of soil texture (sand, silt, and clay contents) (Bouyoucos, 1962). Moreover, pH meter, electrical conductivity (EC), and the wet oxidation method were employed to identify pH, EC, and organic matter (OM), respectively (Nelson and Sommers, 1996). The total nitrogen (N) was assayed using the method described by Bremner (1996). Moreover, available phosphorus (P) and potassium (K) were determined according to the procedure reported by Olsen (1954) and Helmke and Sparks (1996), respectively.

Statistical Analysis

The experiment was set in a randomized complete block design (RCBD) with three replications. Analysis of variance was performed using SPSS software package, and the means were compared using Duncan test ($p \leq 0.05$).

Experimental site, soil preparation, planting, and harvesting

All experiments were done in the Medicinal Plants Farm of Shahid Bakeri Higher Education Center of Miandoab (36.5°N latitude, 46.6°E longitudes, and an altitude of 1314 meters above the sea level). Seeds of ajowan (*Trachyspermum ammi*) were planted in the prepared field at a depth of about 10-mm with a spacing of 4 cm and a row spacing of 50 cm. Seeds were carefully irrigated after planting. During the preparation of the field and the period of plant growth from planting to harvesting, no chemical fertilizers, herbicides, pesticides, and fungicides were used. The plants were harvested at the seed stage for relevant assays.

Table 2
Results of variance analysis of the effects of fertilizer treatments on the quantitative traits of ajowan

		Mean Square					
Source of variation	df	Plant height	Diameter of stem	1000-seed weight	Seed yield	Essential oil %	Essential oil yield
Fertilizer treatment	4	84.527**	2.552**	0.015*	1241.382**	0.931**	62.005**
Replication	2	0.254^{ns}	0.007^{ns}	0.003^{ns}	91.008^{ns}	0.005^{ns}	0.116^{ns}
Error	8	0.776	0.003	0.002	22.258	0.004	0.317

^{ns}, * and ** non- significant and significant at 5 and 1% probability level, respectively.

Essential oil extraction

The essential oil of the air-shade dried ajowan samples were extracted using the hydro-distillation in a Clevenger based on the method described by Tarakemeh et al. (2012). Briefly, the dry samples (50 g) were weighed, powdered, and subjected to Clevenger for 4 hours. The extracted essential oil was then dehydrated using anhydrous sodium sulfate. The collected samples were analyzed by gas chromatography mass spectrometry after being placed in hermetical glass vials at 4° C. The extracted essential oil was calculated by following equation (Tarakemeh et al., 2012):

$$\text{Yield (\%)} = \left(\frac{\text{mass content of essential oil}}{\text{leafs dry weight (mass)}}\right) \times 100$$

Proline analysis

Proline content of leaf tissues was determined using the method described by Rowshan et al. (2014). Briefly, the homogenized fresh tissue of plant in 10 ml of aqueous sulpho-acetic acid (3%) was weighed to 5 g. Filtration of the obtained suspension was done via Whatman filter paper Grade 1. Two ml of acid-ninhydrin and concentrated acetic acid were mixed with equal volume of the filtrated sample in a test tube. Four ml was added to test tube, and then the absorbance of the sample was measured at 520 nm through a spectrophotometer following shaking (UV Jenway 6300). The free proline content was measured using L-proline standard.

Total phenol content

Following the method presented by Gao et al. (2000), the total content of phenolic compound was measured using Folin–Ciocalteu (FC). Then, 0.1 gram of each samples were ground in 2 ml methanol and centrifuged at 5000 g for 10 minutes at room temperature. Afterward, 50 µl of the resulting extract, 450 µl distilled water, and 2.5 ml 10% Folin–Ciocalteu reagent were mixed. The mixture was kept in a dark place for 6 minutes. Then, 2 ml of 7.5% (w/v) Na_2CO_3 was added. The solution was incubated at room temperature in the dark for 1.5 h and the absorbance was recorded at 765 nm with a spectrophotometer. Gallic acid was used as standard and the values were presented as milligram GAE (Gallic acid standard) equivalents per g of dry weight (mg GAE/g dw).

Chlorophylls and carotenoids: Pigments of photosynthetic bio membranes

The extracted chlorophyll and carotenoid contents of the leaf tissues were determined using the method described by Arnon (1949). Before extraction, fresh leaf samples were rinsed in clean deionized water for the removal of surface contaminations. Photosynthetic pigment was extracted from the 4th fully expanded leaves from the top of the plant at the end of the experimental period. Then, 0.1 g of a fresh leaf was ground in 5 ml 80% (v/v) acetone in dark. Afterward, the filtrate slurry was centrifuged at 5,000 × g for 10 min. Then, the absorbance of the sample was determined at 645, 663, and 470 nm against acetone as blank with a spectrophotometer, for Chlorophyll a, Chlorophyll b, and carotenoids concentrations, respectively.

Plant height and diameter of stem

After harvest, stem diameters were measured by a caliper at three points: center and two ends of the plant and the average of the three points were recorded. The plant heights were also measured by a tape measure. The number of branches, and seed yield were determined.

Soluble carbohydrate content

Soluble carbohydrates were determined based on phenol sulfuric acid method. First, 100 mg of the weighed samples were put into a boiling tube containing 5 ml of 2.5 N-HCl. The tubes were placed in a water bath for 3 hours. Upon completion of hydroxylation, the samples were cooled to room temperature. Solid sodium carbonate was used for neutralization of samples. The ultimate content was increased to 100 ml by adding deionized water and centrifuged. In a series of test tubes, 0.2, 0.4, 0.6, 0.8, and 1 ml of the standard were applied. Then, 0.1 and 0.2 ml of the solutions were poured into separate tubes. Each tube volume was increased to 1 ml by adding distilled water. Then, 1 ml and 5 ml of phenol solution and sulfuric acid (96%) were added to each tube, respectively before they were completely shaken. After 10 minutes, the tubes were put in a water bath at a temperature of 27± 2° C for 20 minutes. The absorbance of the samples was recorded at 490 nm. Finally, the total carbohydrate content of the samples was measured via the standard chart. The blank sample was prepared with 1 ml of water instead of phenol solution.

Leaf N, P, and K contents

Nitrogen, phosphorous, and potassium contents in each treatment were measured via the methods described by Hashmi et al. (2012). First, the leaves from each treatment were well washed and dried and then completely powdered. Samples were digested to measure phosphorus and potassium contents. In short, 0.5 g of powdered dry samples and 10 ml of concentrated nitric acid 65% were mixed in digestive tubes. Sample tubes with two tubes as control (nitric acid only) were stored for 12 hours without any treatment. After 12 hours, the tubes were heated for 3 h at 60° C and then, the temperature was slowly augmented up to 110° C. The completion of digestion was determined using the indication of color clarity (yellow amber) of samples. Afterwards, the samples were immediately cooled to room temperature. The ultimate content was increased to 100 ml by adding distilled water. This solution was used to measure the amount of phosphorus and potassium. Measurement of phosphorus was done by vanadium-molybdenum colorimetric method. In the orthophosphate ions in the acidic medium with vanadium-molybdate the yellow complex of vanadium molybdate was formed, which showed the maximum absorption at 430 nm. Flame emission was used to measure potassium. Potassium was first measured using flame photometry and then its calibration curve was drawn (Hashmi et al., 2012). After that, leaf nitrogen content was determined by the Kjeldhal method (Khalid and Shedeed, 2015). One gram of powdered dry samples was used in a Pyrex digestion tube (30 ml of concentration). Then H_2SO_4 was added with caution to 10 g potassium sulfate and 14 mg copper sulfate. The resulting mixture was placed on sand and gently heated with a low flame to make it boil. Then, further heating was applied to turn it into a clear and colorless solution. After cooling and diluting the solution with distilled water, the contents were transferred into an 800 ml Kjeldhal flask. The digestion flask was washed and a piece of catalyst tablet, 100 ml of granulated zinc, and 40% caustic soda were added, respectively. Next, the flask was connected with a condenser to a splash-head distillation apparatus. Then, 25 ml of 0.1 N sulfuric acid was collected in the receiving flask on distillation system. Upon being removed and following the completion of reaction, the solution was titrated against 0.1 N caustic soda solution via methyl red indicator to measure nitrogen.

Results

Analysis of variance indicated the significant effects of fertilizer treatments, on plant height, diameter of stem, 1000-seed weight, seed yield, essential oil %, essential oil yield, proline, chlorophyll a, nitrogen, phosphorous, and

Table 3
Comparison of means for the effects of fertilizer treatments on the quantitative and qualitative traits of ajowan

Fertilizer treatments	Plant height(cm)	Diameter of stem	1000-seed weight (g)	Seed yield (kg.ha^{-1})	Essential oil %	Essential oil yield (lit.ha^{-1})
Control	50.62±7.25c	2.85 ± 0.53d	1.5 ± 0.11c	686.02± 29.53d	1.94 ± 0.52d	13.45 ± 5.04d
Cow manure	55.78 ±5.90b	3.10 ± 0.65c	1.57 ± 0.11c	735.77± 11.38bc	2.50 ± 0.51c	18.43 ± 5.01c
Compost	57.67 ±5.31b	3.42 ± 0.69b	1.55 ± 0.10c	730.01± 17.73c	2.86 ± 0.53b	21.00 ± 4.66b
Vermicompost	64.21 ± 5.22a	4.91 ± 0.75a	1.69 ± 0.07a	744.22± 27.11a	3.31 ± 0.44a	24.68 ± 5.42a
Chemical fertilizer	61.81 ±7.14a	4.72 ± 0.86a	1.64 ±0.06b	739.77± 10.62ab	3.22 ± 0.50a	23.86 ± 3.70a

* Means in each row or column followed by the same lowercase or capital letters are not significantly different (P<0.05) by Duncan's Multiple Range Test.

potassium at 1% probability level (Table 2). The vegetative growth properties of ajowan are given in Table 3. Results showed that all treatments significantly improved vegetative properties compared to the control (P<0.01). The height of plants, number of branches, and diameter of stem were increased (Table 3 and Fig. I.). Results indicated that 1000-Seed weight was strongly affected by vermicompost. The effect of different treatments of fertilizer on seed yield is presented in Table 3.

The essential oil extracted from ajowan treated with fertilizer was yellow (similar to the control). Findings of the essential oil in terms of dry matter (v/w) and oil yield (Lit/ha) are also presented in Table 3. The control sample had an essential oil content of 1.94% whereas the essential oil contents of samples treated with cow manure, compost, vermicompost, and chemical fertilizer were 2.50%, 2.86%, 3.31, and 3.22, respectively.

The changes in proline contents of ajowan are presented in Table 4. The findings indicated that the vermicompost and chemical fertilizer showed the highest proline content.

Analysis of variance showed that fertilizers application significantly changed total phenol, chlorophyll b, and carotenoids contents (Figs. II, III, IV). The maximum content of photosynthetic pigments was observed in vermicompost while the minimum contents were observed in the control.

In addition, the correlations between total soluble sugars (TSS) of the samples are given in Fig.

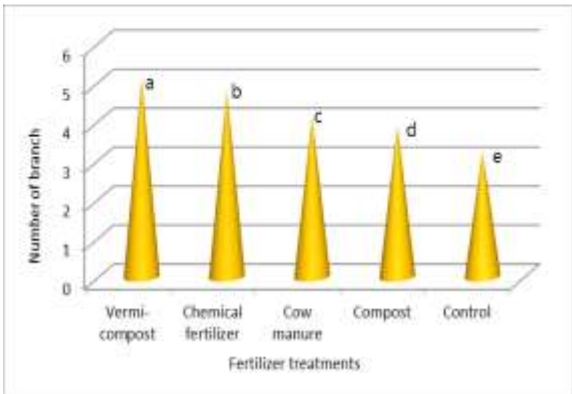

Fig. I. The effect of fertilizer treatments on the number of branches of ajowan; means followed by the same letter(s) are not significantly different at p<0.05 as determined by Duncan test. The data shown are means of three replicates.

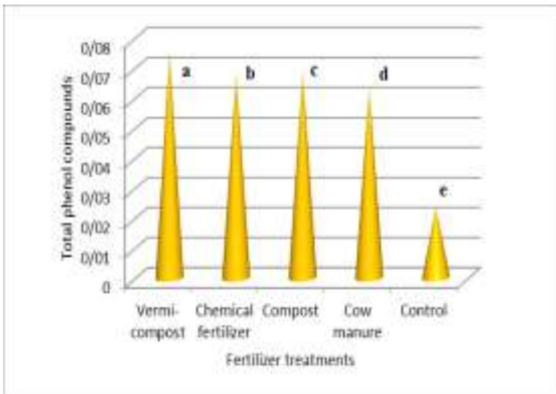

Fig. II. The effect of fertilizer treatments on total phenol content of ajowan; means followed by the same letter(s) are not significantly different at p<0.05 as determined by Duncan test. The data shown are means of three replicates.

Table 4
Comparison of means for the effect of fertilizer treatments on the quantitative and qualitative traits of ajowan.

Fertilizer treatments	Proline	Chlorophyll a	Nitrogen	Phosphorous	Potassium
Control	1.08 ± 0.14c	0.025 ± 0.030d	19.19 ± 0.92e	5.31 ± 0.34d	22.39 ± 1.89c
Cow manure	1.12 ± 0.14bc	0.086 ± 0.049c	20.43 ± 1.15d	5.49 ± 0.57c	22.42 ± 1.27c
Compost	1.16 ± 0.13b	0.093 ± .043b	21.03 ± 1.66c	5.89 ± 0.58b	22.50 ± 1.24c
Vermi- compost	1.27 ± 0.08a	0.125 ± .032a	21.98 ± 1.58a	6.15 ± 0.43a	24.23 ± 1.47a
Chemical fertilizer	1.24 ± 0.07a	0.125 ± .033a	21.22 ± 1.60b	5.85 ± 0.70b	23.78 ± 1.34b

* Means in each row or column followed by the same lowercase or capital letters are not significantly different (P<0.05) by Duncan's Multiple Range Test.

Table 5
Results of variance analysis of the effects of fertilizer treatments on some traits of ajowan

		Mean Square				
Source of variation	df	Proline	Chlorophyll a	Nitrogen	Phosphorous	Potassium
Fertilizer treatment	4	0.025^{**}	0.005^{**}	3.278^{**}	0.332^{**}	2.274^{**}
Replication	2	0.001^{ns}	0.0001^{ns}	0.011^{ns}	0.002^{ns}	$.002^{ns}$
Error	8	0.000	0.0007	0.003	.001	.011

ns, *, and **: non- significant and significant at 5 and 1% probability levels, respectively

V. Results indicated that the fertilizer treatments on ajowan significantly increased TSS content (P<0.05). Also, Vermicompost demonstrated the highest improvement on the nitrogen, phosphorous, and potassium contents of ajowan leaves (Table 4 and Table 5).

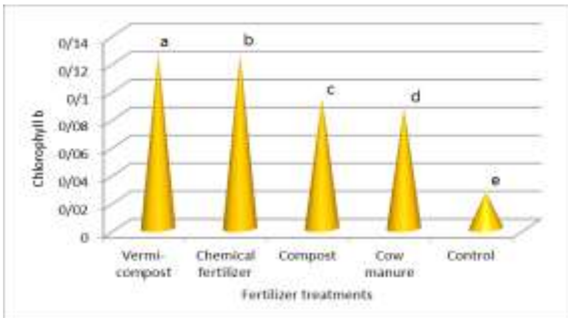

Fig. III. The effect of fertilizer treatments on chlorophyll b content of ajwain; means followed by the same letter(s) are not significantly different at p<0.05 as determined by Duncan test. The data shown are means of three replicates.

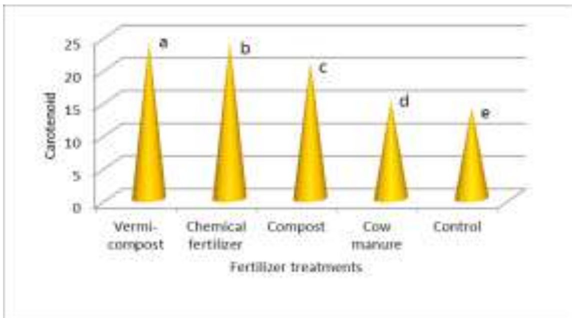

Fig. IV. The effect of fertilizer treatments on carotenoid content of ajowan; means followed by the same letter(s) are not significantly different at p<0.05 as determined by Duncan test. The data shown are means of three replicates.

Discussion

A significant (p≤0.01) positive correlation was found between plant height, diameter of stem, 1000-seed weight, seed yield, essential oil %, proline, Chlorophyll a, Nitrogen, Phosphorous and Potassium (Table 6).

Yadav et al. (2002) also reported an increase in the height of the medicinal herbs due to the use of animal fertilizer. Similarly, Chiane et al. (2014) observed an enhancement in plant height and vegetative growth of ajowan (*Trachyspermum ammi*) after fertilizer treatments. Increases in the number of branches per plant under fertilizer treatments of ajowan and barley plants have been due to improved soil structure and more nutritional value for these plants (Mahdavi et al., 2014). Moradi et al. (2009) studied the effect of various organic and biologic

Table 6
Pearson's correlation coefficients (r) between on some traits of ajowan under fertilizer treatment.

Traits	Plant height (cm)	Diameter of stem	1000-seed weight (g)	Seed yield (kg ha^{-1})	Essential oil %	Essential oil yield	Proline	Chlorophyll a	N	P	K
Plant height(cm)	1										
Diameter of stem	.937**	1									
1000-seed weight (g)	.754**	.805**	1								
Seed yield (kg.ha^{-1})	.821**	.675**	.405	1							
Essential oil %	.972**	.902**	.648**	.856**	1						
Essential oil yield	.977**	.896**	.648**	.873**	.997**	1					
proline	.956**	.955**	.767**	.766**	.947**	.944**	1				
Chlorophyll a	.964**	.878**	.658**	.906**	.977**	.982**	.939**	1			
N	.876**	.807**	.518*	.837**	.941**	.936**	.875**	.953**	1		
P	.933**	.850**	.653**	.764**	.937**	.938**	.910**	.886**	.804**	1	
K	.879**	.980**	.835**	.582*	.818**	.812**	.894**	.789**	.690**	.78**	1

** and *: correlation is significant at the 0.01 and 0.05, respectively.

fertilizers of *Foeniculum vulgare* and found that fertilizer treatment increased the number of lateral branches.

The application of animal fertilizer, especially in deep soils, resulted in increased porosity and improvement of the soil structure and aeration and increased the soil water availability. These factors increase the growth and expansion of roots, absorption of nutrients, and the production of growth hormones. Plant growth and development are improved, and eventually increase plant height (Ghosh et al., 2004). The results of this survey confirmed the results of Sanchez Govin et al. (2005). He showed that the application of biological fertilizers in medicinal plants increased the yield of flowers. Mahfouz and Sharaf-Eldin (2007) also presented similar results for *Foeniculum vulgare.* They found that biological fertilizers had an impact on the enhancement of growth cycle, yield, and monoterpenes biosynthesis and developed content of essential oil in the plants.

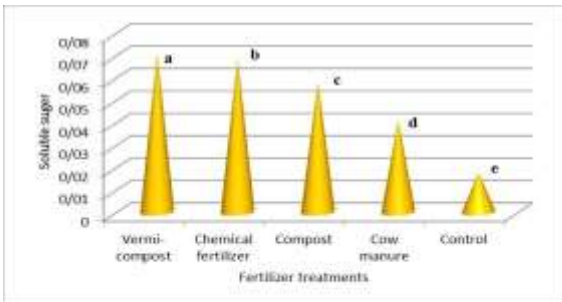

Fig. IV. The effect of fertilizer treatments on soluble sugar content of ajowan; means followed by the same letter(s) are not significantly different at p<0.05 as determined by Duncan test. The data shown are means of three replicates.

Among the qualitative traits of medicinal plants, the main attributes are essential oil percentage and yield. Environmental conditions and soil fertility are the main factors in determining the qualitative characteristics of plants. Manure contains many micronutrients that can increase the amount of effective ingredient and improve its quality in medicinal plants (Kafi. 2002). In short, transgenic approaches have proline accumulation during stress. Occasionally, proline accumulation is considered as part of a developmental program in plant tissues (Verbruggen and Hermans, 2008). During stress, proline prevents degradation of enzymes and macromolecular degradation, interferes with maintaining cell wall strength and purifies the hydroxyls produced under tension in the plant (Haddad and Moshiri, 2011). In addition, Szabados and Savoure in 2010, demonstrated that proline has different uses in herbal physiology. For example, it is used as an important substance to make proteins, it has protective application for herbs just like osmolyte, helps the herb to maintain redox at an effective level; It also contributes to development, regulation of some Intracellular organs like controlling mitochondrial functions, decreasing in stress level and development.

The chlorophyll content of the plants is an important factor because of its significant role in photosynthesis and production of corps (Banerjee et al., 2011). Plants increase the soluble sugars to maintain cellular structure and modulate the osmotic potential (Nedjimi and Davoud, 2006).

The highest content of total soluble sugar (TSS) was observed in vermicompost treatment. The role of the photosynthesis in the leaves has a direct effect on variable total sugar content. In general, this rate enhanced by many factors such as supplementation of a nutrient. Our results matched with the findings reported by Ram Rao et al. (2007) and Mondal et al. (2017) for the mulberry mustard crop. They concluded that bio fertilizers had significant effects on carbohydrate biosynthesis in leaves. There is a direct relationship between sugar content of leaves and physiological processes such as respiration, photosynthesis and, translocation. Thus, vermicompost as a nitrogen source in the soil increased biosynthesis and accumulation of sugar in the leaves. Considering the significant increase of N P K elements in fertilizer treatments, especially in vermicompost treatment, increasing these elements resulted in increased yield and improved physiological characteristics. These treatments improved the physicochemical properties of the soil and created a suitable environment for better growth and higher yields and quality.

Conclusion

In recent years, the ecological characteristics and beneficial effects of vermicompost have been clearly demonstrated. Vermicompost is a kind of green manure that is made of organic matter with useful microorganisms. It contains enough nutrients to produce a healthy product. It is evident that vermicompost can improve physical, chemical and biological properties of soil and it is an excellent organic fertilizer. In fact, an optimized way, not to waste any agricultural residue, is to use them as fertilizer in organic cultivation. This approach leads to a higher population of bacterial and fungal organisms in the soil. This approach not only helps the plants to grow better, but it also helps the soil to be richer. The high rate of agricultural residue and waste should be used as an alternative to chemical fertilizers in order to improve organic agriculture and, it needs higher processing technologies for better results. Therefore, to preserve the environment and produce more valuable medicinal herbs such as ajowan, vermicomposting is recommended because it is described as an excellent soil amendment and a biocontrol agent which makes it the best organic fertilizer and more eco-friendly as compared to chemical fertilizers.

Acknowledgments

The authors would like to thank the grace of God for completing this work. We wish to thank Head of Department of Shahid Bakeri of Miandoab for providing the laboratory facilities.

References

Abadi, ZA., M. G. Sepanlou and **M. Bahmanyar**. 2011. 'The effect of municipal compost application on the amount of micro elements and their absorption in soil and medicinal plant of mint (Menthas)'. *African Journal of Biotechnology*, 10(77), 17716-17725.

Akbarinia, A., A. Ghalavand, FS. Feed Kan, M. B. Rezaiee and **A. Sharifi Ashoorabadi**. 2003. 'Evaluation of effect of integrated manure and chemical fertilizers on yield and composition of essence of ajwain'. *Journal of*

Research and Development, 61, 32–41. (In Persian).

Arnon, D.I.1949.' Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*.' *Plant Physiol*. 24, 1–15.

Bairwa, R., R. Sodha and **B. Rajawat**. 2012. '*Trachyspermum ammi*.' *Pharmacognosy reviews* 6(11), 56.

Baker WH and **T. L. Thompson** .1992. 'Determination of nitrate-nitrogen in plant samples by selective ion electrode', pp. 25-31. IN: C.O. Plank (Ed.), Plant Analysis Reference Procedures for the Southern Region of the United States. Southern Cooperative Series Bulletin 368. University of Georgia, Athens.

Banerjee, A., J. K. Datta, N. Mondal and **T. Chanda.** 2011. 'Influence of integrated nutrient management on soil properties of old alluvial soil under mustard cropping system'. *Communications in soil science and plant analysis*, 42(20), 2473-2492.

Bano, U., A. F. Khan, F. Mujeeb, N. Maurya, H. Tabassum, M. H. Siddiqui, M. Haneef, K. Khwaja Osama and **A. Farooqui.** 2016. 'Effect of plant growth regulators on essential oil yield in aromatic plants'. *Journal of Chemical and Pharmaceutical Research*, 8 (7), 733-739.

Bates, L., R. Waldren and **I. Teare**. 1973. 'Rapid determination of free proline for water-stress studies'. *Plant and soil*, 39(1), 205-207.

Bouyoucos, G. J. 1962. 'Hydrometer method improved for making particle size analyses of soils 1'. *Agronomy journal*, 54(5), 464-465.

Bremner, J. 1996. 'Nitrogen-total'. *Methods of Soil Analysis Part 3—Chemical Methods(methodsofsoilan3)*, 1085-1121.

Chiane, E., A. Mohammadi and **A. Nasab**. 2014. 'Evaluation of combined application of biological and chemical fertilizers on quantitative and qualitative function of *Carum copticum* L. in cultivars with *Trigonella foenum-graecum* L.' *Journal of Agroecology*, 6, 582-594.

Cottenie, A. 1980. 'Soil and plant testing'. F. A. O. Soils Bulletin, No. 38 /2.

Dubois, M ., K. A. Gilles, J. K. Hamilton, P. T. Rebers and **F. Smith**. 1956. 'Colorimetric method for determination of sugars and related substances'. *Analytical chemistry*, 28(3), 350-356.

Esmaielpour, B., M. Rahmanian, O. Heidarpour and **M. H. Shahriari**. 2017. 'Effect of vermicompost and spent mushroom compost on the nutrient and essential oil composition of Basil (*Ocimum basilicum* L.)'. *Journal of Essential Oil Bearing Plants*, 20(5), 1283-1292.

Gandomi, H., S. Abbaszadeh, A. JebelliJavan and **A. Sharifzadeh**. 2014. 'Chemical constituents, antimicrobial and antioxidative effects of *Trachyspermum ammi* essential oil'. *Journal of food processing and preservation* 38(4), 1690-1695.

Gao, X., M. Ohlander, N. Jeppsson, L. Bjork and **V. Trajkovski**. 2000. 'Changes in antioxidant effects and their relationship to phytonutrients in fruits of sea buckthorn (*Hippophae rhamnoides* L.) during maturation'. *Journal of Agricultural and Food Chemistry*, 48(5), 1485-1490.

Ghosh, P., K. Bandyopadhyay, M. Manna, K. Mandal, A. Misra and **K. Hati**. 2004. 'Comparative effectiveness of cattle manure, poultry manure, phosphocompost and fertilizer-NPK on three cropping systems in vertisols of semi-arid tropics. II. Dry matter yield, nodulation, chlorophyll content and enzyme activity'. *Bioresource technology*, 95(1), 85-93.

Haddad, R and **Z. Moshiri**. 2011. 'Effect of silicon in increasing drought tolerance on 2-leaves stage of barley plant'. *Modern genetics*, 5(4), 47-58.

Helmke, P. A and **D. Sparks**.1996. 'Lithium, sodium, potassium, rubidium, and cesium'. *Methods of Soil Analysis Part 3-Chemical Methods*, (methodsofsoilan3), 551-574.

Kafi, M. 2002. 'Technology, production and processing of cumin'. Center of Science for Special Crops. Publication of Ferdowsi University of Mashhad, 200 p. (In Persian).

Kedia, A., B. Prakash, P. K. Mishra, A. K. Dwivedy and **N. Dubey**. 2015. '*Trachyspermum ammi* L. essential oil as plant based preservative in food system'. *Industrial Crops and Products*, 69, 104-109.

Kemble, A and **H. T. Macpherson**. 1954. 'Liberation of amino acids in perennial rye grass during wilting'. *Biochemical Journal*, 58(1), 46.

Khalid, K. A and **M. R. Shedeed**. 2015. 'Effect of NPK and foliar nutrition on growth, yield and chemical constituents in *Nigella sativa* L+. *Journal of Materials and Environmental Science*, 6, 1709-1714.

Litterick, A., L. Harrier, P. Wallace, C. Watson and **M. Wood**. 2004. 'The role of un composted materials, composts, manures, and compost extracts in reducing pest and disease incidence and severity in sustainable temperate agricultural and horticultural crop production—A review'. *Critical Reviews in Plant Sciences*, 23(6), 453-479.

Mahdavi, T., A. GHanbari and **M. R. Asgharipor**. 2014. 'Investigation of Ajowan and barley crop cultivation under the influence of livestock and chemical fertilizers'. *Journal of Plant Ecophysiology*, 4,63-78.

Mahfouz, S and M. **Sharaf-Eldin**. 2007.' Effect of mineral vs. biofertilizer on growth, yield, and essential oil content of fennel (*Foeniculum vulgare* Mill.)'. *International Agrophysics*, 21(4), 361.

Moazeni, M., M. J. Saharkhiz and **A. A. Hosseini**. 2012. 'In vitro lethal effect of ajowan (*Trachyspermum ammi* L.) essential oil on hydatid cyst protoscoleces'. *Veterinary parasitology* 187(1-2), 203-208.

Moein, MR., K. Zomorodian, K. Pakshir, F. Yavari, M. Motamedi and **M. M. Zarshenas**. 2015.'Trachyspermum ammi (L.) Sprague: chemical composition of essential oil and antimicrobial activities of respective fractions'. *Journal of evidence-based complementary & alternative medicine*, 20(1), 50-56.

Mondal, T., J. K. Datta and N.K. **Mondal**. 2017.'Chemical fertilizer in conjunction with bio fertilizer and vermicompost induced changes in morpho-physiological and bio-chemical traits of mustard crop'. *Journal of the Saudi Society of Agricultural Sciences*, 16(2), 135-144.

Moradi, R. 2009. 'Effect of biological and inorganic fertilizers on, yield components and essence of fennel (*Foeniculum vulgare*)'. M.Sc. Thesis of Agroecology, Ferdowsi University of Mashhad. (In Persian).

Nedjimi, B. and **Daoud, Y.** 2006. 'Effect of Na_2SO_4 on the growth, water relations, proline, total soluble sugars and ion content of *Atriplex halimus* subsp. schweinfurthii through in vitro culture. Annuls Biology 28: 35–43.

Nelson, DW and **L. E. Sommers**. 1996. 'Total carbon, organic carbon, and organic matter'. Methods of soil analysis part 3-chemical methods(methodsofsoilan3), 961-1010.

Olsen, SR. 1954. 'Estimation of available phosphorus in soils by extraction with sodium bicarbonate'. United States Department of Agriculture; Washington.

Paul, S and **S. Kang**. 2012. 'Studies on the viability and membrane integrity of human spermatozoa treated with essential oil of *Trachyspermum ammi* (L.) Sprague ex Turrill fruit'. *Andrologia*, 44, 117-125.

Ram Rao, D., J. Kodandaramaiah, M. Reddy, R. Katiyar and **V. Rahmathulla**. 2007. 'Effect of VAM fungi and bacterial bio fertilizers on mulberry leaf quality and silkworm cocoon characters under semiarid conditions'. *Caspian Journal of Environmental Sciences*, 5(2), 111-117.

Rowshan, V., F. Farhadi and **S. H. Najafian**. 2014.'The essential oil of *Dodonaea viscosa* leaves is allelopathic to rosemary (*Rosmarinus officinalis* L.)'. *Industrial Crops and Products*, 56, 241-245.

Ryan, M., D. Wilson, P. Hepperly, J. Travis, N. Halbrendt and **A. Wise**. 2005. 'Compost tea potential is still brewing: Compost tea and disease control'. *Biocycle*, 46(6), 30-32.

Salehi, A., M. Gholamhoseini, R. Ataei, F. Sefikon and A. **Ghalavand**. 2018. 'Effects of zeolite, bio-and organic fertilizers application on german chamomile yield and essential oil composition'. *Journal of Essential Oil Bearing Plants*, 21(1), 116-130.

Sanchez Govin, E., H. Rodríguez González, C. Carballo Guerra and **M. Milanés Figueredo**. 2005. 'Influencia de los abonos orgánicos y biofertilizantes en la calidad de las especies medicinales *Calendula officinalis* L. *Matricaria recutita* L'. Revista Cubana de Plantas Medicinales, 10(1), 0-0.

Szabados, L and **A. Savoure**. 2010. 'Proline: a multifunctional amino acid'. *Trends in plant science* 15(2), 89-97.

Tarakemeh, A., V. Rowshan and **S. H. Najafian**. 2012. 'Essential oil content and composition of *Lavandula angustifolia* Mill. as affected by drying method and extraction time.' *Analytical Chemistry Letters*, 2(4), 244-249.

Verbruggen, N and **C. Hermans**. 2008. 'Proline accumulation in plants: a review'. *Amino acids*, 35(4), 753-759.

Vitali, LA., D. Beghelli, P. C. B. Nya, O. Bistoni, L. Cappellacci, S. Damiano, G. Lupidi, F. Maggi, G. Orsomando and **F. Papa**. 2016. 'Diverse biological effects of the essential oil from Iranian *Trachyspermum ammi*.' *Arabian Journal of Chemistry*, 9(6), 775-786.

Yadav, R., G. Keshwa and **S. Yadva**. 2002. 'Effect of integrated use of FYM, urea and sulphur on growth and yield of isabgol (*Plantago ovata*).' *Journal of Medicinal and Aromatic Plant Sciences*, 25, 668-671.