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# Yield and physiological response of Perilla (*Perilla frutescens*) under different soil fertility treatments

**M. Ghane [1], M. Mohammadi [2 (*)], H. Pirdashti [1]**

[1] *Department of Agronomy, Sari Agricultural Sciences and Natural Resources University, Sari, Iran.*

[2] *Department of Plant Sciences, University of Tennessee, USA.*

*Key words:* bio-fertilizer, chemical fertilizer, DPPH test, inoculation, organic fertilizer, Perilla, rosmarinic acid.



**(*) Corresponding author:**
mmohamm9@utk.edu

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All relevant data are within the paper and its Supporting Information files.

**Competing Interests:**
The authors declare no competing interests.

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**Abstract: Medicinal plants are one of the main natural resources of Iran from ancient times. Perilla is one of the most important medicinal plants of the mint family *Lamiaceae*, since there is no study about adaptability of Perilla in Iran climate conditions and different fertilizer systems, this experiment was conducted in two experimental sites. The experiment was conducted as split-plot factorial based on a randomized complete block design with three replications at two experimental regions. The main factor was three chemical fertilizer levels (control, 50, 100, 200 kg/ha) and subplots were different kinds of organic fertilizer (control, humic acid, and compost application) and inoculation with *Piriform osporaindica* (inoculation and without). Among levels of chemical treatments, 50 and 100 kg/ha lead to a better result. Also, humic acid allows to achieve the highest amount of measured traits between different treatments of organic fertilizer. The highest plant yield (147.2 g/m$^2$) and rosmarinic acid yield per area (3.432 g/m$^2$) was achieved in 100 kg/ha at chemical fertilizer with humic acid and biological fertilizer application and the lowest plant yield (89.86 g/m$^2$) and rosmarinic acid yield per area (1.253 g/m$^2$) was observed in control. Also, the highest stomatal conductance was obtained with application of compost fertilizer (67.67 mmol $H_2O$ m$^{-2}$s$^{-1}$). Integrated application of the studied fertilizers showed the more positive effect on yield and quality of Perilla than individual application of those fertilizers.**

## 1. Introduction

The Perilla is a medicinal plant belonging to the *Labiatae* family and is widely cultivated in Southeast Asian countries (Igarashi and Miyazaki, 2013). A number of studies have shown that advantages of Perilla are related to the metabolites contained therein (Ghimire *et al.*, 2017). To date, very limited information exists regarding the adaptability and management of chemical, organic and bio-fertilizer of Perilla in Iran.

Organic matter effects on physicochemical properties and health of soil. It also affects the efficiency of fertilizer application, pesticides and herbicides. One of the most effective organic fertilizers on the growth of plants and improvement of soil status is humic acid. Humic acid can be obtained from any material such as organic matter, coal, or well-decomposed compost. This material plays an important role in increasing soil moisture, absorbing micronutrients and contributing to carbon sequestration (Spaccini *et al.*, 2002).

Another organic material that plays an important role in soil fertility is compost and it comes from plant and food residues. Some experimental researches have shown the important role of compost in crop production (Adugna, 2016). Compost retains moisture in the soil, slow release nutrients to crops and finally increase the crop yield. Application of compost obtained from plant remains leads to increased fertility, soil nutrients and increases water retention in the soil. Zemanek (2011) also confirmed that application of 50 t/ha and 100 t/ha compost has a positive effect on soil moisture retention.

Moreover, biologic fertilizers assists well in mineralization and channelization of nutrients leading to enhanced plant productivity (Fischer *et al.*, 2007; Ansari *et al.*, 2017). Biologic fertilizers adopt various possible ways to accelerate the rate of crop production (Rizvi *et al.*, 2015; Ansari *et al.*, 2017). They increase and improve plant growth by increasing access to nutrients in the root rhizosphere. These fertilizers provide nutrients through biological processes such as biological nitrogen fixation, phosphorus solubilization, and plant growth stimulation. Also, they help to natural nutrient cycle and build soil organic matter (Kapoor *et al.*, 2015). Use of biologic fertilizers ensures healthy plants growth, while enhancing the sustainability and the vigor of the soil. These biologic fertilizers play a special role in increasing plant nutrition and fertility of soils (Vessey, 2003).

A number of studies have shown that the chemical composition of secondary metabolites in the Perilla plant is influenced by various factors such as soil conditions, temperature, growth season (Kiazolu *et al.*, 2016), geographic region (Ruberto *et al.*, 2002), and phenological stages (Saeb and Gholamrezaee, 2012). Therefore, this study was carried out to evaluate the yield and physiological traits of Perilla cultivars under different fertilizer treatments in two regions. Based on the work, attempt was also made to provide amicable solutions to address the challenges of organic farming with the help of Perilla cultivation in two different locations in dry region of Iran.

## 2. Materials and Methods

The field experiments were conducted at two locations in Esfahan Province: 1-Mashhad Ardehal (latitude 34° North, longitude 51° East, Altitude 1800 m above mean sea level) 2- Sensen, Iran (latitude 33°, longitude 51°, Altitude 945 m above mean sea level) during 2015. Mashhad Ardehal is located in warm and dry condition and Sensen is located in the mountainous and dry region. The meteorological data recorded during the period of plant cultivation are given in Table 1.

Table 1 - Weather characteristics in Mashhad Ardehal and Sensen

| Year | Temperature mean (°C) | | Precipitation mean (mm) | | Humidity mean (%) | |
|---|---|---|---|---|---|---|
| | Sensen | Mashhad Ardehal | Sensen | Mashhad Ardehal | Sensen | Mashhad Ardehal |
| 2015 | 19.8 | 18.4 | 89.3 | 90.8 | 42 | 49 |
| Average of 5 years | 20.86 | 19.88 | 143.36 | 133.42 | 40.6 | 46.6 |

In order to determine the physical and chemical characteristics of experimental fields, two weeks before planting, the soil samples were taken. Physicochemical properties of experimental field soil are presented in Table 2.

This experiment was conducted as split-plot factorial based on a randomized complete block design with three replications at two experimental sites in the year 2015. The main factor was three chemical fertilizer levels (50, 100, 200 kg/ha) plus control, and sub plots were different kinds of organic fertilizer (humic acid, and compost application), plus control; inoculation with *Piriformospora indica* (inoculation and without inoculation) was also evaluated.

Table 2 - Soil analysis of the experimental site

| Parameter | Location | |
|---|---|---|
| | Sensen | Mashhad Ardehal |
| Total Nitrogen (%) | 0.09 | 0.28 |
| Phosphorous availability (mg/kg) | 15.56 | 12.18 |
| Potassium availability (mg/kg) | 245.6 | 209.7 |
| pH | 7.93 | 7.83 |
| EC ($dS.m^{-1)}$) | 2.82 | 0.89 |
| Organic carbon (%) | 0.53 | 1.63 |
| Clay (%) | 14.3 | 10.3 |
| Silt (%) | 33.3 | 43.4 |
| Sandy (%) | 52.4 | 46.3 |

Foliar-applied humic acid (95% purity) was obtained from Humic Strong company (70% w/w, pH 5.17, EC: 4.80 mS/cm) and added to the plots at 4 stages of the plant growth. Compost fertilizer was prepared from Barij Essence Company (including 1.5% nitrogen, 1.1% phosphorous, 0.9% potassium, 50% organic matter). Compost fertilizer was used as a strip one week after plantation.

Perilla seeds were purchased from Barij essence Company, Isfahan, Iran. First of all, the seeds were germinated in the laboratory and then transplanted to the pots with 10 cm diameter and 15 cm height at the 4-leaf stage. The mycorrhizal-like fungus *Piriformospora indica* was prepared in mycology laboratory of Sari University, Iran and then prepared biologic fertilizer was placed two weeks in incubator with 20-25˚C and 50 rpm under dark condition. Before planting of Perilla plants in the field, half of the plants in pots were inoculated with biologic fertilizers and then translocated to the field. Each plot was 2×2 meter and had 4 furrows with 25 plant per plot.

The recommended dose of chemical fertilizer (0-50-100 and 200 kg/ha) in the form of urea, triple super phosphate and sulfate potassium was applied to grow the crop. Nitrogen was applied in three splits, the first along with phosphorus and potassium fertilizer at the time of soil preparation while the second part at the time of transplanting of plants and the third part at the flowering of crops. Date of transplanting of both experimental sites was 20 of March.

After plant growth, different traits were studied at the suitable stage. A furrow irrigation system was applied for both experimental sites. Hand weeding of the experimental area was performed as required. In both experimental sites, after flowering five plants from each plot were harvested from two central rows and then plant height, fresh weight and dry weight of plants were measured. Samples dried in an oven at 80°C for 24 hours and the mean of dry weight for each treatment at each replicate was determined.

*Rosmarinic acid determination by HPLC*

The dried seeds of Perilla were pulverized (60 mesh) for 3 min using an HR 2860 coffee grinder (Philips, Drachten, Netherlands), and each sample (1.0 g) extracted in 30 ml of 80% methanol for 6 h at room temperature in a shaking incubator. The supernatant was centrifuged at 3000g for 3 min and then filtered through a 0.45 lm syringe filter (Whatman Inc., Maidstone, UK) prior to HPLC analysis. For quantification, the peak areas of the isolated compounds were integrated from the HPLC chromatogram at 330 nm using the Dionex software. The stock solutions were prepared by dissolving in methanol to obtain a 1 mg/ml concentration. Calibration curves were obtained with methanol at eight different concentrations (0.5, 1, 2, 5, 10, 25, 50, and 100 g/ml). All calibration curves had coefficients of linear correlation $r^2$>0.998.

*Stomatal conductance*

Twenty days after plantation stomatal conductance was measured in the shade-enclosure with saturated light using Promoter (Model KR1301, KOREA TECH) Stomatal conductance (mmol $H_2O$ $m^{-2}$ $s^{-1}$) was measured at 12:00-14:00 hours on a clear, cloud-less day in fully expanded, healthy, turgid, three flat and uniform in color and size leaves of each samples (Barbieri *et al.*, 2012).

*DPPH radical-scavenging activity*

This test was used for the determination of the free radical-scavenging activity of the extracts (Ebrahimzadeh *et al*., 2008). DPPH test was performed following the method proposed by Ebrahimzadeh *et al*. (2008). Three young fully developed leaves were selected from each replication.

*Polyphenoloxidase (PPO) Activity*

Crude extract was prepared by homogenization of frozen plant sample in buffer medium. Leaves of Perilla plant which were stored at -20°C was used for the enzyme extraction. 10 g of the sample were cut quickly into thin slices and homogenized in 50 mL of 100 mM sodium phosphate buffer (pH 7.0) containing 1 mM ascorbic acid and 0.5% (w/v) polyvinylpyrrolidone for 5 min at 4°C. The homogenate was filtered through three layers of cheesecloth and then the filtrate was centrifuged at 5,000 x g for 15 min, and the supernatant was collected.

Enzymatic activity was assayed by determining the rate of increase in absorbance at 420 nm and 25˚C in a Perkin-Elmer Lambda 15 UV/VIS spectrophotometer (Shimadzu Corp., Tokyo, Japan). The reaction mixture contained 3.0 mL of catechol substrate, the solution freshly prepared in 0.05 M sodium phosphate buffer at pH 6.5 and a fixed quantity of PPO. The reference cuvette contained only the catechol substrate solution. The reaction was conducted at 25°C. The PPO activity was defined as a change of 0.001 in absorbance at the conditions of the assay (Pizzocaro *et al*., 1993).

*Catalase activity assay*

CAT activity was measured by monitoring the $H_2O_2$ decomposition at 240 nm in 3 mL of reaction mixture containing 50 mmol/l phosphate buffer (pH 7.0), 15 mmol/L $H_2O_2$, 100 mL enzyme extract and 0.1% (v/v) Triton X-100 (Aebi, 1984). The activity was expressed in terms of mmol $H_2O_2$ reduced min/mg/protein.

*Statistical analysis*

The data were tested for homogeneity and normality of residuals using the Bartlett tests and Kolmogorov-Smirnov, respectively. A combined ANOVA was used to compare treatments for 2 location using PROC GLM of SAS 9.1 software. Means were separated by application of LSD test when the *F* test proved significant at $P \leq 0.05$ and 0.01.

## 3. Results and Discussion

Combined statistical analysis of two studied location is presented in Table 3. Also, the analysis of variance of Mashhad Ardehal and Sensen locations are shown separately in Table 4 and 5. In Mashhad Ardehal location, the maximum plant yield (157.6 g/m$^2$) was achieved in 100 kg/ha chemical fertilizer plus humic acid treatment and the minimum amount (90.68 g/m$^2$) was produced in zero levels of chemical fertilizer and without application of organic treatment (Fig. 1, Table 3). While in Sensen location, the maximum plant yield (126.9 g/m$^2$) was achieved with the application of 50 kg/ha chemical fertilizer plus humic acid (Fig. 1).

Between treatments of chemical × organic × biologic fertilizer interaction, the maximum plant yield (147.2 g/m$^2$) was produced in 100 kg/ha along with humic acid and application of biologic fertilizer (Table 6). Differences between plant yield (147.2 g/m$^2$ and 89.86 g/m$^2$) showed the importance of fertilizer application in improving yield in this plant. In all of the chemical fertilizer treatments, the plant yield was increased with application of organic and biologic fertilizer, especially with organic fertilizer. So, the integrated fertilizer application was very positive. In the present investigation, application of organic fertilizer especially humic acid significantly increased plant

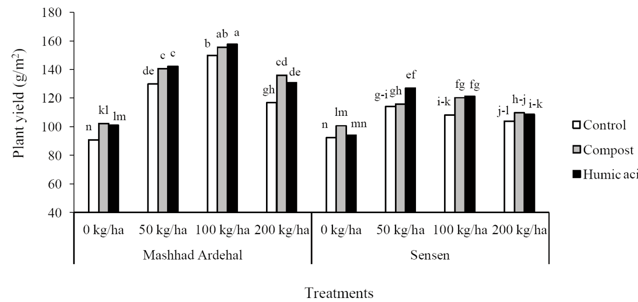


Fig. 1 - Effect of chemical and organic fertilizers on plant yield of Perilla in Mashhad Ardehal and Sensen regions. Different letters in each column denote a significant difference at p≤0.05 (LSD test).

Table 3 - ANOVA of some morphologic, yield and essence traits of Perilla under different fertilizer systems in Sensen and Mashhad Ardehal in Kashan regions

| S.O.V. | d.f. | Means squares | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Plant yield | Rosmarinic acid content | Rosmarinic acid yield | Stomatal conductance | DPPH | Catalase activity | Polyphenol oxidase |
| Location (L) | 1 | 14012.64 ** | 13.80 ** | 8.87 ** | 248.11 ** | 224.60 ** | 0.00357 * | 0.000935 ** |
| Replication (Location) | 4 | 56.75 | 0.70 | 0.01 | 4.02 | 18.56 | 0.00047 | 0.000021 |
| Chemical fertilizer (C) | 3 | 10188.25 ** | 244.44 ** | 13.92** | 1504.81 ** | 99.75 ** | 0.03396 ** | 0.010834 ** |
| L×C | 3 | 1899.70 ** | 27.75 ** | 2.53** | 184.57 ** | 19.74 | 0.01396 ** | 0.000478 ** |
| Ea | 12 | 28.48 | 1.18 | 0.04 | 5.74 | 44.51 | 0.00063 | 0.000072 |
| Organic fertilizer (O) | 2 | 1435.58 ** | 27.93 ** | 1.69** | 76.66 ** | 75.18 * | 0.00178 | 0.000454** |
| Biologic fertilizer (B) | 1 | 308.17 ** | 8.41 ** | 0.53** | 213.84** | 0.24 | 0.00003 | 0.000146 * |
| C × O | 6 | 73.04 | 4.75 ** | 0.06* | 8.46 | 47.80 * | 0.00170 * | 0.000217 ** |
| C × B | 3 | 130.19 * | 1.05 | 0.06 | 2.01 | 2.82 | 0.00257 ** | 0.000022 |
| O × B | 2 | 236.97 ** | 1.31 | 0.09* | 2.47 | 19.55 | 0.00014 | 0.000008 |
| L × O | 2 | 71.26 | 4.79 ** | 0.17** | 13.49 | 2.83 | 0.00253 * | 0.000275 ** |
| L × B | 1 | 88.64 | 4.42 * | 0.22** | 1.92 | 22.75 | 0.00026 | 0.000003 |
| L × C × O | 6 | 83.62 * | 2.89 ** | 0.03 | 10.55 | 5.98 | 0.00244 ** | 0.000180 ** |
| L × C × B | 3 | 18.09 | 0.16 | 0.001 | 3.44 | 0.20 | 0.00178 * | 0.000009 |
| L × O × B | 2 | 95.96 | 0.77 | 0.11* | 9.61 | 0.13 | 0.00075 | 0.000010 |
| C × O × B | 6 | 191.38 ** | 2.38 * | 0.11 ** | 2.76 | 18.59 | 0.00068 | 0.000004 |
| L × C × O × B | 6 | 66.33 | 1.42 | 0.04 | 3.05 | 6.50 | 0.00009 | 0.000006 |
| Eb | 80 | 37.44 | 0.79 | 0.02 | 5.84 | 19.15 | 0.00059 | 0.0000224 |
| CV (%) | - | 5.11 | 4.69 | 6.66 | 3.64 | 2.42 | 5.48 | 3.06 |

*, ** significant at 5% and 1% probability levels, respectively.

yield in all levels of chemical fertilizer. However, there was not clear trend about application of biologic fertilizer. Previously, researchers (Ciarkowska *et al.*, 2017) had highlighted the important role of humic acid, especially in root formation.

Rosmarinic acid concentrations in Perilla were significantly affected by location, chemical and organic fertilization (Table 3) and, at both locations and all chemical fertilization levels, the humic acid application increased rosmarinic acid content in Perilla. This fact exhibited the importance of humic acid on rosmarinic acid content in Perilla. The maximum rosmarinic acid (25.01 mg.g DM) was achieved in Mashhad Ardehal along with using 100 kg/ha chemical fertilizer and application of humic acid (Fig. 2).

Application of organic fertilizer especially humic acid with/without biologic fertilizer in all chemical fertilizer levels increased rosmarinic acid concentration in Perilla. The highest rosmarinic acid (23.48 mg.g DM) was acheved in 100 kg/ha chemical fertilizer plus humic acid and without application of biologic fertilizer (Table 6). Similarly, with plant yield, rosmarinic acid concentration was affected by fertilizer application.

These results are in agreement with Hendawy *et al.* (2015) on *Mentha piperita*. They reported foliar application of humic acid increased growth characteristics and finally possessed the best oil percentage and yield in mint plant. Zaghloul *et al.* (2009) reported also the application of humic acid increased oil content of *Thuja orientalis.*

At both of studied location and with/without application of biologic fertilizer, the maximum rosmarinic acid yield was achieved by using humic acid. The maximum rosmarinic acid yield (2.967 g/m$^2$) was observed in Mashhad Ardehal and application of

Table 4 - ANOVA of some morphologic, yield and essence traits of Perilla under different fertilizer systems in Mashhad Ardehal in Kashan regions

| S.O.V. | d.f. | Means squares | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Plant yield | Rosmarinic acid content | Rosmarinic acid yield | Stomatal Conductance | DPPH | Catalase activity | Polyphenol oxidase |
| Replication | 2 | 61.95 | 0.46 | 0.01 | 5.24 | 24.06 | 0.0006 | 0.000040 |
| Chemical fertilizer (C) | 3 | 10082.10 ** | 195.60 ** | 13.42** | 1256.35 ** | 540.52 ** | 0.0411 ** | 0.00749 ** |
| Ea | 6 | 10.21 | 1.61 | 0.04 | 5.48 | 58.46 ** | 0.0003 | 0.00013 ** |
| Organic fertilizer(O) | 2 | 1049.35 ** | 27.60 ** | 1.46** | 42.86 ** | 581.28 ** | 0.0012 | 0.00071** |
| Biologic fertilizer (B) | 1 | 363.69 ** | 12.52 ** | 0.71** | 87.60 ** | 13.86 | 0.0002 | 0.00005 |
| C × O | 6 | 50.03 * | 3.84 ** | 0.07** | 13.03 ** | 11.17 | 0.0006 | 0.00037 ** |
| C × B | 3 | 42.09 | 0.63 | 0.03 | 2.73 | 1.39 | 0.0027 ** | 0.00001 |
| O × B | 2 | 240.40 ** | 1.60 | 0.18** | 1.28 | 8.78 | 0.0006 | 0.00001 |
| C × O × B | 6 | 89.83 ** | 1.67 | 0.08 ** | 5.60 | 5.38 | 0.0009 * | 0.000005 |
| Eb | 40 | 21.24 | 0.86 | 0.02 | 3.16 | 14.71 | 0.0004 | 0.00003 |
| CV (%) | - | 3.56 | 4.79 | 5.65 | 2.63 | 2.32 | 4.48 | 3.64 |

*, ** significant at 5% and 1% probability levels, respectively.

Table 5 - ANOVA of some morphologic, yield and essence traits of Perilla under different fertilizer systems in Sensen in Kashan regions

| S.O.V. | d.f. | Means squares | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Plant yield | Rosmarinic acid content | Rosmarinic acid yield | Stomatal Conductance | DPPH | Catalase activity | Polyphenol oxidase |
| Replication | 2 | 51.56 | 0.96 | 0.01 | 2.80 | 13.07 | 0.00030 | 0.000003 |
| Chemical fertilizer (C) | 3 | 2005.86 ** | 76.59 ** | 3.03** | 433.02 ** | 326.18 ** | 0.00677 ** | 0.003819 ** |
| Ea | 6 | 46.75 | 0.76 | 0.04 | 5.99 | 30.56 | 0.00090 | 0.000009 |
| Organic fertilizer(O) | 2 | 457.49 ** | 5.13 ** | 0.39** | 47.28 ** | 521.70 ** | 0.00307 * | 0.000019 |
| Biologic fertilizer (B) | 1 | 33.13 | 0.31 | 0.03 | 128.16** | 9.14 | 0.00005 | 0.000095 ** |
| C × O | 6 | 106.63 | 3.80 ** | 0.01 | 5.98 | 36.55 | 0.00351 ** | 0.000025 |
| C × B | 3 | 106.20 | 0.58 | 0.02 | 2.72 | 1.62 | 0.00156 | 0.000019 |
| O × B | 2 | 92.53 | 0.48 | 0.02 | 10.79 | 10.91 | 0.00030 | 0.000006 |
| C × O × B | 6 | 167.87 * | 2.15 * | 0.06 ** | 0.21 | 19.72 | 0.00065 | 0.000006 |
| Eb | 40 | 53.64 | 0.74 | 0.02 | 8.51 | 23.59 | 0.00077 | 0.000012 |
| CV (%) | - | 6.67 | 4.59 | 7.88 | 4.48 | 2.98 | 6.37 | 2.28 |

*, ** significant at 5% and 1% probability levels, respectively.

Table 6 - Means comparison of some morphologic, yield and essence traits of Perilla under different fertilizer systems in Sensen and Mashhad Ardehal in Kashan regions

| Chemical fertilizer | Organic fertilizer | Biologic fertilizer (Inoculation) | Plant yield ($g/m^2$) | Rosmarinic acid content (mg/g D.M.) | Rosmarinic acid yield ($g/m^2$) |
|---|---|---|---|---|---|
| 0 kg/ha | Control | (-) | 93.12 lm | 14.40 lm | 1.34 l |
| | | (+) | 89.86 m | 13.94 m | 1.25 l |
| | Compost | (-) | 99.40 kl | 15.31 l | 1.52 k |
| | | (+) | 103.40 jk | 17.29 jk | 1.67 ij |
| | Humic acid | (-) | 99.91 kl | 16.44 k | 1.64 jk |
| | | (+) | 95.17 lm | 17.31 jk | 1.65 jk |
| 50 kg/ha | Control | (-) | 115.70 gh | 19.52 def | 2.25 gh |
| | | (+) | 128.40 de | 19.38 defg | 2.54 ef |
| | Compost | (-) | 129.50 de | 19.56 def | 2.52 ef |
| | | (+) | 126.90 de | 20.11 ced | 2.54 e |
| | Humic acid | (-) | 127.90 de | 18.92 fgh | 2.42 efg |
| | | (+) | 141.20 ab | 20.37 cd | 2.89 bc |
| 100 kg/ha | Control | (-) | 131.40 cd | 21.05 bc | 2.79 d |
| | | (+) | 126.70 de | 21.58 b | 2.75 d |
| | Compost | (-) | 138.00 bc | 21.7 b | 3.02 bc |
| | | (+) | 137.70 bc | 21.63 b | 3.00 bc |
| | Humic acid | (-) | 131.70 cd | 23.48 a | 3.11 b |
| | | (+) | 147.20 a | 23.02 a | 3.43 a |
| 200 kg/ha | Control | (-) | 107.20 ij | 17.52 ij | 1.88 i |
| | | (+) | 113.50 hi | 18.4 ghi | 2.09 h |
| | Compost | (-) | 126.20 def | 18.2 hij | 2.30 g |
| | | (+) | 119.40 fgh | 19.13 egh | 2.29 g |
| | Humic acid | (-) | 116.90 gh | 19.33 efg | 2.27 gh |
| | | (+) | 122.70 efg | 19.07 fgh | 2.35 fg |

Different letters in each column denote a significant differences at p≤0.05 (LSD test).

humic acid beyond biologic fertilizer (Fig. 3). Also, between chemical × organic × biologic fertilizer treatments, the maximum rosmarinic acid yield (3.432 $g/m^2$) was observed in 100 kg/ha along with humic acid and biologic fertilizer application (Table 6). Integrated application of chemical, organic and biological fertilizers improved soil physical and chemical properties and resulted in the increase in availability of nutrients and ultimately the yield and quality of plants. Darzi *et al.* (2007) reported the application of mycorrhiza and vermicompost and phosphate solubilizing biologic fertilizers determined increased essential oil yield in fennel.

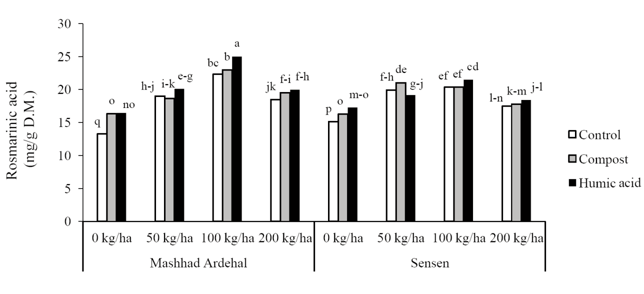


Fig. 2 - Effect of chemical and organic fertilizer on rosmarinic acid content of Perilla in Mashhad Ardehal and Sensen regions. Different letters in each column denote a significant difference at p≤0.05 (LSD test).

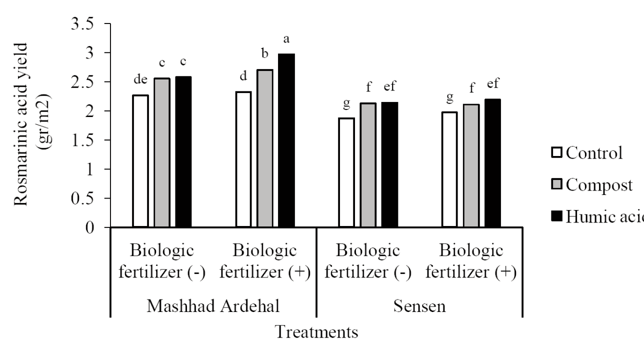


Fig. 3 - Effect of organic and biologic fertilizer on rosmarinic acid yield of Perilla in Mashhad Ardehal and Sensen regions. Different letters in each column denote a significant difference at p≤0.05 (LSD test).

*Stomatal conductance*

Stomatal conductance is one of the most important factor in determining the photosynthesis amount. The study of stomatal conductance between location and chemical fertilizer treatments showed the highest stomatal conductance (75.88 mmol $H_2O$ $m^{-2}$ $s^{-1}$) in Mashhad Ardehal with application of 100 kg/ha chemical fertilizer (Fig. 4). At both of studied location especially in Mashhad Ardehal, application of chemical fertilizer increased the stomatal conductance of Perilla leaves. Increasing of stomatal conductance increases the photosynthesis activities and finally improved the plant yield (Atteya, 2003). The results of stomatal conductance are agreement with result of plant yield. This result showed the increasing of stomatal conductance can be useful to increasing of plant yield especially with 100 kg/ha application of chemical fertilizer.

The highest stomatal conductance was obtained with application of compost fertilizer (67.67 mmol $H_2O$ $m^{-2}$ $s^{-1}$) (Table 7). Organic fertilizers can provide better moisture conditions by increasing the absorption and preservation of water and the availability of water and food, and this can contribute to the favorable conditions for photosynthesis in the plant. If there is enough stomatal conductance, $CO_2$ gas will enter the stomata more easily and sufficient photosynthesis will be performed. Also means comparison showed inoculation with mycorrhiza improved stomatal conductance as compared to without inoculation (Table 7).

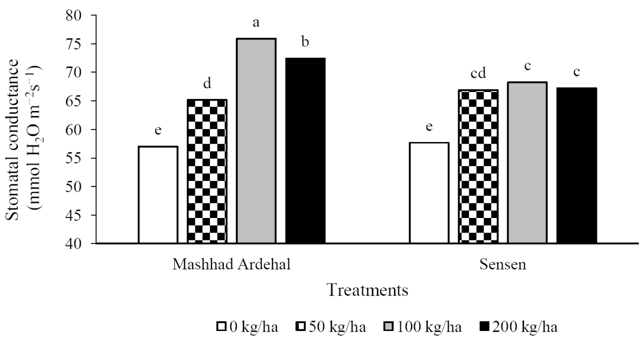


Fig. 4 - Effect of chemical fertilizer on stomatal conductance of Perilla in Mashhad Ardehal and Sensen regions. Different letters in each column denote a significant difference at p≤0.05 (LSD test).

The physiological effects of mycorrhizal fungi symbiosis include aboveground modifications of water relations and physiological status in terms of leaf water potential, relative water content, stomatal conductance, $CO_2$ assimilation, and efficiency of photosystem II as compared to non-mycorrhizal plants (Barzana *et al.*, 2012). Mycorrhizal fungi increase the contact surface with soil and moisture around the plant roots by 10 to 1,000 times, thus increasing the plant's ability to use the resources in its surroundings (Sharma, 2002).

*DPPH test*

Many researchers have used DPPH test to express the antioxidant status of plants (Dasgupta and De, 2007; Sahu *et al.*, 2013). The results showed the antioxidant activity of Perilla plant in Mashhad Ardehal (165.29 ug/ml) was higher than Sensen (162.79 ug/ml) location (Table 7). Perilla is very sensitive to free radicals productions. In a study in Korea on different species of Perilla, different species for DPPH test showed a significant difference (Choi *et al.*, 2002).

Increasing levels of chemical fertilizer reduced the plant's efficiency in inhibiting free radicals. The highest amount of DPPH test was obtained (169.47 µg/ml) under control condition and the lowest (55.85 µg/ml) was obtained from the highest level of fertilizer application (200 kg/ha) (Table 7). Omar *et al.*, (2012) reported the antioxidant activity determined using the DPPH was high with the application of organic fertilizer compared to chemical fertilizer in cassava tubers.

Organic fertilizers, especially compost (277.17 µg/ml), increased the antioxidant activity and inhibited free radicals (Table 7). Uthairatanakij *et al.* (2017) reported an increase in antioxidant activity and inhibition of free radicals due to the application of organic fertilizers. Organic fertilizers with plant sources activated defense mechanisms against pests, diseases and other tensions (Brandt and Molgaard, 2001).

Table 1 - Weather characteristics in Mashhad Ardehal and Sensen

| Treatments | Stomatal Conductance (mmol $H_2O$ $m^{-2}s^{-1}$) | DPPH (ug/ml) | Polyphenol oxidase (µmol/min) |
|---|---|---|---|
| *Location* | | | |
| Mashhad Ardehal | 67.61 a | 165.29 a | 0.156 a |
| Sensen | 64.98 b | 162.79 b | 0.152 b |
| *Chemical fertilizer* | | | |
| 0 kg/ha | 57. 36 d | 169.48 a | 0.130 c |
| 50 kg/ha | 65.99 c | 161.73 c | 0.152 b |
| 100 kg/ha | 72.05 a | 166.41 b | 0.167 a |
| 200 kg/ha | 69.80 b | 158.55 d | 0.168 a |
| *Organic fertilizer* | | | |
| Control | 66.03 b | 158.54 b | 0.152 b |
| Compost | 67.68 a | 167.27 a | 0.158 a |
| Humic acid | 65.19 b | 166.31 a | 0.153 b |
| *Biologic fertilizer (Inoculation)* | | | |
| (-) | 65.08 a | 164.08 a | 0.153 b |
| (+) | 67.52 b | 164.00 a | 0.155 a |

Different letters in each column denote a significant differences at p≤0.05 (LSD test).

*Catalase activity*

The highest catalase activity (0.5332 nmol $H_2O_2$/min) was observed in Mashhad Ardehal region with the application of 100 kg/ha fertilizer and without application of biologic fertilizer. The lowest amount was obtained from Mashhad Ardeal area without using of chemical and biologic fertilizer. Also, in the Sansen location, the highest catalase activity was obtained from 200 kg/ha chemical fertilizer and biologic fertilizer application (Fig. 5).

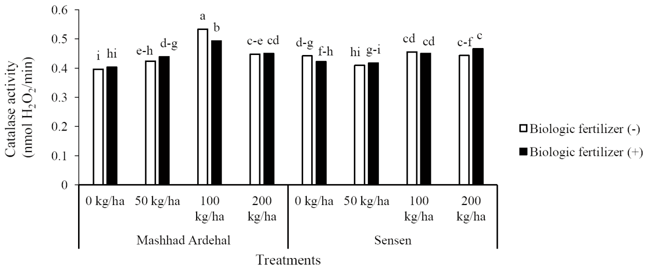


Fig. 5 - Effect of chemical and biologic fertilizer on catalase activity of Perilla in Mashhad Ardehal and Sensen regions. Different letters in each column denote a significant difference at p≤0.05 (LSD test).

Among the chemical and organic fertilizer treatments in two studied locations, the highest catalase activity was obtained from 100 kg/ha of chemical fertilizer with compost application in Mashhad Ardehal region (0.5182 nmol $H_2O_2$/ min) and the lowest amount (0.338 nmol $H_2O_2$/ min) was obtained without using of chemical and organic fertilizer control in Mashhad Ardehal location (Fig. 6). Logan *et al.* (1999) stated that nitrogen had a significant effect on the activity of enzymes involved in photosynthesis, such as Ribulose-1,5-bisphosphate. In another study the researchers reported increased nitrogen levels caused to production of antioxidant enzymes like APX, SOD, CAT, and POD in the *Populus yunnanensis* plants (Lin *et al.*, 2012). Our result indicated that sources of fertilizer had a significant influence on the level of catalase activity in field grown Perilla.

*Polyphenol oxidase activity*

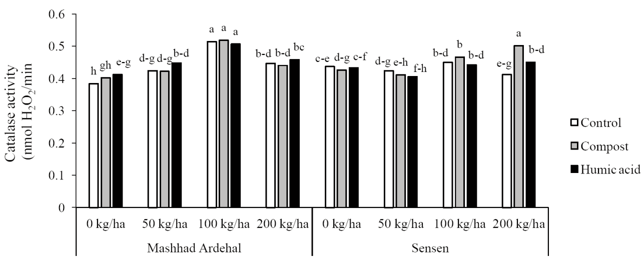


Fig. 6 - Effect of chemical and organic fertilizer on catalase activity of Perilla in Mashhad Ardehal and Sensen regions. Different letters in each column denote a significant difference at p≤0.05 (LSD test).

Among the treatments of chemical and organic fertilizer in different locations, similar to catalase activity, the highest polyphenol oxidase activity was obtained from 100 kg/ha with compost application in Mashhad Ardeal location (0.193 µmol/min) and the lowest amount (0.129 µmol/min) was obtained from without application of chemical and organic fertilizer in Mashhad Ardehal area (Fig. 7). At all levels of chemical fertilizer in both locations, application of organic fertilizers increased the activity of polyphenol oxidase activity, and in most cases, organic matter compost was superior to humic acid. Also, the application of biologic fertilizer led to an increase in polyphenol oxidase activity (Table 7). Similar to our results, the application of biologic fertilizer increased the amount of polyphenol oxidase activity in triticale (Kheirizadeh-Arough *et al.*, 2016).

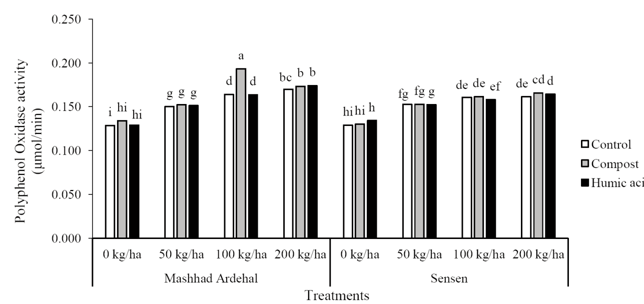


Fig. 7 - Effect of chemical and organic fertilizer on polyphenol oxidase activity of Perilla in Mashhad Ardehal and Sensen regions. Different letters in each column denote a significant difference at p≤0.05 (LSD test).

## 4. Conclusions

In the present experiment, the results showed that proper nutritional management of Perilla medicinal plant has a special role in improving quantitative and qualitative traits. Among individual fertilizer treatments, 50 and 100 kg/ha of fertilizer and among organic fertilizers, humic acid played a more effective role in improving the studied indices, but the application of biologic fertilizer separately did not have a very significant effect on this plant. Integration with other fertilizers has been shown to be more effective. Mashhad Ardehal area has better conditions for cultivating Perilla due to its soil characteristics and climatic characteristics. Also, the combined application of different fertilizer sources in comparison with the single application of each of them in both studied regions significantly improved the growth characteristics, as well as essential oil yield and biochemical indices of Perilla. Furthermore, organic fertilizers like compost and humic acid discharge nutrients very slowly to the plants. Hence, an integrated approach, combining application of compost with an application of chemical fertilizer is a good strategy for increasing crop productivity. This will reduce the cost of chemical fertilizer and improve soil fertility.

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