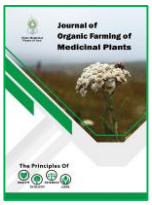
Journal of Organic Farming of Medicinal Plants: September 2022; 1 (2): 79-84
Available online at http://www.jofmp.areeo.ac.ir



Original Article

Total phenolic and flavonoid content of Satureja sahandica plant under the influence of animal manure and enriched straw in dry conditions

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ARTICLE INFO

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Received: 22 April 2024
Accepted: 13 May 2024

Keywords:
Flavonoid
Soil fertility
Plant density
Antioxidant enzymes

ABSTRACT

Satureja sahandica is an exclusive species of Iran. The aerial parts of this plant are widely used in the food and pharmaceutical industries. Soil fertility management is one of the main factors determining the growth and quantitative and qualitative performance of medicinal plants. According to the statistics presented in 2024, Iran is in a year of drought that multiplies the necessity of using the climate capacity correctly. Agriculture in dry farming conditions is very important considering the available water capacities in the world. Because something like 41% of the world includes dry land. These experiments were carried out in the form of split plots in the form of a randomized complete block design in the steppe region (Humand area of Damavand city). The experimental treatments included organic manure (cow, straw enriched with ammonium sulfate and control treatment), planting density (low density of 26666 plants per hectare, medium density of 40000 plants per hectare, and high density of 80000 plants per hectare) in 2017 and 2018. Sahandi Savory seeds were obtained from the Research Institute of Forests and Rangelands of Iran (RIFR). They were planted in culture trays filled with a mixture of peat moss and vermiculite (1:1 volume) under greenhouse conditions. Two-month-old seedlings were brought to the farm from the greenhouse in April 2017. The average annual rainfall was 333 mm. This research showed that increasing soil fertility can make a significant change in the increase of phenol and flavonoids.

1. Introduction

Savory (*S.sahandica*) is an exclusive species of Iran (Yousefzadi et al.; 2012). The aerial parts of this plant are widely used in food and pharmaceutical industries and can replace artificial preservatives as a natural antioxidant (Hajhashemi et al.;2002). The vegetative growth of this Sahandi spice starts from the end of March and its flowering continues from the middle of June to the end of August. If it is harvested or trapped, its flowering continues until early autumn (Akbarinia et al.;2009). Harvesting medicinal plants at the wrong time not only reduces the amount of the obtained product, but also the harvested product will not have the desired quality. Because the function of the target organ as well as the amount of secondary metabolites of a medicinal plant is different in different stages of plant growth and development (Omidbaigi;2005). Plants have a set of antioxidants to control the amount of free oxygen radicals and to protect their plant cells in stressful conditions (Borsani et al.;2001).Biological activities of plant extracts, especially their anti-aggregation and free radical activities (Brunetti et al.;2013) as well as antimicrobial activity and capacities (Pistelli & Giorgi.;2012) and anticancer (Batra & Shama.;2013)Due to the presence of complex compounds, they contain phenol and flavonoids (Gopčević et al.;2019). Phenols are a large group of biologically active compounds (over 8000 compounds) that are among the ubiquitous secondary metabolites in plants (Dreosti;2000). Phenols are classified into two basic groups, simple phenols and polyphenols, based on the number of phenol subunits (Marinova et al.;2005). The group of simple phenols contains phenolic acids or phenols with a carboxyl group, which determines their functional properties. There are at least two phenol rings in polyphenol. Flavonoids belong to this group. More than 4000 flavonoids have been identified in different plant species (Harborne & Tumer.;1984).The integrated

management of plant nutrition is called an operation that uses nutrients, water, products and plant cover management methods in the soil bed, according to the cultivation and the specific agricultural system, with the aim of improving and preserving soil fertility, land productivity and reducing destruction in environment is done (Mohebi.;2016).The integrated management of nutrients is done with the aim of optimizing soil conditions, according to its chemical, physical, biological and hydrological properties, in order to increase the productivity of the farm, so that soil degradation is as low as possible (Feller et al.; 2012). Soil fertility management is one of the main factors determining the growth and quantitative and qualitative performance of medicinal plants (Omidbaygi.;1995). In high densities, the competition for moisture, food and light increases, which results include the reduction of stem diameter and the increase of plant height (Gozubenli et al.;2003). One of the important factors to obtain the highest seed yield in corn is to determine the appropriate density according to the climatic conditions of each region and the characteristics of the cultivars to be planted (Akintoye et al.;1997). The changing pattern of the hydrological cycle is one of the issues related to climate change that deserves urgent attention (Donat et al.,2016). Evaporation, transpiration is related to water and soil balance and has a key role in the interactions of climate, soil and vegetation (Yang et al,2007). Water management in dry farming agriculture systems is important in addition to providing the water needed for food production, in order to create resistance to the risks and uncertainties related to water use. The availability of water is different in different regions, and the competition to obtain water can become very intense in regions where natural rainfall decreases, such as arid and semi-arid regions (Lovelli;2019). Rainfed agriculture and dry farming agriculture are terms that are often used as synonyms, but this is a mistake, in both cases irrigation is omitted, but by omitting this aspect, their meaning is profoundly different (Stewart.; 2016,a). But the latest methods are to conserve water and the sustainable performance of crops, limit fertilizers and other inputs and act to limit erosion (Stewart; 2016, b). Agriculture in dry farming conditions is very important considering the available water capacities in the world, because something like 41% of the world includes dry lands (Lovelli.;2019) that In order to develop and cultivate them, we should turn to dry farming agriculture.

2. Materials and Methods

This experiment was carried out based on split plots in the form of randomized complete blocks design in the Humand area of Damavand city. The experimental treatments included organic manure (cow, straw enriched with ammonium sulfate and control treatment), planting density (low density of 26666 plants per hectare, medium density of 40000 plants per hectare and high density of 80000 plants per hectare) in 2017 and 2018. Sahandi Savory seeds were obtained from the Research Institute of Forests and Rangelands of Iran (RIFR). They were planted in culture trays filled with a mixture of peat moss and vermiculite (1:1 volume) under greenhouse conditions. Two-month-old seedlings were brought to the farm from the greenhouse in April 2017. The average annual temperature of the farm is 11°C, minimum -24°C in January-February and maximum 37°C in July-August. The average annual rainfall was 333 mm. Soil pH and EC (0-20 cm) were 8.3 and 0.8 dsm-1, respectively (Table 1). The results of the physicochemical analysis of the soil of savory planting bed in dry conditions are as described in Table 1.

Table.1-Physico-chemical analysis of the soil of savory planting bed in dry conditions

Features	Amount
clay (percent)	5/35
sand (percent)	17
silt (percent)	5/47
soil pattern	Rosy Silty
CEC (m.e/100 gr)	26
soil acidity	48/7
Electrical conductivity (decsiemens/meter)	0.522
organic carbon (percentage)	0.98
Total nitrogen (percentage)	0.072
Absorbable potassium (mg/kg)	800

The meteorological information of the farm for conducting the experiment is as described in Table 2.

Table.2- Meteorological information of planting area

Year	Average temp(°C)	Annual rainfall (mm)
2016	12.38	321.5
2017	11.95	487

In order to improve soil fertility, the consumption of rotted cow manure required for the implementation of the project was determined based on 30 tons per hectare. To apply fertilizer treatment, we took 36 kg of rotted cow manure for each sub-plot and spread it evenly in the created furrows. Then we covered the manure with pile soil. The amount of straw needed to implement each plan was based on 10 tons per hectare. In order to carry out the treatment of enriched straw, the consumption of 12 kilograms of chopped wheat straw was uniformly distributed in the created grooves of each sub-plot.And the consumption of 240 grams of ammonium sulfate (based on 2 kilograms for every 100 kilograms of straw in the silo) after dissolving in 20 liters of water was sprayed uniformly on the straws of the floor. Then it was covered with pile soil. After applying the desired treatments and planting, annual statistics were taken.

Phenolic compounds are measured by methods that use Folin-Ciocalteu as a reagent and gallicacid as a standard. Phenolic content was measured by spectrophotometer, according to Ouchikh (2011) method. In this experiment, 2 grams of the sample was homogenized with 8 milliliters of 80% ethanol and centrifuged at 12,000 x g for 20 minutes, and then 0.5 milliliters of the supernatant was removed using a sampler and poured into 15 milliliter flasks. , then 500 microliter Folin-Ciocalto was added to the contents of Falcon and after 2 minutes, one milliliter of 7% sodium carbonate was added to the reaction mixture and the final volume was brought to 6 milliliters using distilled water. The falcons were placed in a 30°C bain-marie bath (dark conditions) for 90 minutes. The absorption of the samples was also measured in a spectrophotometer at a wavelength of 725 nm. This method was used for all gallic acid standard solutions and standard calibration curve drawing. The amount of flavonoid was measured by aluminum chloride colorimetric method in this method, 0.5 ml of extract solution with 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml a liter of distilled water was mixed. After keeping the samples at room temperature for 30 minutes, the absorbance of the mixture was read at a wavelength of 415 nm. Querstein standard was used to draw the curve (Chang, et al., 2002). The amount of enzyme activity was measured by the method of Ranieri et al. (2003). As a result of the reaction between ascorbate peroxidase and ascorbic acid and H2O2, dehydroascorbate is produced, which is read at a wavelength of 290 nm. The reaction medium contained 600 microliters of 0.1 mm EDTA, 1500 microliters of 50 mm phosphate buffer (pH = 7), 400 microliters of 0.5 mm ascorbic acid, 400 microliters of 30% H2O2, and 50 microliters of enzyme extract. Enzyme activity measurement was recorded for 7 minutes with 20 second intervals.Ascorbate peroxidase also converts H2O2 into water using ascorbate, which acts as an electron donor, according to the following equation:

$$\text{Ascorbate } (C_6H_8O_6) + H_2O_2 \longrightarrow \text{Dehydroascorbate } (C_6H_6O_6) + 2\,H_2O$$

The specific activity of the enzyme was reported as micromoles of oxidized ascorbate per minute per milligram of protein.

$$\left(Activity\left(U/_{ml}\right) = \frac{\Delta A_{290} \times l \times V_t \times d_f}{\varepsilon \times l \times t \times V_s}\ \mu\text{mol ascorbate oxidized min}^{-1}\ \text{mg}^{-1}\ \text{protein}.\right)$$

The activity of catalase enzyme (CAT) was measured at 25 degrees Celsius using a spectrophotometer and according to the method of Aebi (1984). A spectrophotometer made in Japan at a wavelength of 240 nm was used to measure enzyme activity.The solutions and materials used included 3000 microliters of phosphate buffer (pH = 7) 50 millimolar, 5 microliters of 30% H2O2 and 50 microliters of enzyme extract.And enzyme activity was recorded for 5 minutes in 20 second intervals.Catalase enzyme converts H2O2 into O2 and H2O according to the equation below without the need for a regenerating agent.

$$2\,H_2O_2 \longrightarrow 2H_2O + O_2$$

The specific activity of the enzyme was reported as micromoles of H_2O_2 dissolved per minute per milligram of protein.

$$\left(Activity\left(U/_{ml}\right) = \frac{\Delta A_{240} \times l \times V_t \times d_f}{\varepsilon \times l \times t \times V_s}\ \mu\text{mol of H2O2 decomposed min}^{-1}\ \text{mg}^{-1}\ \text{protein}\right)$$

Superoxide dismutase activity was measured according to the method of Dhindsa et al. (1981). The activity of this enzyme is evaluated photo metrically. The main reaction buffer included 100 mm phosphate buffer (pH=7.8), 12 mm methionine, 75 µm nitro blue tetrazolium, 100 µm EDTA, and 0.025% Triton X-100. 290 microliters of the main buffer was added to each well. Then 5 µM of 2 µM riboflavin buffer was added to the reaction mixture and the device was calibrated at a wavelength of 560 nm. 10 microliters of protein extract was used to measure each sample. This reaction is evaluated based on the rate of photo regeneration of nitro blue tetrazolium and the ability of superoxide dismutase enzyme to inhibit this reaction. In order to measure polyphenol oxidase, 0.05 g of fresh leaf tissue was homogenized in a cold Chinese mortar and in an ice container with 2 ml of 0.1 M phosphate buffer with an acidity of 6.8 and then it was centrifuged for 15 minutes at 13,000 revolutions per minute at a temperature of 4 degrees Celsius. The upper phase of the obtained extract was used to measure the activity of the polyphenol oxidase enzyme (Lio and Hang; 2000). After measuring and calculating the mentioned traits, the obtained results were analyzed. Analysis of variance and comparison of means was done with the help of SAS software. Excel software was used to draw the figures. The mean comparison of traits was done using Duncan's test at the probability level of 5%.

3. Results and Discussion

The results of analysis of variance showed that the effect of year on flavonoid yield was significant at 1% level (P≤0.01), but it had no significant effect on phenol. The effect of fertilizer on phenol and flavonoids was significant at 1% level (P≤0.01). The effect of density on phenolic and flavonoid traits was also significant at 1% level (P≤0.01). The interaction effect of year and fertilizer on phenol and flavonoid was significant at 1% level (P≤0.01). The interaction effect of year and density, the interaction effect of fertilizer and density,

Table 3- The results of variance analysis of phenol, flavonoid and antioxidant enzymes of sahandi Savory plant.

S.O.V	DF	TPC	TFC	APX	SOD	CAT	PPO
Year	1	6.897	** 55.206	** 1988.972	** 32.666	** 7.141	** 0.007
Rep (year)	2	9.955	0.968	1978.97	0.166	0.003	0.00002
Fertilizer	2	** 339.676	** 10.583	** 9551.779	** 233.185	** 1.726	** 0.006
Year x Fertilizer	2	** 20.678	** 3.350	** 14577.962	** 20.222	0.090	0.0002
Rep (year x fertilizer)	8	2.657	0.267	17.417	1.203	0.013	0.00019
Density	2	** 26.423	** 2.100	** 3468.991	** 41.129	** 0.987	** 0.0015
Year x density	2	5.664	0.483	** 5354.678	4.166	** 0.335	0.0004
Fertilizer x density	4	1.465	0.244	** 6309.165	0.824	0.147	0.0008
Year × Fertilizer × Density	4	2.400	0.104	2948.533	1.638	0.061	0.00013
CV%	-	4.567	5.385	4.785	4.789	4.198	8.725

**, *: Significant at the %1 and 5% probability levels, respectively.

Table 4- Essential oil compassion of sahandi savory under manure fertilizer, Straw fertilizer and plant density (HPD: high plant density, MPD: medium plant density, LPD: low plant density) in 2017.

Compound	RI	Control			Manure application			Straw fertilizer application		
		HPD	MPD	LPD	HPD	MPD	LPD	HPD	MPD	LPD
α-Thujene	934.61	1.11	1.05	0.54	0.88	0.68	0.54	0.51	0.68	0.53
α-Pinene	946.09	0.75	0.78	0.57	0.65	0.57	0.53	0.31	0.51	0.52
Myrcene	976.79	1.84	1.65	1.15	1.43	1.26	1.25	1.30	1.40	1.16
α-Terpinene	1043.62	1.38	0.88	0.50	1.34	1.16	0.68	0.77	0.73	0.56
p-Cymene	1053.41	47.57	56.37	48.68	43.13	44.36	48.55	26.92	50.81	50.26
ɤ-Terpinene	1084.13	13.22	8.46	6.21	14.75	12.71	7.41	12.25	0.13	6.34
Cis-Sabinene hydrate	1100.00	0.18	0.25	0.35	0.19	0.24	0.23	0.59	0.43	0.46
Thymol	1320.15	26.53	23.48	31.70	27.13	30.95	32.26	36.39	30.38	28.89
Carvacorol	1330.04	1.12	1.02	1.52	3.27	1.11	1.80	1.35	1.39	1.77
Thymol acetate	1363.79	0.39	0.16	0.46	0.40	0.44	0.49	0.68	0.28	0.96
E - Caryophyllene	1476.54	0.85	0.78	0.70	0.92	0.78	0.39	1.41	0.56	0.59
Spathulenol	1649.09	0.15	0.17	0.36	0.31	0.44	0.37	0.33	0.31	0.87
Caryophllene Oxide	1659.41	0.55	0.86	1.55	1.08	1.17	1.07	1.26	1.31	2.41

Table 5- Essential oil compassion of sahandi savory under manure fertilizer, Straw fertilizer and plant density (HPD: high plant density, MPD: medium plant density, LPD: low plant density) in 2018

Compound	RI	Control			Manure application			Straw fertilizer application		
		HPD	MPD	LPD	HPD	MPD	LPD	HPD	MPD	LPD
α-Thujene	937.56	1.36	1.08	1.00	1.08	0.99	0.90	0.93	0.88	0.68
α-Pinene	946.29	0.98	0.71	0.76	0.76	0.76	0.69	0.66	0.64	0.56
Myrcene	1010.34	0.30	0.21	0.26	0.26	0.27	0.25	0.21	0.22	0.18
α-Terpinene	1045.05	1.79	1.68	1.45	1.45	1.97	1.22	1.35	1.36	1.76
p-Cymene	1054.08	47.49	43.49	45.74	48.74	36.03	42.09	44.50	42.38	29.87
ɤ-Terpinene	1084.53	18.84	18.23	15.32	15.32	22.01	14.03	14.74	15.59	20.74
Cis-Sabinene hydrate	1100.00	0.14	0.16	0.16	0.16	0.15	0.22	0.24	0.29	0.31
Thymol	1322.00	21.59	26.05	24.04	24.04	29.86	31.14	28.75	30.37	36.56
Carvacorol	1326.82	1.11	1.06	0.97	0.97	0.82	1.29	1.16	1.18	1.05
Thymol acetate	364.32	0.88	0.98	0.62	0.62	1.07	0.88	0.86	1.60	2.77
E - Caryophyllene	1480.12	0.69	0.75	0.77	0.77	0.85	0.84	0.76	0.95	1.10
Spathulenol	1654.17	0.51	0.28	0.34	0.34	0.37	0.31	0.33	0.50	0.54
Caryophllene Oxide	166.11	0.47	0.69	0.80	0.80	0.62	0.89	0.90	0.97	1.04

and the interaction effect of year and fertilizer and density were not significant. The results of analysis of variance showed that the effect of year on the enzyme ascorbate peroxidase, catalase, and polyphenol oxidase and superoxide dismutase was significant at 1% level (P≤0.01).

The effect of fertilizer on all enzymes of ascorbate peroxidase, catalase, and polyphenol oxidase and superoxide dismutase was significant at 1% level (P≤0.01).

The effect of condensation on ascorbate peroxidase, superoxide dismutase, polyphenol oxidase and catalase were also significant at 1% level (P≤0.01). The interaction effect of year and fertilizer on ascorbate peroxidase and superoxide dismutase was significant at 1% level (P≤0.01) and not significant on catalase and polyphenol oxidase.

The interaction effect of year and density on catalase and ascorbate peroxidase was significant at 1% level (P≤0.01) and it had no significant effect on polyphenol oxidase and superoxide dismutase. The interaction effect of density and fertilizer was significant only on ascorbate peroxidase at 1% level (P≤0.01) and had no significant effect on catalase, polyphenol oxidase and superoxide dismutase.

The interaction effect of year, fertilizer and density on the enzyme ascorbate peroxidase, catalase, and polyphenol oxidase and superoxide dismutase had no significant effect (Table-3).

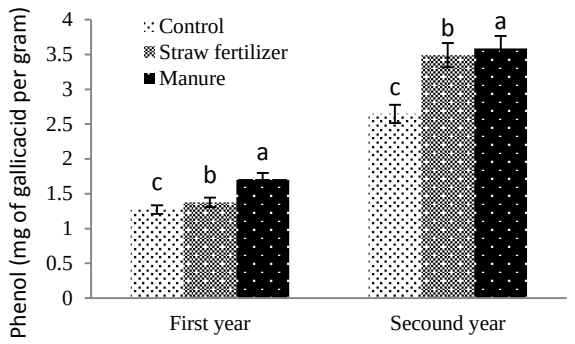

Figure 1- The results of the comparison of the mean interaction effect of year and fertilizer on the phenolic compounds of Sahandi Savory plant.

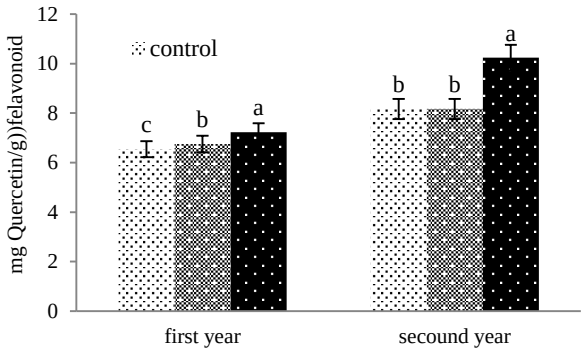

Figure 2- The results of comparing the mean interaction effect of year and fertilizer on the flavonoid compounds of Sahandi Savory plant.

5. Conclusion

This research showed that increasing the soil fertility can make a significant change in the increase of phenol and flavonoids. Based on the results of this investigation, cow manure caused a significant difference in plant flavonoid compounds. The highest amount of flavonoid was observed in the treatments of cow manure.

6. Acknowledgments

This work was supported by the, shar-e-Qods Branch, Islamic Azad University, Tehran, Iran and Research Institute of Forests and Rangelands, Tehran, Iran

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