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Biochemical Changes of Bakhtiari Savory Affected by Organic Fertilizer and Plant Density under Dryland Farming Conditions

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Abstract

Organic fertilizers and optimal density are known to alleviate the undesirable effects of biotic and abiotic stresses in plants. This research aimed to study the effect of density and organic fertilizers on the biochemical status of Bakhtiari savory (*Satureja bachtiarica* Bunge.) under dryland farming conditions. Hence an experiment was conducted as a split-plot based on a randomized complete block design with three replications at Homand rangeland research station of Damavand in crop years 2018-19. Organic fertilizer as the main factor was included three levels of cattle manure (30 t ha^{-1}), enriched wheat straw (10 t ha^{-1}) enriched with ammonium sulfate, and control (without fertilizer). Also, the plant density as a sub-factor was allocated with three levels of low plant density (LPD, 26666 plant ha^{-1}), medium plant density (MPD, 40000 plant ha^{-1}), and high plant density (HPD, 80000 plant ha^{-1}). The results revealed that cattle manure and HPD increased glycine-betaine (GB) and anthocyanin. The maximum Carotenoid was observed at HPD with control and also in cattle manure and MPD. The highest total soluble sugar content (TSS) was achieved with cattle manure in the second year. The most remarkable antioxidant capacity (DPPH) was measured in control, LPD, and the second year. Also, increased total protein amount was found at MPD and cattle manure compared with LPD and control. This research could widely improve the current knowledge of management use of organic fertilizers and optimal density in *Satureja bachtiarica* species under dryland farming conditions.

1. Introduction

With several features, including different climates, diverse landscapes, such as plains and mountain ranges, Iran became one of the most vibrant medicinal herbs (Heshmati., 2012). In addition, it has a high biodiversity of species, ecologies, and environments that provide good natural resources for medicinal and aromatic plants (Dolatkhahi *et al.*, 2014). For instance, the *Satureja* L. genus has around 30 varieties whose placement center is established in the Mediterranean region. Totally 14 species of this genus are developing in the western, northern, and interior parts of Iran, which *S. bachtiarica* is one of them that could be suitable for dryland farming (Khadivi-Khub *et al.*, 2014). Dryland agriculture includes precise farming techniques for growing crops without irrigation. Dryland agriculture is related to dryland areas, identified by an incredible wet season followed by a warm, dry season. They are also related to arid conditions or with areas that are drought or having scarce water resources. Additionally, dryland farming is being developed for these areas (Li *et al.*, 2020).

The application of fertilizer in suitable amounts and improvement of biological conditions of the soil, and the use of resistant cultivars are effective ways to diminish the conflicting effects of drought tension (Tabrizi *et al.*, 2011). One of the main pillars in sustainable farming is utilizing organic fertilizers in crop environments to eliminate or significantly lessen the application of chemical fertilizers (Emami Bistganii *et al.*, 2018). Organic fertilizers comprise various plant-derived substances that vary from green or dried plant matter to herbivore manures and rural by-products. The main content of these organic fertilizers ranges

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considerably among elements, and readily biodegradable corporealities make better nutrient sources (Pandey and patra 2016). Among agronomic determinants, plant frequency in a vigorous area is fundamental for optimal environmental resources such as water, sunlight, and nutrients and plays a critical function in controlling competition between plants in nature (Ghiasy-Oskoee *et al.*, 2018). Intraspecific competition for insufficient supplies rises when the plant density exceeds the optimal limit (Jiang *et al.*, 2013). As a result, it seems essential to select the optimal plant density to achieve the greatest quantitative and qualitative yield. Crop production is influenced by internal and external competition factors of plants. The plant density and plant distribution have a crucial role in maximizing dry matter production by internal and external plant competition (Emami Bistgani *et al.*, 2012; Gardner *et al.*, 2017).

The use of organic fertilizer could increase antioxidant capacity in *S. hortensis* L. (Alizadeh *et al.*, 2010). Usually, one of the tasks that plants do in the face of stress such as nutrient deficiency, salinity stress, drought, and high temperature is the synthesis and accumulation of osmotic preservatives including soluble sugars, amino acids, and Glycine-betaine, which could be plant response to stress (Jogawat., 2019). Nutrient in cattle manure plays a vital role in carotenoids structure and is also the most critical element in protein synthesis. Cattle manure application could increase carotenoid and plant protein under optimal conditions (Xu and Mou 2016). The results of (Saeed *et al.*, 2015) showed that carotenoids are essential in increasing photosynthesis as auxiliary pigments and play other vital roles, such as protecting thylakoid membranes and preventing chlorophyll photo-oxidation. It was assumed that Anthocyanins might preserve photosynthetic cells from unwelcome consequences of the intense spotlight. Anthocyanin's photoprotective roles have seemingly received more attention in recent years than any other functional hypothesis (Gould *et al.*, 2008). Therefore, the current research was conducted to study the role of organic manure and fertilizers and different plant densities to improve some biochemical traits in medicinal plants of Bakhtiari savory (*S. bachtiarica* Bunge.) under dryland farming system.

2. Materials and Method

The operations were handled at Homand rangeland research station of Damavand, Iran, for two crop years in 2017-18 and 2018-19 under dryland farming conditions (latitude 350 40/ N, longitude 5205/ E, altitude 1960 m asl). Annual maximum and minimum temperatures were 35℃ and -15℃ respectively. Annual mean temperature was 12℃ and the farm slop was 4%. The climate of the study area is semi-cold, and the average annual precipitation is about 330 mm. The experimental design was a split-plot based on an RCBD with three replications. The main plots were included three levels: cows manure (30-ton ha^{-1}), embellished wheat hay (10-ton ha^{-1}, enriched with ammonium sulfate), and control (without fertilizer). The subplots had three levels: low plant density (LPD) (26666 plant ha^{-1}), medium plant density (MPD) (40000 plant ha^{-1}), and high plant density (HPD) (80000 plant ha^{-1}). The row distance of each subplot was 50 cm and on the row were 25, 50, and 75 cm. Border rows were not included for any sampling. The seedlings were provided in winter and then transferred into the field. Hence concerning weather forecasts, the seedlings were transferred to the main field before the onset of rain. The mean total precipitation during the experiment was 340 mm, and the mean adequate rainfall in spring was 187 mm. Other fertilizers and pesticides were not applied throughout the study, and removing weeds was manually conducted as needed. Branches harvesting were conducted at flowering stage and in the end of summer. The experimental field's soil properties and the chemical analyses of cattle manure properties are presented respectively in Tables 1 and 2.

2.1. Determination of Total Soluble Sugar Content (TSS)

The TSS determination was done based on the approach described by (Dubois, 1951). Green leaves (0.1 g) were combined with 5 ml of ethanol (80%) to experiment tubes, located in a water bath, and boiled at 80 °C for one hour. Then 1 ml of the specimen extract was added into another set of test tubes mixed with 1 ml each of phenol (18%) and distilled water and then left at room temperature for 1 hour. Subsequently, 5 ml of sulphuric acid were combined, and the whole compound was vortexed. The reading of the absorbance of solutions was done at 490 nm wavelength on the UV spectrophotometer (Biochem, 2100). Ethanol (80%) was used as the clean sample. TSS was explained as g per 100 g FW.

2.2. Determination of Anthocyanins

Discs with 1 cm diameter of leaves were used to measure the concentration of anthocyanins in acidic methanol solution (HCl: methanol, 1:99, v/v) (Havaux and Kloppstech., 2001). After centrifugation (4000 rpm for 10 minutes), the determination of solutions' absorption ranges was done at 530 nm wavelength via a UV-1700 spectrophotometer (Shimadzu, Tokyo, Japan).

2.3. Extraction of Chlorophyll and Total Carotenoid

Modified (Litch Tonler's., 1987) method was used to measure chlorophyll and total carotenoid. First, leaf specimens were powdered with liquid nitrogen, after that 10 ml of acetone was added to 100 mg of it. For separation, the resulting extract was placed in a centrifuge (German model Hettich-Mikro 200R) for 10 minutes at 6000 rpm (Lichtenthaler., 1987).

2.4. Measurement of Chlorophyll and Total Carotenoid

By using (PG Instruments ItdT80 + UV / VIS), the reading of absorbance quantity of the extracted specimens was done at three wavelengths of 470, 646, and 663 nm. The amount of total carotenoid, total chlorophyll, chlorophyll a, and chlorophyll b were calculated using the adsorption rate of the samples in these three wavelengths and the Equations 1-4.

$$Chl_a\ (mg/g) = 12.25\ A_{663} - 2.79 A_{646} \quad \text{(Eq. 1)}$$

$$Chl_b\ (mg/g) = 21.5 A_{646} - 5.1 A_{663} \quad \text{(Eq. 2)}$$

$$Tchl\ (mg/g) = Chl_a + Chl_b \quad \text{(Eq. 3)}$$

$$C_{x+c}\ (mg/g) = (1000 A_{470} - 1.8\ Chl_a - 85.02\ Chl_b)/198 \quad \text{(Eq. 4)}$$

2.5. Measurement of Glycine-Betaine (GB)

Determination of Glycine-betaine (GB) was conducted by using the dry leaf powder. The powdered plant sample (0.5 g) was added to 20 ml of deionized water and was shaken at 25 °C for 48 hours. Then the solution was filtered and stored in the freezer until its decomposition. Melted samples were diluted 1:1 with 2 N sulfuric acid. Exactly 0.5 ml of liquid in a test tube was cooled in the ice water for 1 hour. Potassium iodide reagent- cold iodine (0.2 ml) was added, and the solution was moderately mixed with the vortex device. All solutions were stored at 0-4 °C for approximately 16 h. After this period, specimens were centrifuged at 10 000×g for 15 min at the temperature of 0 °C. Its supernatant was cautiously evacuated with a 1 ml micropipette. Since the solubility of the peridotite specimens in the acid reaction combination significantly increases with higher temperature, the sample tubes must be stored cool till the peridotite combination is token from the acid media. The peridotite crystals were added into 9 ml of 1,2-dichloroethane (reagent grade). For the effect of complete solubility in solvent production, intense vortex mixing was performed. The absorbance was determined after 2.0-2.5 h at 365 nm wavelength with an UV-visible spectrophotometry device. Reference standards of GB (50-200 μg ml^{-1}) were set in 2 N sulfuric acid, and for sample estimation, the procedure provided by (Sairam *et al*., 2002) was followed.

2.6. Radical Scavenging Activity Evaluation (Antioxidant Capacity) (DPPH)

Antioxidant capacity of extracts, was measured from the way of DPPH free radical neutralization property, by using the spectrophotometric method, in the wavelength of 517 nm (Brand-William *et al*., 1995). For this aim, the DPPH solution was added in extracted samples. Then, it was kept under the dark condition for 30 minutes and, after that reading was done via a spectrophotometer device at wavelength 517 nm. The level of antioxidant capacity for extracts was calculated in the form of percentage (%).

2.7. Measurement of Total Protein Concentration

The total protein content of the solution was determined by using the method provided by Bradford (Bradford., 1976) at a wavelength of 595 nm. In addition, standard bovine serum albumin (BSA) protein was used to measure soluble protein concentration in leaf tissue.

2.8. Statistical Analysis

The SAS software (ver. 9.3) was used to analysis of gathered data (at the 5% probability level), and graphs were prepared from Excel. The Duncan's multiple range tests was used for the mean comparison.

Table 1 Soil properties of field study in two depths (0-20 cm and 20-40 cm)

Year	Depth	pH	EC (ds/m)	OC (%)	N (%)	P (mg/kg)	K (mg/kg)	Sand (%)	Silt (%)	Clay (%)
2018	0-20 cm	8.1	0.8	1.1	0.97	18	270	21	46	33
	20-40 cm	8.3	0.4	1.1	0.98	17	255	21	42	37
2019	0-20 cm	8.1	0.9	1.3	0.96	21	273	20	47	33
	20-40 cm	8.2	0.4	1.2	0.95	19	259	21	43	36

Table 2 Chemical analysis of cattle manure properties

Fertilizer	Ash (%)	DM (%)	S (%)	N (%)	P (%)	K (%)	Ca (%)	OM (%)	Total K (mg/kg)	Total P (mg/kg)	EC (ds/m)	pH
Cattle manure	14	92	1.78	2.1	1	0.5	1.2	39	2583.97	1299.5	16.4	8.2

Table 3 ANOVA for biochemical traits of Bakhtiari savory medicinal plant

S.O.V	Df	TSS	Anthocyanin	Carotenoid	GB	DPPH	Total protein
Year	1	0.177 ns	0.00089 ns	0.00036 ns	8679.2**	83.12*	2.986 ns
Rep*Year	2	0.086	0.00009	0.00009	0.082	0.907	0.267
Fertilizer	2	1.345**	0.00390**	0.00163**	245.98**	122.35**	3.406 **
Year*Fer	2	0.421**	0.00004 ns	0.00003 ns	0.307 ns	12.24 ns	0.241 ns
Rep*Year*Fer	8	0.022	0.00081	0.00007	5.070	1.351	0.072
Den	2	0.244**	0.01551**	0.00180**	358.32**	37.64**	1.282**
Year*Den	2	0.064 ns	0.00017 ns	0.000007 ns	0.257 ns	9.685 ns	0.036 ns
Fer*Den	4	0.024 ns	0.00220*	0.00103**	11.216**	9.435 ns	0.103 ns
Year*Fer*Den	4	0.024 ns	0.00021 ns	0.00006 ns	0.187 ns	10.04 ns	0.154 ns
Error	24	0.028	0.00057	0.00007	1.987	3.712	0.120
%CV	-	5.92	5.22	2.47	2.19	5.49	4.28

(TSS: Total soluble sugar, GB: Glycine-betaine, DPPH: Radical scavenging activity evaluation (antioxidant capacity)).
*, ** respectively means significant at the level of 5%, 1% and ns indicates non-significant

Table 4 The effect of fertilizer on biochemical traits of Bakhtiari savory

fertilizer	DPPH %	Total protein %
Cattle Manure	32.11 b	8.62 a
Wheat Straw	36.22 a	7.88 b
Control	36.94 a	7.85 b

(DPPH: Radical scavenging activity evaluation (antioxidant capacity))

Table 5 The effect of plant density on biochemical traits of Bakhtiari savory

Plant Density	DPPH %	Total protein %
High Density	33.83 b	8.10 b
Medium Density	34.77 b	8.39 a
Low Density	36.66 a	7.86 c

(DPPH: Radical scavenging activity evaluation (antioxidant capacity))

Table 6 The correlation among the studied traits

	TSS	Anthocyanin	Carotenoid	GB	DPPH
Anthocyanin	0.289 *	-	-	-	-
Carotenoid	0.447 **	0.297 *	-	-	-
GB	0.387 **	0.304 *	0.379 **	-	-
DPPH	-0.461**	-0.206	-0.460 **	0.098	-
Total protein	0.563 **	0.246	0.429 **	0.532 **	-0.240

(TSS: Total soluble sugar, GB: Glycine-betaine, DPPH: Radical scavenging activity evaluation (antioxidant capacity)).

3. Results

3.1. Total Soluble Sugar (TSS)

Total soluble sugar (TSS) content in Bakhtiari savory was influenced by studied treatments significantly ($P \leq 0.01$, Table 3). In the second year, cattle manure (30 t ha^{-1}) increased the TSS content by 28.5% compared with control (without fertilizer). The highest TSS content (3,38%) was observed at cattle manure application and in the second year (Fig. 1). Under these conditions, the correlation coefficient between TSS and DPPH was negative and significant ($P \leq 0.01$, Table 6).

3.2. Anthocyanin

The effects of the organic fertilizer, plant density, and their interaction were significant on anthocyanin content ($P \leq 0.05$, Table 3). The use of cattle manure (30 t ha^{-1}) and HPD (80000 plant ha^{-1}) increased the anthocyanin content. The highest content of anthocyanin (0.52 mg g^{-1} FW) was detected in the cattle manure application and HPD. In addition, anthocyanin content was improved by cattle manure and HPD by about 18% in comparison with control (without fertilizer) and LPD (26666 plant ha^{-1}, Fig. 2).

3.3. Carotenoids

The results indicated that the amount of carotenoid was affected by organic fertilizers and plant density ($P \leq 0.01$, Table 3). The highest carotenoid content (0.371 mg quercetin/g DW) was detected in control (without fertilizer) plants sowed in HPD (80000 plant ha^{-1}), which had no difference with cattle manure and MPD (40000 plant ha^{-1}). However, the minimum carotenoid was observed in enriched wheat straw (10 t ha^{-1}) enriched with ammonium sulfate and LPD (26666 plant ha^{-1}) as 0.331 mg quercetin/g DW (Fig. 3). Also, a positive and significant correlation coefficient was observed between carotenoid and total protein ($P≤0.01$, Table 6).

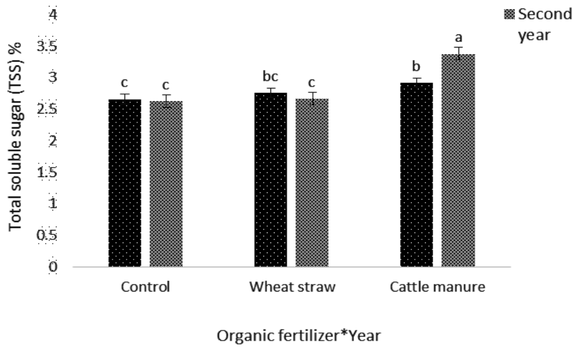

Fig. 1 The interaction of organic fertilizer and year on TSS (Total soluble sugar) of Bakhtiari savory medicinal plant. (control: no application, wheat straw: enriched wheat straw).

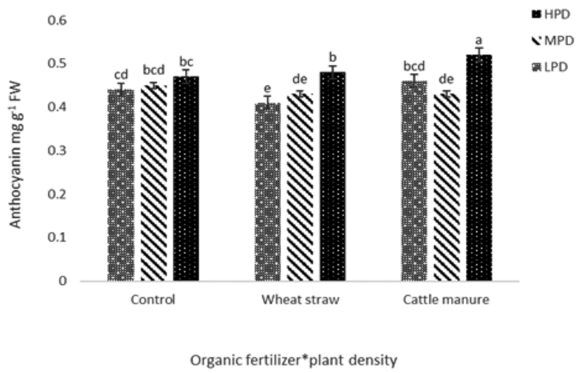

Fig. 2 The interaction of organic fertilizer and plant density on Anthocyanin of Bakhtiari savory medicinal plant. (control: no application, wheat straw: enriched wheat straw) (LPD: low plant density, MPD: medium plant density, HPD: high plant density).

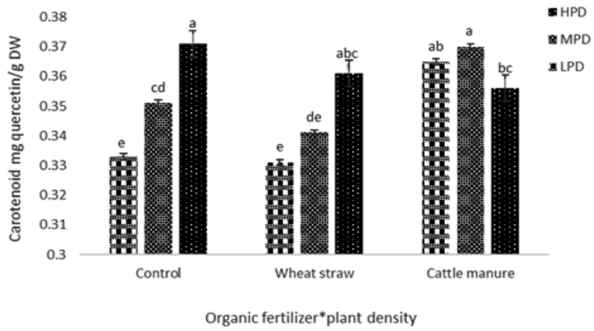

Fig. 3 The interaction of organic fertilizer and plant density on carotenoid of Bakhtiari savory medicinal plant (control: no application, wheat straw: enriched wheat straw) (LPD: low plant density, MPD: medium plant density, HPD: high plant density).

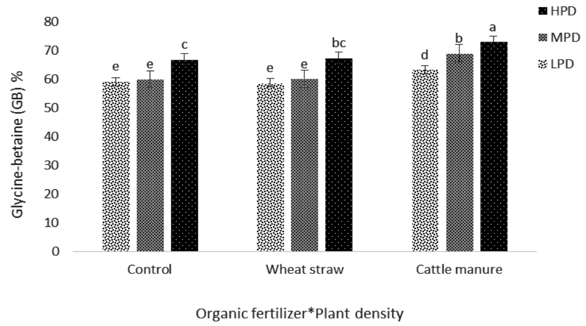

Fig. 4 The interaction of organic fertilizer and plant density on GB (Glycine-betaine) of Bakhtiari savory medicinal plant (control: no application, wheat straw: enriched wheat straw) (LPD: low plant density, MPD: medium plant density, HPD: high plant density).

3.4. Glycine-Betaine (GB)

Glycine-betaine (GB) was affected by organic fertilizers and plant density ($P≤0.01$, Table 3). GB increased with cattle manure (30 t ha^{-1}) application and HPD (80000 plant ha-1). The highest GB was found in cattle manure and HPD to be 73.06 μg ml^{-1}. The lowest GB was found in enriched wheat straw (10 t ha^{-1}) enriched with ammonium sulfate and LPD (26666 plant ha^{-1}) as 58.76 μg ml^{-1}, which had no difference with control (without fertilizer) and LPD. In total the lowest GB decreased about 20% in comparison with the highest GB (Fig. 4). In addition, the correlation coefficient between total protein and GB was positive and statistically significant ($P≤0.01$, Table 6).

3.5. Radical Scavenging Activity Evaluation (Antioxidant Capacity) (DPPH)

The effects of year, organic fertilizer, and plant density were remarkable on plant radical scavenging activity evaluation (antioxidant capacity) (DPPH) ($P≤0.01$, Table 3). The most significant activities of DPPH were measured in control (without fertilizer), also enriched wheat straw (10 t ha^{-1}) enriched with ammonium sulfate, LPD (26666 plant ha^{-1}), and in the second year. The lowest activities of DPPH were observed at cattle manure (30 t ha^{-1}), HPD (80000 plant ha^{-1}), and in the first year (Tables 4, 5). In this investigation, the correlation coefficient between DPPH and TSS and carotenoid was negative and significant ($P≤0.01$, Table 6).

3.6 Total Protein

Total protein concentration was affected by organic fertilizer application and plant density ($P≤0.01$, Table 3). Increased total protein concentration was observed by cattle manure (30 t ha^{-1}) application. Cattle manure increased the total protein concentration by 9.8%, compared with control (without fertilizer) (Table 4). Total protein concentration significantly increased by

MPD (40000 plant ha^{-1}). In the Bakhtiari savory with LPD (26666 plant ha^{-1}), we observed a decreased total protein concentration by 6.7 % compared with MPD (Table 5).

4. Discussion

The optimum TSS content was obtained by the cattle manure treated plants in the second year. Due to its vital micro and macro elements for plant growth, such as nitrogen, phosphorus, potassium, and zinc. Also, this fertilizer could improve the soil properties such as soil moisture-holding capacity, aeration, structure, and nutrients (Wang *et al*., 2017). Additionally, manure could have a vital role in enhancing TSS content by providing a segment of these elements and improving the solubility of some nutrients and elements such as zinc (Clemente *et al*., 2007; Saki *et al*., 2019). The improving effects of organic fertilizer on TSS content also have been reported in other plants (Najm *et al*., 2012). Also, in this study, increased TSS content was observed in the second year in comparison with the first year. Plants can adapt to the new environmental condition over time; As a result, the second year has more potential for nutrients uptake, thereby improving TSS content. In addition, it could be noticed that the decomposition of cattle manure happens in the second year, resulting in more nutrients remain for plants.

Cattle manure has a significant role in anthocyanin content because of its high potential in improving the nutrients uptake and water holding capacity. Reduced anthocyanin content decreases plant growth and photosynthesis, in which a close and positive correlation was observed between anthocyanin content and carotenoid (Table 6). Anthocyanin decreased at LPD (26666 plant ha^{-1}) and control but increased at HPD (80000 plant ha^{-1}) and cattle manure. The reduction of anthocyanin at LPD is due to decreased soil moisture induced by a high light rate. In dryland farming conditions, the open canopy with sunlight rate could adversely influence the soil characteristics such as nutrients, moisture, and texture (Surakod *et al*., 2017).

By cattle manure application, the plant's nitrogen uptake has increased, and due to the direct relationship between the photosynthetic pigment content and the application of manure, the carotenoid content has been improved. It was acclaimed that the organic fertilizer application by decreasing nitrogen leaching and increasing its supply, production of growth stimulants, increase of microbial population in soil, and also improving access and providing more efficient nutrients absorption, have led to increased leaf chlorophyll synthesis and carotenoid content (Amirnia *et al*., 2019; Rahimi *et al*., 2019).

It seems that glycine betaine accumulation in plants treated with cattle manure and sowed at the high plant density, could be protect the plant by balancing the osmotic conditions. The GB compounds could be involved in protection of macromolecules (nucleic acids, proteins, and lipids) and reactive oxygen species (ROS) scavenging, and act as main sources of nitrogen and carbon (Umezawa *et al*., 2006). The present review indicates the role of GB in preserving plants under stressful environments such as dryland farming conditions. Biosynthetic genes of GB have also been extensively utilized for improving abiotic tension tolerance and resistance in plants (Chen and Murata 2011).

An increase in Antioxidant capacity (DPPH) in control (without fertilizer) and enriched wheat straw treatments showed that lack of cattle manure reduced soil moisture and its nutrients. Therefore, these treatments caused decreased plant resistance to drought stress, accumulated ROS, and eventually increased the DPPH content. An increase in the antioxidant potential of medicinal plants treated with cattle manure has been reported in *S. Hortense's* (Alizadeh *et al*., 2010), *Brassia olaracea* (Naguib *et al*., 2012), and *Foeniculum vulgate* (Salama *et al*., 2015). An increase in DPPH in LPD (26666 plant ha^{-1}) treatment showed that LPD compared with HPD (80000 plant ha^{-1}) reduced plant number per unit area and increased weed growth. Hence under this condition (LPD), the plant competed for moisture and soil nutrients absorption, and these conditions increased oxidative stress and increased the accumulation of DPPH in the plant. Also, increasing the amount of DPPH indicated that environmental conditions were not favorable due to lower density selection. In this cause, LPD, while reducing soil moisture and increasing drought stress, reduced plant resistance and increased DPPH. In general, how the plant adapts to drought stress has been complicated, and it was affected by internal stress tolerance mechanisms and external environmental factors (Shamim *et al*., 2009). The plant's antioxidant activity increases over time due to the plant's biochemical activity during periods when the plant is exposed to environmental stress parameters such as humidity, temperature, and light (Naghuib *et al*., 2012).

It seems that the decrease of total protein within the control treatment (without fertilizer) could be due to a fast decrease in nutrients. One reason for the high protein could be the cattle manure application and fast absorption of nitrogen, and the increase in nitrogen concentration by the plant. Nitrogen has a significant role in the structure of chlorophyll and, on the other hand, is the most critical element in the synthesis of proteins and increasing of it to a certain extent, under certain conditions, increases the amount of protein (Xu and Mou 2016).

This research investigated the effect of organic fertilizers and different plant densities on the improvement of Bakhtiari savory (*S. bachtiarica* Bunge.) biochemical traits in dryland farming conditions. The results indicated that we could improve biochemical traits by applying cattle manure (30 t ha^{-1}) and high plant density (80000 plant ha^{-1}). In general, the management of organic fertilizers and plant density could be a promising strategy for developing and cultivating medicinal plants such as Bakhtiari savory in dryland farming conditions.

References

1. Alizadeh A, Khoshkhui M, Javidnia K, Firuzi O, Tafazoli E, Khalighi A. Effects of fertilizer on yield, essential oil composition, total phenolic content and antioxidant activity in *Satureja hortensis* L. (Lamiaceae) cultivated in Iran. J Med Plant Res. 2010; 4(1): 033-040.
2. Amirnia R, Ghiyasi M, Moghaddam SS, Rahimi A, Damalas CA, Heydarzadeh S. Nitrogen-fixing soil bacteria plus mycorrhizal fungi improve seed yield and quality traits of lentil (*Lens culinaris* Medik). J Soil Sci Plant Nutr. 2019; 19(3): 592-602.
3. Bradford MM. A rapid and sensitive method for the quantification of microgram quantities of proteins utilising the principle of protein-dye binding. Anal Biochem. 1976; 72(1-2): 248-54.
4. Brand-Williams W, Cuvelier ME, Berset CLWT. Use of a free radical method to evaluate antioxidant capacity. LWT - Food Sci Technol. 1995; 28(1): 25-30.
5. Chen TH, Murata N. Glycinebetaine protects plants against abiotic stress: mechanisms and biotechnological applications. Plant Cell Environ. 2011; 34(1): 1-20.
6. Clemente R, Paredes C, Bernal MP. A field experiment investigating the effects of olive husk and cow manure on heavy metal availability in a contaminated calcareous soil from *Murcia* (Spain). Agric Ecosyst Environ. 2007; 118(1-4): 319-326.
7. Dolatkhahi M, Dolatkhahi A, Nejad JB. Ethnobotanical study of medicinal plants used in Arjan–Parishan protected area in Fars Province of Iran. Avicenna J Phytomedicine. 2014; 4(6): 402.
8. Dubois M, Gilles K, Hammiltron JK, Robers PA, Smith F. A colorimetric method for the determination of sugars. Nature. 1951; 168(4256): 167-167.
9. Emami Bistgani Z, Siadat A, Torabi M, Bakhshandeh A, Alami Saeed KH, Shiresmaeili H. Influence of plant density on light absorption and light extinction coefficient in sunflower cultivars. Res. Crops. 2012; 13(1):174-179.
10. Emami Bistgani Z, Siadat SA, Bakhshandeh A, Pirbalouti AG, Hashemi M, Maggi F, Morshedloo MR. Application of combined fertilizers improves biomass, essential oil yield, aroma profile, and antioxidant properties of Thymus daenensis Celak. Ind Crops Prod. 2018; 121: 434-440.
11. Gardner FP, Pearce RB, Mitchell RL. Physiology of crop plants. Scientific publishers; 2017.
12. Ghiasy-Oskoee M, Agha Alikhani M, Sefidkon F, Mokhtassi-Bidgoli A, Ayyari M. Blessed thistle agronomic and phytochemical response to nitrogen and plant density. Ind Crops Prod. 2018; 122: 566-573.
13. Gould K, Davies K, Winefield C. Anthocyanins Biosynthesis, Functions, and Applications. Springer Science & Business Media. 2008; 387pp.
14. Havaux M, Kloppstech K. The protective functions of carotenoid and flavonoid pigments against excess visible radiation at chilling temperature investigated in Arabidopsisnpq Mutants Planta. 2001; 213(6): 953-966.
15. Heshmati GA. Vegetation characteristics of four ecological zones of Iran. Int. J Plant Prod. 2012; 1(2): 215-224.
16. Jiang W, Wang K, Wu Q, Dong S, Liu P, Zhang J. Effects of narrow plant spacing on root distribution and physiological nitrogen use efficiency in summer maize. Crop J. 2013; 1: 77-83.
17. Jogawat A. Osmolytes and their Role in Abiotic Stress Tolerance in Plants. *Molecular plant abiotic stress:* biol. Biotechnol. 2019; pp.91-104.
18. Khadivi-Khub A, Salehi-Arjmand H, Hadian J. Morphological and phytochemical variation of Satureja bachtiarica populations from Iran. Ind Crops Prod. 2014; 54: 257-265.
19. Li GX, Xu BC, Yin LN, Wang SW, Zhang SQ, Shan L. Dryland agricultural environment and sustainable productivity. Plant Biotechnol. Rep. 2020; 14(2): 169-176.
20. Lichtenthaler HK. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. Methods Enzymol. 1987; 148:350-382.
21. Saeed B, Das M, Khurana P. Overexpression of β-carotene hydroxylase1 (BCH1) in Indian mulberry, Morus indica cv. K2, confers tolerance against UV, high temperature and high irradiance stress induced oxidative damage. Plant Cell, Tissue and Organ Culture (PCTOC). 2015; 120(3) :1003-1014.
22. Naguib AEMM, El-Baz FK, Salama ZA, Hanaa HAEB, Ali HF, Gaafar AA. Enhancement of phenolics, flavonoids and glucosinolates of Broccoli (*Brassica olaracea, var. Italica*) as antioxidants in response to organic and bio-organic fertilizers. J Saudi Soc Agric Sci. 2012; 11(2): 135-142.
23. Najm AA, Hadi MRHS, Fazeli F, Darzi MT, Rahim A. Effect of integrated management of nitrogen fertilizer and cattle manure on the leaf chlorophyll, yield, and tuber glycoalkaloids of Agria potato. Commun. in Soil Sci. Plant Anal. 2012; 43: 912-923.
24. Pandey V, Patel A, Patra DD. Biochar ameliorates crop productivity, soil fertility, essential oil yield and aroma profiling in basil (*Ocimum basilicum* L.). Ecol. Eng. 2016; 90:361-366.
25. Rahimi A, Moghaddam SS, Ghiyasi M, Heydarzadeh S, Ghazizadeh K, Popović-Djordjević J. The influence of chemical, organic and biological fertilizers on agrobiological and antioxidant properties of Syrian cephalaria (*Cephalaria syriaca* L.). Agriculture. 2019; 9(6): 122-135.
26. Sairam RK, Veerabhadra Rao K, Srivastava GC. Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. Plant Sci. 2002; 163(5):1037-1046.

27. Saki A, Mozafari H, Karimzadeh-Asl k, Sani B, Mirza M. Plant Yield, Antioxidant Capacity and Essential Oil Quality of *Satureja Mutica* Supplied with Cattle Manure and Wheat Straw in Different Plant Densities. Commun. in Soil Sci. Plant Anal. 2019; 50(21): 2683-2693.
28. Salama ZA, El Baz FK, Gaafar AA, Zaki MF. Antioxidant activities of phenolics, flavonoids and vitamin C in two cultivars of fennel (*Foeniculum vulgare* Mill.) in responses to organic and bio-organic fertilizers. J Saudi Soc Agric Sci. 2015; 14(1): 91-99.
29. Shamim A, Rashid A, Muhammad YA, Ashraf M, Ejaz AW. Sunflower (*Helianthus annuus* L.) response to drought stress at germination and seedling growth stages. Pak J Bot. 2009; 41(2): 647-654.
30. Surakod VS, Ashwathama VH, Patil MB, Patil PV, Muktumsab T. Influence of crop geometry (*Pennisetum glaucum* L.) R. Br.) on relative water content of pearl millet under dryland conditions. J Ecol Environ. 2017; 34(3):876-878.
31. Tabrizi L, Koocheki A, Rezvani Moghaddam P, Nassiri Mahallati M, Bannayan M. Effect of irrigation and organic manure on Khorasan thyme (*Thymus transcaspicus* Klokov). Arch. Agron. Soil Sci. 2011; 57(3): 317–326.
32. Umezawa T, Fujita M, Fujita Y, Yamaguchi-Shinozaki K, Shinozaki K. Engineering drought tolerance in plants: discovering and tailoring genes to unlock the future. Curr. Opin. Biotechnol. 2006; 17(2):113-122.
33. Wang X, Ren Y, Zhang S, Chen Y, Wang N. Applications of organic manure increased maize (*Zea mays* L.) yield and water productivity in a semi-arid region. Agric. Water Manag. 2017; 187: 88-98.
34. Xu C, Mou B. Short-term Effects of Composted Cattle Manure or Cotton Burr on Growth, Physiology, and Phytochemical of Spinach. Hort Sci. 2016; 51(12): 1517-1523.