

Effect of organic manures on nutritional quality of chicory under water deficit

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ABSTRACT

Drought stress and organic manure have vigorous role in plant growth. In order to alleviate effects of drought on chicory (*Chicorium intybus* L.), poultry and livestock manures were used at different levels of drought which induced according to evaporate level from evaporation pan. Aim of this research was to improve the chicory nutritional and pharmaceutical suitability under drought. Results suggested significant effects of both manures on chicory mineral accumulation and phytochemicals. The highest concentration of potassium (K), phosphorus (P) and manganese (Mn) were obtained with poultry application at moderate drought, but no significant difference with livestock manure on potassium in severe drought. Livestock manure usage increased zinc (Zn) concentration in severe drought significantly. Both manures increased total phenolic compounds in severe drought significantly, but total carbohydrate significantly affected by poultry manure. Therefore, chicory inulin and chicoric acid under drought conditions were significantly affected by both poultry and livestock manures.

Keywords: Livestock manure, poultry manure, mineral accumulation, phytochemical, drought stress, *Chicorium intybus*.

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INTRODUCTION

Common chicory (*Cichorium intybus* L.) is a typical Mediterranean vegetative herb with variegation of utilization (Castellini et al., 2007; Mulabagal et al., 2009; Sinkovič et al., 2015). Chicory is an edible herb with prevalent applications in many areas of Iran includes raw vegetable and salad, cooking edible, medicinal usage and livestock forage (IHPC, 2002; Bahmani et al., 2014). High levels of necessary nutritive minerals such as potassium, calcium, magnesium, sulfur, zinc and sodium, as well as phenols and vitamins particularly A and C are accessible in chicory (Jurgonski et al., 2011; Carazzone et al., 2013). Vegetable and forage production can be affected by many environmental factors so that in recent years drought stress extension is an important challenge for this crops production, drought stress lead to serious damage in plants root growth, water and nutrient uptake decreases and causes to yield reduce and also oxidative stress incitement with drought stress (Rahimian Boogar et al., 2014; Mârza et al., 2015; Zhao et al., 2015; Daryanto et al., 2016).

Plants accessibility to mineral nutrients particularly K, P and Ca are necessary for tolerance to drought (Feng et al., 2015; Zhao et al., 2015). In addition, plants absorbancy of nutritional elements are depend to various factors such as the element natures, soil conditions (as: soil texture; clay and organic matter content, pH) and soil amendment quality (Ben Achiba et al., 2009). Exogenous organic matter has different effects on instigation the mineral elements diffusion in the soil and their availability for plants (Zhang et al., 2015; Vanden Nest et al., 2016; Salehi et al., 2016). The cattle manure application lead to increases of potassium availability (as a necessary element in drought tolerance) and plant nourishment in the soil in compare with mineral fertilizers (Pires et al., 2008). Furthermore, application of organic manures and compost increase the soil organic carbon and P-CaCl₂, and P leaching in compare with mineral fertilized soils (Vanden Nest et al., 2014), fulvic and humic synthesis (Vanden Nest et al., 2016) and epitomize the high content of organic materials in plants sowing bed (Ukale

et al., 2016). In addition to macro-aggregates stability, the manure application increase the soil porosity and better infiltration of water, so increase water-holding capability and soil water content, and water use efficiency (Rasool et al., 2008; Karami et al., 2012; Liu et al., 2013; Ibrahim et al., 2015).

Application of organic manures is useful to produce the high nutritive value product. Regarding the negative effects of drought on plants nutritional quality, application of organic manure can be alleviate destructive effects of drought on chicory. The objective of this research was kept the suitable nutritional content and edible value of chicory, as well as support the physiology of growth under drought stress for improve the pharmaceutical indices.

MATERIALS AND METHODS

Plants and experiment condition

This research was carried out at the research farm of agricultural faculty in Zabol University, Sistan & Balochestan, Iran. The weather of Zabol is hot and dry and summer is windy. Average of annual rainfall is 63 millimeters. Chicory seeds (type) were cultivated in March and plantlets were exposed to drought stress 30 days after planting.

Manure treatments

Manure treatments were applied in soil before seed sowing, applications of poultry and livestock manure added at rate of 30 t ha⁻¹ to soil with control treatment. Soil analysis was performed before planting (Table 1).

Water stress treatment

Water stress was applied from mid-May until the mid of July by limiting irrigation. Drought stresses were determined by application of evaporation pan (class-A). Three levels of water evaporation at surface of evaporate pan (Class A) was detected for irrigation intervals; 60 mm evaporation or well-watered (WW), 90 mm evaporation or moderate drought stress (MD) and 120 mm evaporation or severe drought stress (SD).

Mineral nutrient assay and analysis

Plant samples were harvested in mid-July for analyze. Samples were washed with distilled water and dried in oven at 70°C for 48 h. The oven-dried subsamples were grounded, then nitrogen (N) concentration was assayed with Kjeldahl method (Domini et al., 2009), and A Perkin Elmer Analyst 700 atomic absorption

spectrometer with deuterium background corrector was applied for nutrient measurement in this experiment. Samples were ashed according to the standard method that prepared by ASTM C114-09 (ASTM, 2009). Ashed samples were dissolved in 0.1N HCl and then content increases to 100 ml. This extract was used for the quantification of Mn, Fe and Zn. Concentrations of P and K were detected with Spectrophotometer (BTS-45) according to the Motomizu et al. (1988) method and Flame Photometer (corning 400) according to the method described by ASTM (2009), respectively.

Analytical reagents; HNO₃, H₂SO₄, H₂O₂, HF, HClO₄ and HCl (E. Merck) were used. The standard solutions of elements were created by extenuating a stock solution of 1000 mg L⁻¹ (Mn) supplied by Sigma and (Zn, Fe) by Aldrich. Therefore, a standard stock solution for potassium (1000 mg L⁻¹) was created by dissolving 0.1907 g of potassium chloride (Merck) in 0.1M hydrochloric acid and make adjustments as the final volume of 100ml, and standard solution of phosphorus (100 mg L⁻¹) was prepared by dissolving 0.2197 g of potassium dihydrogen phosphate (KH₂PO₄) in water (100 ml), therefore add 8 ml Sulphuric acid (H₂SO₄) and then dilute it into 500 ml. Double deionized water was used to prepare all stoke solutions for calibration.

Extraction the total phenol and chicoric acid

Total phenol was extracted according to the method of Ferioli and D'Antuono (2012) and Vitali et al. (2009) with some modification. Both leave and stem of chicory were harvested as described in the above experimental material and used liquid nitrogen tank for transport samples to lab. One gram of freeze-dried samples was crushed and homogenized by 15 ml of methanol: HCl conc: water (86:2:10 v/v/v) in a centrifuge tube and was shaken with a vortex for one minute, and ultrasonic bath was used for 15 minutes at room temperature and then centrifuged at 665 ×g for 10 min at 5°C (Sigma 3 K 30, Germany), separated the supernatant layer of extract as extractable phenolic extraction. Finally, total phenol was measured with Folin-Ciocalteu colorimetric method (Apak et al., 2008), results were presented as milligrams of gallic acid equivalent (GAE) per 100 gram of fresh weight according to the method of Cefola et al. (2016), and calibration curve of gallic acid was plotted with eight points, from 25 to 500 mg/L with R² = 0.99.

Extraction of total carbohydrate and inulin

Ten gram of fresh root was homogenized in 30ml of ice-cold 50 mM sodium acetate buffer (pH 5) with a blender for 1 min. According to the modified method of van Arkel et al. (2012), then this mixture was used for carbohydrate and inulin analysis Timmermans et al. (1994) and Van den Ende et al. (1996).

Statistical analysis

Experiments were conducted in a randomized complete block design with three replications. Data were analyzed by factorial ANOVA at a significance level of $P \leq 0.05$ using SPSS v.22, and means were compared using LSD test.

Table 1. Physical and chemical properties of the soil at 0-30 cm depth.

Soil texture	Loam	Clay	Sand	Fe	Mn	Zn	P	K	N	Organic matter	pH	Electrical conductivity
	(%)											
Sandy clay	27.04	31.1	86.41	3.8	3.2	2.8	18	194	0.06	0.59	8.2	2.5

RESULTS

Mineral content

Nutritious content of chicory was affected by water stress and soil fertility. So, two organic manure applications improve the nutritious value of chicory in water stress condition in compare with control condition, but poultry manure had the highest significant effects (Table 2).

Nitrogen

Nitrogen content was affected by water stress levels ($F_{2, 27} = 81.27$, $P < 0.01$), and organic manure treatments ($F_{2, 27} = 3018.66$, $P < 0.01$) in herbal part of chicory, this effect was not similar in all water stresses or manure treatments and peaked at poultry manure in three irrigation condition (Table 2). The magnitude of nitrogen differed between water stress and fertilizer treatments ($F_{4, 27} = 19.28$, $P < 0.01$). The poultry manure treatment resulted in the greatest increase in nitrogen content in chicory (Table 2).

Potassium

Potassium content significantly increased in chicory plant under water stress ($F_{2, 27} = 25.63$, $P < 0.01$) and organic manure treatment ($F_{2, 27} = 62.54$, $P < 0.01$), although this increase was not same for all treatments of water stress and fertilizer statistically the highest pointed in poultry and livestock manure at SD, and poultry manure at MD (Table 2). The degree of this increase in potassium content was not different between drought stress and organic manure ($F_{4, 27} = 1.42$).

Phosphorus

Drought stress lead to significant decrease in phosphorus content of chicory plant ($F_{2, 27} = 32.09$, $P < 0.01$), so application of all organic fertilizer treatments ($F_{2, 27} = 69.36$, $P < 0.01$) had significant effect on improving the phosphorus content in chicory. The lowest content of phosphorus is existing in SD condition. However, poultry organic fertilizer has the highest significant effect on phosphorus accumulation in all condition (Table 2). However, the rate of phosphorus decreases is similar between drought stress and organic fertilizer treatments ($F_{2, 27} = 0.86$), interaction of drought stress with organic fertilizers is not significant.

Zinc

All treatments of drought stress and organic fertilizer

were effected on zinc content of chicory, according to this result water stress ($F_{2, 27} = 269.57$, $P < 0.01$), and organic fertilizer ($F_{2, 27} = 104.57$, $P < 0.01$) increased zinc content significantly. Though this effect was not same for all treatments of water stresses and organic fertilizer, and peaked at SD with organic livestock manure (Table 2). The interaction of water stress and manure treatments ($F_{4, 27} = 11.71$, $P < 0.01$) has a significant effect on process of zinc increasing (Table 2).

Iron

Iron content in chicory plant was affected by all treatments of drought stress ($F_{2, 27} = 268.20$, $P < 0.01$), and organic manure treatments ($F_{2, 27} = 310.26$, $P < 0.01$). Significantly, this effect was not like in all treatments and highest content obtained at poultry manure at WW (Table 2). However, drought and organic manure interactions ($F_{4, 27} = 1.86$) was not significant (Table 2).

Manganese

Manganese content was reduced significantly in chicory plant under drought stress ($F_{2, 27} = 43.04$, $P < 0.01$) and improved by organic manure treatment ($F_{2, 27} = 192.69$, $P < 0.01$). Although the effects of all treatments of water stress and fertilizer is not same and the poultry organic fertilizer has the highest effect on manganese content increases under WW and MD condition (Table 2). Interaction between drought stresses with organic fertilizer ($F_{4, 27} = 3.99$, $P < 0.05$) is significant.

Biochemical content

Total phenol

The chicory total phenol significantly increased by drought stress ($F_{2, 27} = 46.90$, $P < 0.01$) and organic manure treatment ($F_{2, 27} = 28.51$, $P < 0.01$), also effect of drought stress and organic fertilizer treatments on increases of phenolic compound was not alike and highest content of phenol pointed in both of organic manure at SD (Table 3). The magnitude of phenolic increases was not depending to interaction of drought stress with organic manure ($F_{4, 27} = 2.74$).

Chicoric acid

Chicoric acid were significantly affected by drought stress ($F_{2, 27} = 181.52$, $P < 0.01$) and organic manure treatment ($F_{2, 27} = 63.06$, $P < 0.01$), however effects of different treatments of drought and fertilizer was not same on

Table 2. The effects of organic manures treatments on chicory mineral content under drought stress.

Drought stress	Organic fertilizer	N (%)	P (%)	K (%)	Fe (ppm)	Zn (ppm)	Mn (ppm)
Well-Watered (WW)	Poultry	2.21 ^{a†}	1.13 ^a	0.96 ^{cd}	145.53 ^a	48.44 ^e	41.19 ^a
	Livestock	0.70 ^d	0.92 ^b	0.93 ^{cd}	132.05 ^c	53.11 ^d	30.90 ^b
	control	0.55 ^f	0.81 ^{cd}	0.53 ^f	126.46 ^d	42.25 ^f	18.07 ^d
Moderate Drought (MD)	Poultry	1.91 ^b	1.06 ^a	1.30 ^{ab}	135.69 ^b	56.76 ^c	40.09 ^a
	Livestock	0.63 ^{de}	0.86 ^{bc}	1.13 ^{bc}	125.97 ^d	58.94 ^{bc}	25.92 ^c
	control	0.47 ^g	0.70 ^{ef}	0.66 ^{ef}	116.90 ^e	51.17 ^{de}	13.23 ^e
Severe Drought (SD)	Poultry	1.70 ^c	0.88 ^{bc}	1.50 ^a	126.43 ^d	60.23 ^b	27.91 ^{bc}
	Livestock	0.60 ^{ef}	0.73 ^{de}	1.40 ^a	116.86 ^e	73.81 ^a	19.74 ^d
	control	0.37 ^h	0.63 ^f	0.76 ^{de}	109.42 ^f	60.96 ^b	11.65 ^e
Significance							
Drought stress (DS)		***	***	***	***	***	***
Organic fertilizer (OF)		***	***	***	***	***	***
DS × OF		***	ns	ns	ns	***	*

† Different letters in each column indicate significant difference between means.

*** significant at 0.001; *significant at 0.05; ns not significant.

Table 3. Effects of organic manures treatments on chicory biochemical content under drought stress.

Drought stress	Organic fertilizer	Total phenol (mg 100 g ⁻¹ FW)	Chicoric acid (mg 100 g ⁻¹ FW)	Total carbohydrate (mg g ⁻¹ FW)	Inulin (mg g ⁻¹ DW of root)
Well-Watered (WW)	Poultry	196 ^{bct}	36.76 ^e	6.82 ^f	73.36 ^a
	Livestock	184 ^c	43.46 ^d	6.48 ^f	74.64 ^a
	Control	165 ^d	35.44 ^e	6.37 ^f	70.00 ^b
Moderate Drought (MD)	Poultry	201 ^b	63.34 ^b	8.69 ^d	64.23 ^d
	Livestock	193 ^{bc}	76.96 ^a	8.45 ^{de}	65.48 ^{cd}
	Control	187 ^{bc}	53.71 ^c	7.85 ^e	52.89 ^f
Severe Drought (SD)	Poultry	237 ^a	55.62 ^c	14.25 ^a	67.55 ^{bc}
	Livestock	225 ^a	57.49 ^c	12.74 ^b	69.17 ^b
	Control	194 ^{bc}	42.06 ^d	10.53 ^c	58.26 ^e
Significance					
Drought stress (DS)		***	***	***	***
Organic fertilizer (OF)		***	***	***	***
DS × OF		ns	***	***	***

† Different letters in each column indicate significant difference between means.

*** Significant at 0.001; *significant at 0.05; ns not significant.

chicoric acid increases and highest content of chicoric acid obtained in livestock manure at MD (Table 3). The greatness of chicoric acid increases was depending on interaction of drought stress with organic manure ($F_{4, 27} = 7.38$, $P < 0.01$). HPLC analyzes of chicory extract carried out for determination the chicoric acid in different treatment (Figure 1).

Total carbohydrate

Total carbohydrate content was affected by drought stress ($F_{2, 27} = 586.84$, $P < 0.01$), organic manure ($F_{2, 27} = 44.32$, $P < 0.01$) and interaction of drought stress with organic manure ($F_{4, 27} = 17.16$, $P < 0.01$), significantly. Therefore, the effects of drought stress and organic

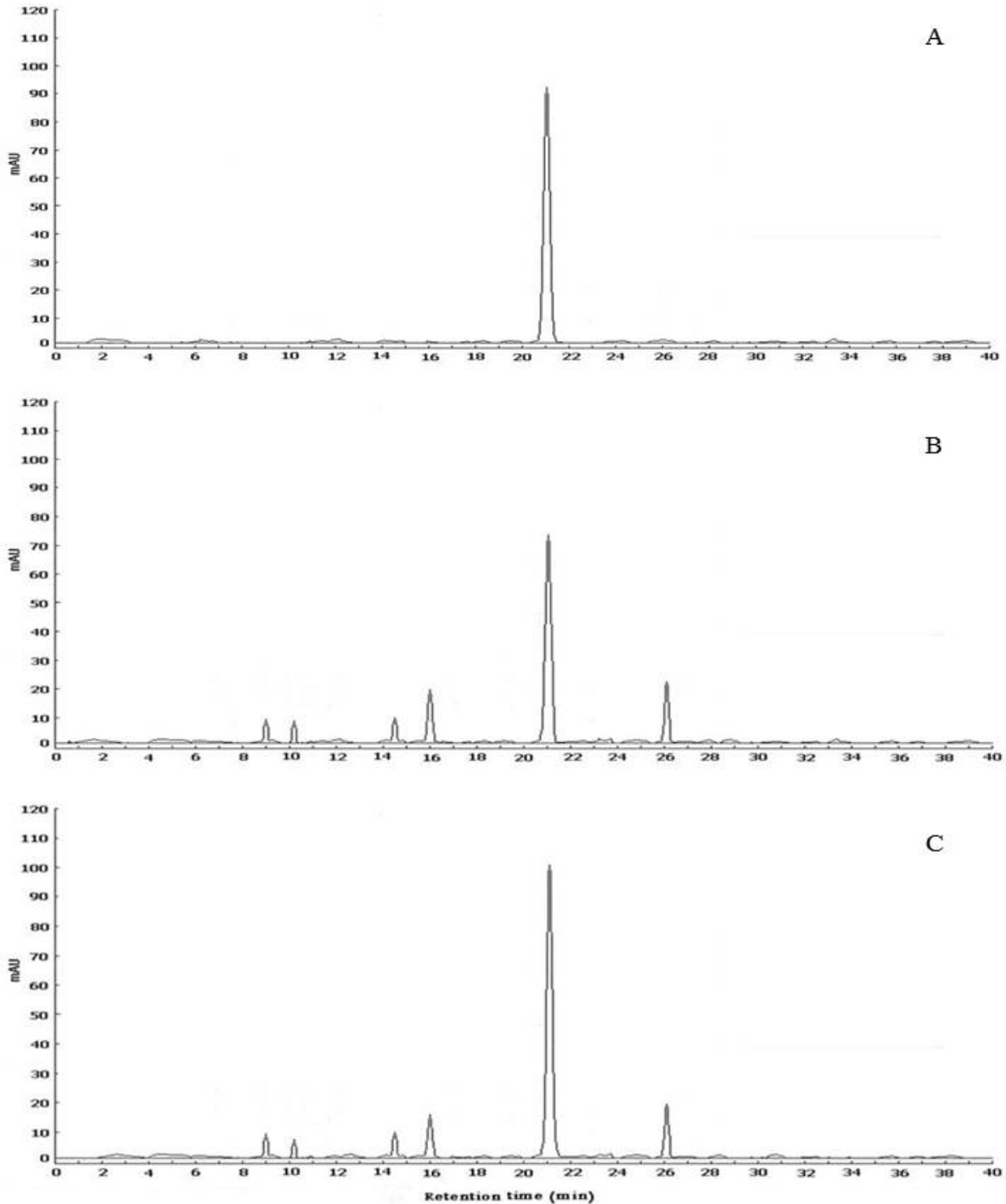


Figure 1. HPLC chromatogram for chicoric acid. A; standard of chicoric acid (40 micrograms per ml). B; peak of chicoric acid at poultry manure treatment. C; peak of chicoric acid at livestock manure treatment.

manure were not the same for all treatments (Table 3). According to these results, the highest carbohydrate content accumulated at the SD condition than MD and WW condition, as well as the poultry manure have higher effect on carbohydrate increases under drought than livestock treatment though both organic manure have had

the significant effects than control.

Inulin

The root inulin concentrations were significantly affected

by drought stress ($F_{2, 27} = 155.18$, $P < 0.01$) and organic fertilizer ($F_{2, 27} = 111.02$, $P < 0.01$), so this effect is not same for drought and organic fertilizers in all treatment (Table 3). As well as, interaction of the two factors ($F_{4, 27} = 8.35$, $P < 0.01$) have a significant effect on root inulin content.

DISCUSSION

This research showed a positive effect of organic manure on the mineral nutrient and chemical compound of chicory grown in drought stress condition. The positive responsiveness of chicory to application of poultry and livestock manure under drought stress confirms the crucial role of manure usage for growth supplementation in the low water environments. In the present study, we observed that applications of different manure for alleviating the effects of drought stress on chicory were showed the significant effect of both manure and their interaction with drought on nutrient content, chicoric acid and root inulin. In WW condition the accumulation of N, P, Fe and Mn concentration was higher in above ground part of chicory than other measured nutritious when poultry manure used. Although both of organic manure treatments had the significant effects on accumulation of mineral nutrient in chicory than control treatment, this results in accordance with the effects of manure fertilization on increases the N and P in above ground part of various plants (Demir et al., 2010; Vieira Pacheco et al., 2017; Herencia et al., 2011). Therefore, under WW condition both organic manure have low effect on K and Zn accumulation in chicory plants which is related to water availability and the effect of water on nutrient releases in soil and nutrient absorption by plants, but not related to the amount of nutrient in organic manure. This result is alike with the effect of cattle manure on K accumulation in yellow passion fruit (Vieira Pacheco et al., 2017), and Zn content in leaves of chicory (Haag and Minami, 1998).

In the MD condition, the P, K and Mn had the highest concentration in above ground part of chicory than other measured nutritious, as well as poultry manure had higher effect on the accumulation of P, K and Mn in compare with livestock manure. Though, the effect of both usage manure was significant than control treatment. Howbeit, in the SD condition the K and Zn had a higher concentration than other mineral nutrient and livestock manure had the highest effect on the K and Zn accumulation under SD condition. The high concentration of K in plants which were grown under drought stress is alignment with the role of K as a mechanism for contribute to osmotic adjustment (Nio et al., 2011; Feng et al., 2015). In this regard, Zhao et al. (2010) reported high concentration of K in high-drought-tolerant genotypes of barley under drought stress. Furthermore, organic fertilizers have positive effects on increases the

cation exchange capacity in soil and lead to increase the level of available K (Herencia et al., 2011). Moreover, Demir et al. (2010) were shown the significant effects of poultry manure on concentrations of Zn in tomato leaf, that accordance with previous study application of two organic manure causes to Zn accumulation in chicory leaf. Moreover, application of several organic manures increases the Zn content in soil (Uprety et al., 2009).

Some biochemical characteristic of chicory includes; total phenolic compound, chicoric acid, total carbohydrate and inulin were affected by drought stress, in accordance with this result previous studies were shown the total phenol increased under drought stress (Oh et al., 2010; Gharibi et al., 2016). Furthermore, both organic manures were applied under WW, MD and SD condition improved phenolic compound and other biochemical characteristic, so that the highest concentration of total phenol was obtained under SD condition with both manure applications. Previous studies in accordance with our result were reported the total phenol increases in organic farming (Oliveira et al., 2013; Vinha et al., 2014), and recently Sunday (2016) showed that the significant effects of poultry manure on phenols increases as phytochemical compound in the Roselle. Chicoric acid was determined as a main phenolic compound in chicory, it has shown significant difference in all treatment and this effect was related to drought stress and organic manure treatments. And the highest accumulation of chicoric acid was obtained at MD condition with livestock treatment. Formerly, Galieni et al. (2015) were reported drought stress improved the accumulation of chicoric acids in the bound forms.

Carbohydrates content in vegetative parts of different plants were recognized as a mechanism for response to drought stress (Iglesias et al., 2002; Pedroso et al., 2014; Nyarukowa et al., 2016). According to this study, carbohydrate scrutiny showed a higher concentration of carbohydrate obtained in herbal parts of chicory at SD condition and poultry manure treatment than other treatments which this result is accordance with effects of drought on carbohydrate concentrations in drought sensitive plant cultivars (Iordachescu and Imai, 2008; Nyarukowa et al., 2016). In addition, Xie et al. (2014) showed that manure amendments significantly increase neutral sugar concentrations in bulk soil in free and occluded particulate organic matter and the neutral sugars can be attributed to plant. Also, other previous studies have shown the high content of carbohydrate compound in dairy manure and poultry litter (Meyer et al., 2002; Wang et al., 2011; He and Olk, 2011). Moreover, drought can influence the inulin metabolism as a main reserve carbohydrate in chicory (De Roover et al., 2000), in addition the inulin accumulation in chicory showed a time followed sigmoid curve and some stresses such as drought and nutritional stress perhaps can still be important factors in inulin degradation (van Arkel et al., 2012). In accordance to previous studies, present

research showed which inulin content in chicory root was significantly affected by both treatments of drought and organic manure, and the highest concentration of inulin obtained in WW and both poultry and livestock manures.

Conclusion

Nutritional quality of edible herbs is important for consumers' health especially in plants that grow in arid and semi-arid areas. Water deficit is a major limiting factor in plant ability to uptake nutrition and causes poor nutritional production. Application of organic manures improved stoke of nutritious substrate and accessible water in the soil, through with this mechanism organic manure can increase in nutritious qualitative index and scale of chicory biomass production. Due to world warming and land aridity, different organic manure sources suggest to investigate for alleviate negative effects of water deficit on edible plants which produce in water stress condition.

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