Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Original article http://dx.doi.org/10.1016/j.apjtb.2016.08.009

Evaluation of proline, chlorophyll, soluble sugar content and uptake of nutrients in the German chamomile (*Matricaria chamomilla* L.) under drought stress and organic fertilizer treatments

Amin Salehi[1*], Hamidreza Tasdighi[1], Majid Gholamhoseini[2]

[1]*Agronomy and Plant Breeding Department, Yasouj University, Yasouj, Iran*

[2]*Oil Seed Crops Department, Seed and Plant Improvement Institute, Agricultural Research, Education and Extension Organization (AREEO), Iran*

ARTICLE INFO

Article history:
Received 18 Nov 2015
Received in revised form 10 Dec 2015, 2nd revised form 11 Jan, 3rd revised form 15 Jan, 4th revised form 27 Jan 2016
Accepted 15 Apr 2016
Available online 26 Aug 2016

Keywords:
German chamomile (*Matricaria chamomilla*)
Drought stress
Osmotic adjustment
Organic fertilizer
Vermicompost

ABSTRACT

Objective: To investigate the effect of drought stress and organic fertilizer on German chamomile (*Matricaria chamomilla*) nutrient uptake, leaf chlorophyll content and osmotic adjustment under field conditions.

Methods: This experiment was carried out through a randomized complete block design with a split factorial arrangement of treatments in three replications. The main plots were subjected to the following irrigation treatments: irrigation after 60, 90, 120, 150 and 180 mm evaporation from Class A pan. The sub-plots were treated with three vermicompost doses (0, 5 and 10 t/ha).

Results: Although drought stress reduced the nutrient percentages in the shoots, application of vermicompost enhanced the nutrient percentages, particularly when the plants were subjected to moderate to severe drought stress conditions. Moreover, the results of this study showed that the interaction between irrigation treatments and vermicompost rates on leaf chlorophyll content was significant. Comparison between the combined treatments indicated that under normal irrigation and moderate drought stress conditions chamomile plants received 5 and 10 t/ha vermicompost showed significantly higher leaf chlorophyll content comparing to the control treatment.

Conclusions: Totally, organic fertilization by vermicompost could partly alleviate the effect of drought stress on chamomile by increasing N, P and K uptake and leaf soluble sugar, especially in stressed treatments.

1. Introduction

Two species of chamomile, German chamomile (*Matricaria recutita*) and Roman chamomile (*Chamaemelum nobile*) are the most important medicinal plants worldwide. This plant has been used for thousands of years in traditional Egyptian, Roman and Greek medicine to treat different diseases such as chest colds, anxiety, insomnia and psoriasis [1]. Nowadays, one third of human demands for drugs is acquired from plants [2]. Increasing demand of pharmaceutical factories for primary materials and, more importantly, conservation of natural genetic resources, lay emphasis on the production as well as research on production and processing of medicinal and spice plants.

Plants confront several environmental stresses which can affect their growth, metabolism, and function depending on the sensitivity of species and the stage of development. As one of the most important environmental aspects, drought is responsible for the majority of global yield loss, especially in regions with low and erratic rainfall [3]. Irrespective of the grain yield loss, drought stress amplifies adverse effects of other abiotic stresses such as nutrient deficiency. Water deficit adversely affects many physiological processes such as photosynthesis, assimilate transmission into the grain, cell expansion, and division and nutrient accumulation and transfer [4]. When soil water potential decreases, plants should be able to reduce the water potential so that they reach to the desired slope to retain water absorption. Osmotic regulation mechanism, moving the flow of water from

*Corresponding author: Amin Salehi, Agronomy and Plant Breeding Department, Yasouj University, Yasouj, Iran.
Tel: +98 912 6776836
Fax: +98 74 3322484
E-mail: aminsalehi@yu.ac.ir

Foundation Project: Supported by Yasouj University (Grant No. 88/8705).
Peer review under responsibility of Hainan Medical University. The journal implements double-blind peer review practiced by specially invited international editorial board members.

the soil into the roots, through which plants reduce osmotic potential in the cells by accumulating some active ions or organic compounds plays significant roles. Sodium, potassium, calcium and chloride are the most abundant electrolytes in osmo-regulator solutions. Additionally, organic compounds such as proline and carbohydrates play a crucial role in cell osmo-regulation [5]. It should be taken into account that water crisis is becoming more serious due to population growth and global climate change which are the major threats for food security and environmental sustainability [6]. Since significant amount of water is consumed in agriculture, more attention should be paid to increasing water use efficiency [7].

Recently, low input cropping systems and innovation of modern management of resources are important objectives of sustainable agriculture, therefore, the use of organic fertilizers instead of chemical fertilizers is one step towards sustainability. Vermicomposting is a natural process in which certain species of earthworms (especially *Eisenia fetida* or *Eudrilus eugeniae*) are used for efficient decomposition of organic waste. Application of vermicompost to soil not only provides the required nutrients to plant but also boosts soil water content and biological processes. Organic matter, especially vermicompost, affects crop growth and yield directly by supplying nutrients and indirectly by modifying soil physical properties that can improve the root environment and stimulate plant growth [8]. According to previous studies, the use of vermicompost offers a great source of available elements and also enhances nutrient uptake by roots [9,10]. Generally, vermicompost application promotes humus formation in the soil.

Recently, medicinal plants cultivation has been increased eight times during the last two decades across the world. However, there is not enough information about the effects of irrigation regimes and organic fertilizer on proline, chlorophyll and soluble sugar content as well as nutrient uptake by German chamomile. Therefore, this experiment was carried out to explore the effects of organic fertilizer application under different irrigation regimes on some traits of German chamomile.

2. Materials and methods

The field experiment was carried out at the Eghlid Agriculture Center, Fars Province, Iran (31°13′ N, 52°55′ E, 2 300 m above sea level), in 2014. The study site has an average annual rainfall of 300 mm and average annual temperature of 25 °C based on long-term meteorological data (30 years). The soil of experimental site was classified as clay loam based on the soil texture triangle. More details of the soil and vermicompost properties are presented in Table 1.

First, seed bed was prepared using plow and disk. After that, the experimental plots were established. Each plot consists of four rows with 30 cm distance between rows. Prior to seed sowing, certain amounts of vermicompost were spread onto the soil surface and incorporated into the top 15 cm of the soil manually. Chamomile seeds were sown during the first week of May. The plant density of 33 plants per square meter was achieved by sowing seeds on the rows 10 cm. Irrigation was performed immediately after seed sowing. A closed irrigation system was implemented to avoid runoff. The treatments were randomized based on randomized complete block design arranged in split-plot with three replicates. Irrigation regimes (irrigation after 60, 90, 120, 150 and 180 mm evaporation from Class A pan) and vermicompost rates (0, 5 and 10 t/ha) were allocated to main and sub-plots, respectively. Weeds were controlled manually during the growing season. Drought stress continued until 80% flowering of the field. To determine nutrient concentrations [nitrogen (N), phosphorus (P) and potassium (K)] in shoots, 20 g of sample was taken from each plot. The plant samples were oven-dried at 70 °C for 72 h and then powdered by an electric mill. To determine N content in shoot, Kjeltec Auto 1030 Analyser (Tecator, Sweden) was used. In addition, P content was determined using a 6505 JenWay spectrophotometer following colorimetrically method, K content was determined using flame-photometer (JenWay PFP7 flame-photometer).

Chlorophyll content was measured at the flowering stage using Arnon proposed method [11]. Soluble sugar was determined at flowering stage using anthrone method [12]. About 0.5 g of fresh sample were placed in a 25 mL of cuvette and then 10 mL distilled water was added. Samples were heated at 100 °C for 1 h, and then filtered into 25 mL volumetric flasks. Reaction mixture (7.5 mL) contained 0.5 mL extracts, 0.5 mL mixed reagent (1 g anthrone + 50 mL ethyl acetate) and 5 mL H_2SO_4 (98%), plus 1.5 mL distilled water. The mixture was heated at 100 °C for 1 min and absorbance was read at 630 nm [13]. Sucrose solutions were used as standard samples.

Proline accumulation was determined by extracting fresh samples in 3% sulfosalicylic acid. The extract was heated in water bath for 10 min and then filtered through filter paper. Two milliliters of extract was mixed into 6 mL assay media containing 2 mL ninhydrin solution and 2 mL acetic acid. After that, all samples were incubated at 100 °C for 30 min and cooled by room temperature. The colored product was extracted by adding 4 mL toluene. Finally absorbance of organic layer was measured at 520 nm [14].

The data were subjected to SAS 8.1 and analyzed using ANOVA. Probability levels of 1% and 5% ($P \leq 0.01$ or 0.05) were used to test the significance among the treatments. When a F-test indicated statistical significance, the protected least significant difference (LSD) was used to split the means of the main effect. Interaction effects were divided by slicing method.

3. Results

3.1. Shoot N content

Effects of drought stress and vermicompost were significant on the N content of shoot; furthermore, the drought stress × vermicompost interaction was significant for this trait (Table 2). A comparison of means showed that irrespective of the irrigation regimes, application of 10 t/ha vermicompost caused a significant increase in the N content of shoot. The maximum content of N (1.30% in dry matter) was observed for those plots that received I_1 irrigation treatment with the highest vermicompost rate, and the minimum content of N (0.55% in dry matter) was obtained from application of the lowest levels of irrigation (I_5) without vermicompost (Figure 1).

Table 1

Soil and vermicompost properties.

Properties	Soil	Vermicompost
Soil texture	Silty clay	–
pH	7.500	7.1
Electrical conductivity (dS/m)	0.900	4.9
Organic matter (%)	1.600	12.2
Nitrogen (%)	0.180	1.1
Phosphate (%)	0.001	1.3
Potash (%)	0.030	1.2

Table 2

ANOVA for the effects of different treatments on the measured traits.

SOV	*df*	N	P	K	LC a	LC b	LP	LSS
Replication	2	*	–	–	*	*	–	–
Irrigation (I)	4	**	**	**	**	**	**	**
Whole-plot error	8	0.0070	0.0009	0.0820	0.0760	0.0370	0.0470	0.0490
Vermicompost (V)	2	**	*	**	**	**	**	**
I × V	8	**	–	–	–	*	**	–
Split-plot error	8	0.00008	0.00004	0.02600	0.01700	0.01500	0.00500	0.00900
CV (%)		4.06	3.14	6.07	1.17	3.71	2.08	2.81

–: Not significant. $^{*}P \leq 0.05$, $^{**}P \leq 0.01$. SOV: Source of the variation; CV: Coefficient of variation; LC a: Leaf chlorophyll *a* content; LC b: Leaf chlorophyll *b* content; LP: Leaf proline content; LSS: Leaf soluble sugar content.

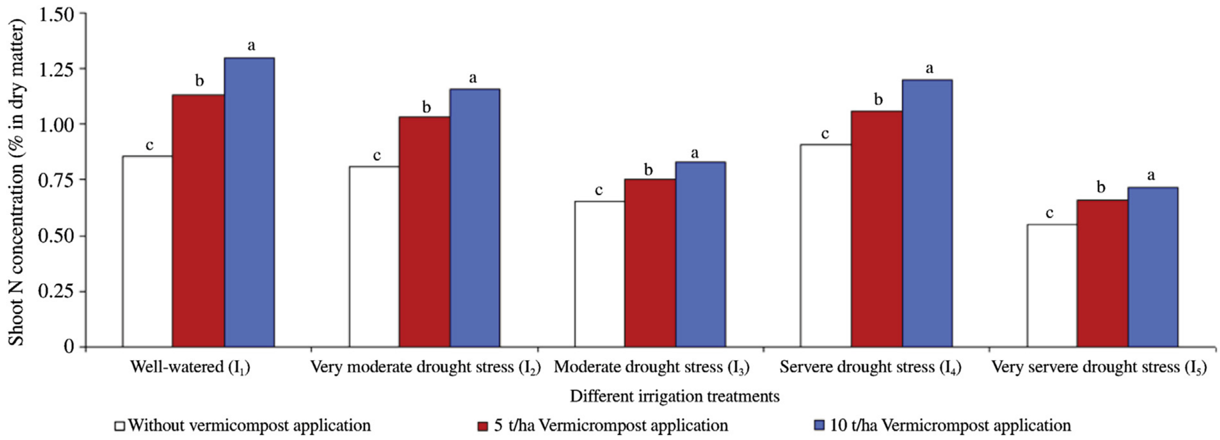

Figure 1. Interaction effect of irrigation treatments × vermicompost rates on chamomile shoot N concentration. Means followed by the different letter are significantly different ($P \leq 0.05$).

3.2. Shoot P content

P content of shoot was significantly affected by irrigation regimes and the vermicompost rates (Table 2). Mean comparisons showed that the reduced water availability in the I_3–I_5 irrigation treatments caused significant decrease in P concentration (Table 3). In addition, according to the results, shoot P content was found to be higher in V_{10} treatment than V_0 and V_5 treatments (Table 3).

3.3. Shoot K content

Drought stress and vermicompost application led to a decrease and increase, respectively, in the shoot K concentration. However, drought stress × vermicompost application interaction was not significant on K content of shoot (Table 2). Compared to well-irrigated treatment (I_1), K concentration of the stressed plants decreased and the relative percentages of reduction were 16%, 42%, 46% and 47% in $I_{2–5}$ irrigation treatments, respectively (Table 3). Drought stress diminished K content of shoot, by contrast vermicompost application increased the K concentration (Table 3), particularly when the plants were irrigated with I_1 treatment (data not shown).

3.4. Leaf chlorophyll a and b contents

As expected, a reduction in water availability (from I_1 to I_5 treatment) led to significant reduce in leaf chlorophyll *a* content (Table 3). In contrast, the V_{10} fertilizer treatment (10 t/ha vermicompost) had the highest leaf chlorophyll *a* content (11.64 mg/g fresh leaf weight), whereas the V_0 treatment (without

Table 3

Mean comparisons of irrigation and vermicompost treatment main effects on chamomile traits.

Traits treatments	P concentration (% in dry matter)	K concentration (% in dry matter)	LC a (mg/g fresh leaf weight)	LSS (mg/g fresh leaf weight)
Irrigation treatments				
Well-watered (I_1)	0.233^{a}	3.87^{a}	14.52^{a}	8.82^{e}
Very moderate drought stress (I_2)	0.218^{b}	3.26^{b}	14.10^{b}	9.09^{d}
Moderate drought stress (I_3)	0.217^{b}	2.24^{c}	11.26^{c}	10.99^{c}
Severe drought stress (I_4)	0.207^{c}	2.05^{c}	8.66^{d}	12.67^{b}
Very severe drought stress (I_5)	0.190^{d}	2.04^{c}	8.24^{e}	13.07^{a}
Vermicompost treatments				
0 (V_0)	0.207^{b}	2.39^{b}	11.01^{c}	10.72^{b}
5 t/ha (V_5)	0.211^{ab}	2.79^{a}	11.42^{b}	10.81^{b}
10 t/ha (V_{10})	0.214^{a}	2.90^{a}	11.64^{a}	11.25^{a}

Means within each column of each section followed by the different letter are significantly different ($P \leq 0.05$). LC a: Leaf chlorophyll *a* content; LSS: Leaf soluble sugar content.

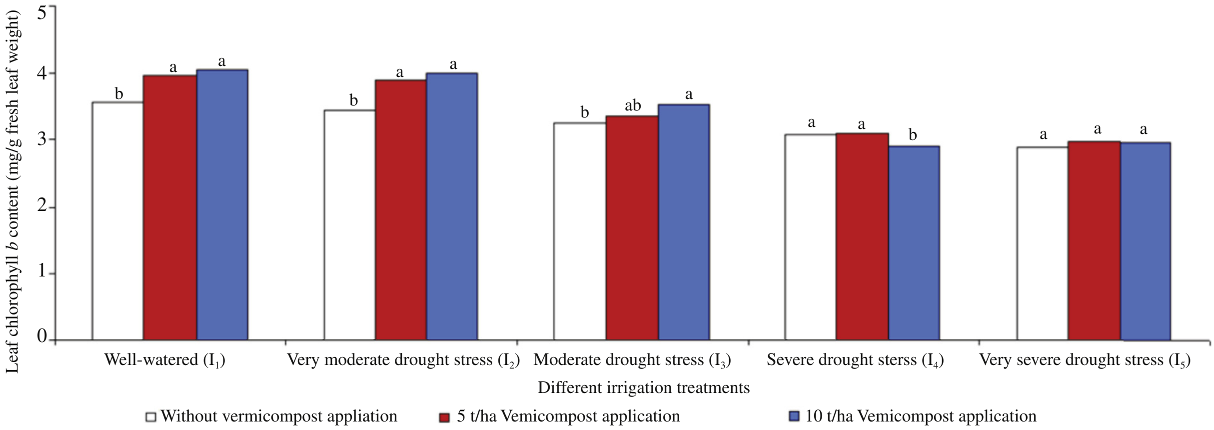

Figure 2. Interaction effect of irrigation treatments × vermicompost rates on chamomile leaf chlorophyll *b* content. Means followed by the different letter are significantly different ($P \leq 0.05$).

vermicompost application) had the lowest leaf chlorophyll *a* content (11.01 mg/g fresh leaf weight). On the other hand, leaf chlorophyll *a* content in the V_{10} treatment was 2% and 6% higher than in the V_0 and V_5 treatments, respectively (Table 3).

Moreover, the interaction between drought stress and vermicompost rates on the leaf chlorophyll *b* content was significant. Under well-irrigated treatment (I_1 treatments) and moderate drought stress (I_2 and I_3 treatments), when plants received 5 and 10 t/ha vermicompost (V_5 and V_{10} treatments), chlorophyll *b* content was significantly increased compared with control treatment (Figure 2).

3.5. Leaf proline content

A decrease in water availability under low irrigation regime (I_2–I_5 irrigation treatments) enhanced the leaf proline content (Figure 3). Although, the leaf proline content increased due to moderate and severe drought stress, its impact was more noticeable under severe and very severe stress conditions (I_4 and I_5 irrigation treatments). Drought stress ($I_{2–5}$ irrigation treatments) compared with well-irrigated treatment (I_1 treatment), in which plots did not receive vermicompost, significantly enhanced the content of leaf proline by 11%, 19%, 47% and 63% in I_2–I_5 treatments, respectively. In contrast, the leaf proline content of the chamomile plants received 10 t/ha vermicompost was less affected by water deficiency and low irrigation, which increased the content of leaf proline only by 6%, 8%, 40% and 37% in I_2–I_5 treatments, respectively (Figure 3). In all irrigation treatments, the leaf proline content was significantly higher in the V_0 treatment than V_5 and V_{10} treatments (Figure 3).

3.6. Leaf soluble sugar content

Results of this study indicated that an increase in drought stress intensity (from I_1 to I_5 irrigation treatment) led to increase leaf soluble sugar content (Table 3). In addition, the obtained results showed that V_0 treatment had the lowest leaf soluble sugar content, being 5% lower than for V_{10} (which had a maximum leaf soluble sugar content) and 1% lower than for V_5 (Table 3).

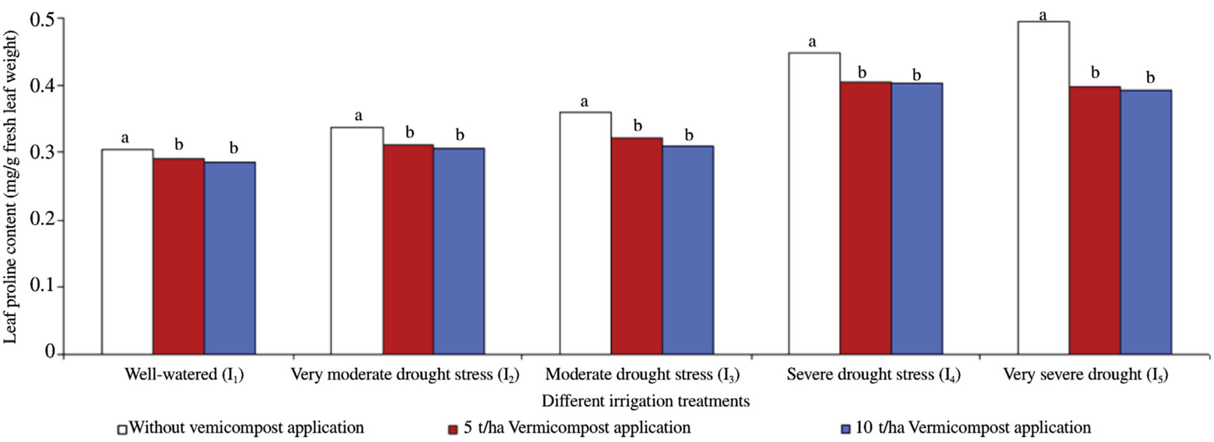

Figure 3. Interaction effect of irrigation treatments × vermicompost rates on chamomile leaf proline content. Means followed by the different letter are significantly different ($P \leq 0.05$).

4. Discussion

In spite of the drought stress, vermicompost could increase N content, particularly when I_1–I_4 irrigation treatments were applied. It can be concluded that reduction in transpiration on account of water shortage, especially due to I_3–I_5 irrigation treatments, could either reduce water mass flow through the soil or N uptake by plants. Also, it seems that the application of vermicompost by increasing water retention and sustaining release of nutrients, especially N [15], improves soil physicochemical properties and reduces the stress effects and therefore increases the absorption of N. It has been reported that vermicompost application could enhance N uptake by plants even under water deficit stress conditions [16]. As a consequence of the enhanced and steady uptake of N due to vermicompost application, plants which received organic fertilizers frequently provide greater yields than those which even received chemical fertilizers.

Mass flow, diffusion and contact exchanges are three major mechanisms of element absorption in higher plants. Among them, diffusion is the most important for P absorption. Diffusion coefficient highly depends on soil water potential and decreases while soil water potential reduces. Thus, P uptake is highly affected by soil water potential and under water shortage conditions, P uptake by plants will be decreased.

Previous findings suggest that the presence of organic matters in the soil increases P solubility [17,18]. There are several mechanisms which can lead to enhanced availability and absorption of P due to organic matters: (i) the application of organic matters rich in P can increase P content in soil; (ii) organic matter decomposition helps to increase organic acids content in soil by which P fixation is reduced [19]; (iii) increase in microbial activity on account of organic matters can increase P availability into the soil through forming weak-acids (H_2CO_3) and releasing P from primary phosphorus-containing minerals. It has also been stated that leaf P content affects the stomatal behavior under stress conditions, possibly by affecting the osmotic potential of guard cell or by wall stiffening governing the stomatal movements [20]. Therefore, it is not surprising if vermicompost treated plants show higher performance compared to control plants, even under stressful environments.

Drought stress diminished K concentration in the shoots, by contrast vermicompost application increased the K concentration, particularly when the plants were irrigated with I_1 treatment. Reduced transpiration rate due to water shortage conditions decreased water mass flow into the soil, prohibiting the K uptake by plants. On the other hand, it is widely stated that organic fertilizer application provides positive effects on the physicochemical characteristics of soil and enhances nutrients uptake by plants [9,10]. The favorable effects of organic fertilizers, especially vermicompost, might be due to their potential to encourage microbial activity, enhance nutrient availability and increase plant photosynthesis. Moreover, it seems that adding vermicompost to the soil not only increases nutrients availability directly, but also operates as a slow-release fertilizer to provide N, P and K to the chamomile steadily. Potassium plays a crucial role in plant metabolism, specifically where drought stress is the main issue. Potassium actuates different enzymes involved in plant growth and plays a key role in stomatal movements and protein synthesis [21]. Therefore, it is reasonable that improved nutrient uptake (P and K) by using vermicompost, especially V_{10} treatment, has been considered as a practical approach for amplifying drought resistance in chamomile.

Reducing the amount of chlorophyll *a* affected by the drought stress is related to increase oxygen radicals in cells. Free radicals cause peroxidation and therefore chlorophyll pigments degradation. It seems that the reduction of chlorophyll *a* concentration under drought conditions is mainly because of the chlorophylls enzyme activity, peroxidase and phenolic compounds, resulting in degradation of chlorophyll [22].

Results showed that with increased drought stress, leaf chlorophyll *b* content decreased and by using of vermicompost the leaf chlorophyll *b* content increased. According to previous findings, there is a significant correlation between leaf N content and chlorophyll [23,24]. Leaf chlorophyll content is a good index to detect N status in plants in order to determine required amount of N fertilizer to gain high N use efficiency with maximum plant performance [13]. In general, it can be supposed that the vermicompost as organic fertilizer can increase chlorophyll and carotenoids content by increasing the amount of N availability for plant and followed by that the ability to absorb more sunlight and produce more assimilates and finally enhancing plant growth and yield. Higher leaf chlorophyll content in vermicompost plots compared with control plots after water deficiency conditions, suggests that higher photosynthetic capacity, attributed to better drought resistance of chamomile treated with organic fertilizers.

Accumulation of different active ions, sugars and amino acids like proline is responsible for osmotic adjustment in plant cells [25]. Osmotic adjustments maintain turgor pressure, control cell expansion and growth as well as stomatal aperture, photosynthesis, and water flow during water shortage periods [26]. Our results showed that drought stress compared to the well-watered treatment, in which plots did not receive vermicompost, significantly enhanced chamomile leaf proline content. In contrast, the proline content of the chamomile plants received 10 t/ha vermicompost was less affected by water deficiency and low irrigation. In all irrigation treatments, leaf proline content was significantly higher in the V_0 treatment than V_5 and V_{10} treatments. It has been reported that high levels of leaf proline can protect plants against severe drought stress and increase drought stress tolerance [26,27]. The obtained results showed that vermicompost application decreased the leaf proline content of chamomile plants compared with non-vermicompost treatment. The results indicated that vermicompost application improved chamomile drought resistance, which did not associate with leaf proline concentration, by contrast with increasing uptake of nutrients (N, K and P). Moreover, it should be noted that the increase in leaf proline concentration may be an incidental change associated with tissue injury. It appears that decline in soil bulk density [28], enhancement in soil water holding capacity (data not shown) and improvement of soil microbial liveliness [29] due to use of vermicompost can also account for chamomile drought tolerance enhances.

Leaf osmotic adjustment by organic solutes such as soluble sugar in the stressed plants has been previously known as a resistance mechanism to water deficit stress [13]. The results of this study indicated that an increase in drought stress intensity led to increase leaf soluble sugar concentration. The accumulation of soluble sugars in drought stressed plants is controlled by several mechanisms affecting soluble sugar formation and transfer in leaves [30]. Increasing the leaf soluble sugars concentration has been found to be correlated with enhancement of the relative leaf water content [25,31]. In addition, soluble sugars play a pivotal role in osmotic adjustment in plants [32]. Also, greater sugar concentration in leaves of plants treated with vermicompost, especially 10 t/ha, might be due to increased leaf water potential and leaf area as well as reduced chlorophyll photooxidation activity. No one has reported the influence of vermicompost application on the leaf soluble sugar content of chamomile leaf under stress and non-stress conditions. In addition, higher leaf soluble sugars concentration in treated plants with the highest rate of vermicompost (V_{10} treatment) than other treatments (V_0 and V_5) following water deficiency conditions, suggesting conservation of better photosynthetic capacity, has been associated with more drought resistance of the plants received organic fertilizers. The treated plants with 10 t/ha vermicompost also showed less stressed-induced accumulation of the proline than other plants, therefore, they were able to keep normal nitrogen metabolism and photosynthesis.

Drought stress in addition to the negative effects on grain yield, causes institution or exacerbation of other stresses such as nutrient deficiency. According to the results, drought stress and vermicompost application had a significant impact on proline, chlorophyll, carbohydrate and nutrient uptake in German chamomile. It can be possible that with proper nutrient management and application of organic fertilizers, particularly vermicompost, medicinal plant resistance to drought stress will be improved by increasing nutrient uptake. It seems that due to exposure to the country's arid and semi-arid region, cultivation of German chamomile in drought conditions can be extended by vermicompost fertilization.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

Authors are highly grateful to Yasouj University for providing funding resources for conducting this study (Grant No. 88/8705).

References

[1] Andrzejewska J, Woropaj-Janczak M. German chamomile performance after stubble catch crops and response to nitrogen fertilization. *Ind Crops Prod* 2014; **62**: 350-8.
[2] Agatonovic-Kustrin S, Babazadeh Ortakand D, Morton DW, Yusof AP. Rapid evaluation and comparison of natural products and antioxidant activity in calendula, feverfew, and German chamomile extracts. *J Chromatogr A* 2015; **1385**: 103-10.
[3] Kadam NN, Xiao G, Melgar RJ, Bahuguna RN, Quiñones C, Tamilselvan A, et al. Agronomic and physiological responses to high temperature, drought, and elevated CO_2 interactions in cereals. *Adv Agron* 2014; **127**: 111-56.
[4] Devnarain N, Crampton BG, Chikwamba R, Becker JVW, O'Kennedy MM. Physiological responses of selected African sorghum landraces to progressive water stress and re-watering. *S Afr J Bot* 2016; **103**: 61-9.
[5] Gholamhoseini M, Ghalavand A, Dolatabadian A, Jamshidi E, Khodaei-Joghan A. Effects of arbuscular mycorrhizal inoculation on growth, yield, nutrient uptake and irrigation water productivity of sunflowers grown under drought stress. *Agric Water Manag* 2013; **117**: 106-14.
[6] Qiu GY, Yin J, Geng S. Impact of climate and land-use changes on water security for agriculture in Northern China. *J Integr Agric* 2012; **11**: 144-50.
[7] Huang J, Ridoutt BG, Xu CC, Zhang HL, Chen F. Cropping pattern modifications change water resource demands in the Beijing metropolitan area. *J Integr Agric* 2012; **11**(11): 1914-23.
[8] Singh R, Gupta RK, Patil RT, Sharma RR, Asrey R, Kumar A, et al. Sequential foliar application of vermicompost leachates improves marketable fruit yield and quality of strawberry (*Fragaria × ananassa* Duch.). *Sci Hortic* 2010; **124**: 34-9.
[9] Karmegam N, Daniel T. Effect of biodigested slurry and vermicompost on the growth and yield of cowpea [*Vigna unguiculata* (L.)]. *Environ Ecol* 2000; **18**(2): 367-70.
[10] Zhang X, Dong W, Dai X, Schaeffer S, Yang F, Radosevich M, et al. Responses of absolute and specific soil enzyme activities to long term additions of organic and mineral fertilizer. *Sci Total Environ* 2015; **536**: 59-67.
[11] Arnon DT. Copper enzymes in isolation chloroplast phenoloxidase in *Beta vulgaris*. *Plant Physiol* 1949; **24**: 1-15.
[12] Li HS. *Principles and techniques of plant physiological biochemical experiment*. Beijing: Higher Education Press; 2000.
[13] Wu QS, Xia RX. Arbuscular mycorrhizal fungi influence growth, osmotic adjustment and water stress conditions. *J Plant Physiol* 2006; **163**: 417-25.
[14] Troll W, Lindsley J. A photometric method for the determination of proline. *J Biol Chem* 1955; **215**: 655-60.
[15] Guo L, Wu G, Li Y, Li C, Liu W, Meng J, et al. Effects of cattle manure compost combined with chemical fertilizer on topsoil organic matter, bulk density and earthworm activity in a wheat-maize rotation system in Eastern China. *Soil Till Res* 2016; **156**: 140-7.
[16] Yang L, Zhao F, Chang Q, Li T, Li F. Effects of vermicomposts on tomato yield and quality and soil fertility in greenhouse under different soil water regimes. *Agric Water Manag* 2015; **160**: 98-105.
[17] Herenica JF, Ruiz-Porras JC, Melero Sánchez S, Morillo González E, Maqueda Porras C. Comparison between organic and mineral fertilization for soil fertility levels, crop macronutrient concentrations, and yield. *Agron J* 2007; **99**: 973-83.
[18] Gholamhoseini M, Ghalavand A, Khodaei-Joghan A, Dolatabadian A, Zakikhani H, Farmanbar E. Zeolite-amended cattle manure effects on sunflower yield, seed quality, water use efficiency and nutrient leaching. *Soil Till Res* 2013; **126**: 193-202.
[19] Wen Z, Shen J, Blackwell M, Li H, Zhao B, Yuan H. Combined applications of nitrogen and phosphorus fertilizers with manure increase maize yield and nutrient uptake via stimulating root growth in a long-term experiment. *Pedosphere* 2016; **26**: 62-73.
[20] Augé RM. Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza* 2001; **11**: 3-42.
[21] Evelin H, Kapoor R, Giri B. Arbuscular mycorrihizal fungi in alleviation of salt stress: a review. *Ann Bot* 2009; **104**: 1263-80.
[22] Ramírez DA, Yactayo W, Gutiérrez R, Mares V, De Mendiburu F, Posadas A, et al. Chlorophyll concentration in leaves is an indicator of potato tuber yield in water-shortage conditions. *Sci Hortic* 2014; **168**: 202-9.
[23] Feng W, He L, Zhang HY, Guo BB, Zhu YJ, Wang CY, et al. Assessment of plant nitrogen status using chlorophyll fluorescence parameters of the upper leaves in winter wheat. *Euro J Agron* 2015; **64**: 78-87.
[24] Hermanson RW, Pan C, Perillo R, Stevans R, Stockle C. *Nitrogen use by crops and the fate of nitrogen in the soil and vadose zone – a literature search*. Pullman: Washington State University; 2000.
[25] Königshofer H, Löppert HG. Regulation of invertase activity in different root zones of wheat (*Triticum aestivum* L.) seedlings in the course of osmotic adjustment under water deficit conditions. *J Plant Physiol* 2015; **183**: 130-7.
[26] Ruiz-Lozano JM. Arbuscular mycorrhizal symbiosis and alleviation of osmotic stress. New perspectives for molecular studies. *Mycorrhiza* 2003; **13**: 309-17.
[27] Cvikrová M, Gemperlová L, Martincová O, Vanková R. Effect of drought and combined drought and heat stress on polyamine metabolism in proline-over-producing tobacco plants. *Plant Physiol Biochem* 2013; **73**: 7-15.
[28] Zhang Y, Li C, Wang Y, Hu Y, Christie P, Zhang J, et al. Maize yield and soil fertility with combined use of compost and inorganic fertilizers on a calcareous soil on the North China Plain. *Soil Till Res* 2016; **155**: 85-94.
[29] Xin X, Zhang J, Zhu A, Zhang C. Effects of long-term (23 years) mineral fertilizer and compost application on physical properties of fluvo-aquic soil in the North China Plain. *Soil Till Res* 2016; **156**: 166-72.
[30] Arabzadeh N. The effect of drought stress on soluble carbohydrates (sugars) in two species of *Haloxylon persicum* and *Haloxylon aphyllum*. *Asian J Plant Sci* 2012; **11**: 44-51.
[31] Karimi M, Ahmadi A, Hashemi J, Abbasi A, Tavarini S, Guglielminetti L, et al. The effect of soil moisture depletion on Stevia (*Stevia rebaudiana* Bertoni) grown in greenhouse conditions: growth, steviol glycosides content, soluble sugars and total antioxidant capacity. *Sci Hortic* 2015; **183**: 93-9.
[32] Zhou Q, Yu BJ. Accumulation of inorganic and organic osmolytes and their role in osmotic adjustment in NaCl-stressed vetiver grass seedlings. *Russ J Plant Physiol* 2009; **56**: 678-85.