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Evaluation of photosynthesis, physiological, and biochemical responses of chickpea (*Cicer arietinum* L. *cv*. Pirouz) under water deficit stress and use of vermicompost fertilizer

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Abstract

One goal in the face of drought stress conditions is to increase growth and yield through the reduction of negative effects of stress. Vermicompost can play an effective role in plant growth and development and in reducing harmful effects of various environmental stresses on plants due to its porous structure, high water storage capacity, having hormone-like substances, plant growth regulators, and high levels of macro and micro nutrients. This study considered the physiological, biochemical, and photosynthetic responses of the chickpea to different combinations of vermicompost and water stress in a greenhouse environment. Two factors were involved, addition of vermicompost to soil at four ratios: control (100 wt% (weight percentage) soil); 10 wt% vermicompost+90% soil; 20 wt% vermicompost+80 wt% soil; 30 wt% vermicompost+70 wt% soil weight percentage, and treatment of water stress at three levels including 75, 50, and 25% of field capacity. The results showed that vermicompost had a significant effect on all traits under stress and non-stress conditions. Application of vermicompost in soil, especially at the levels of 20 and 30 wt% significantly increased all studied traits under non-stress conditions. Under moderate stress conditions, vermicompost at 30 wt% treatment resulted in a significant increase in the photosynthetic pigments, CO_2 assimilation rate, internal leaf CO_2 concentration, transpiration, the maximal quantum yield of photosystem II (PSII) photochemistry (F_v/F_m), concentrations of Ca and K in root and leaf tissues, proline and soluble protein contents in root tissues. Peroxidase (POX) and catalase (CAT) enzyme activities decreased significantly with increasing proportions of vermicompost, but the activity of superoxide dismutase was not significantly different. In conclusion, the above results showed that vermicompost fertilizer had a positive effect on physiological, biochemical, and photosynthetic responses of chickpea under non-stress and moderate stress conditions, but no positive effect was determined under severe water stress.

Keywords: organic fertilizer, photosynthetic features, water stress, gas exchange

1. Introduction

Chickpea (*Cicer arietinum* L.) grows in a wide range of weather conditions from the subtropical to Mediterranean regions of western Asia, northern Africa, and southern and southwestern Europe (Toker *et al*. 2007; Canci and Toker 2009a, b). The chickpea plays an important role in increasing the yield of arable land according to features such

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as nitrogen fixation, root induction depth, and effective use of rainwater (Ganjeali *et al*. 2011; Hosseinzadeh *et al*. 2016).

Environmental stresses are the most important factor that limits crop production of chickpea. The most critical environmental stresses that have a negative effect on crop production are water and heat stresses (Rahbarian *et al*. 2011; Amiri *et al*. 2017). Although, chickpea in comparison with other legumes is more resistant to water stress but water stress is the main factor in reducing the physiological and morphological characteristics (Ganjeali and Nezami 2008). Responses of plants to water stress are different and depend on the intensity and period of stress. These responses are of two types: 1) Water shortage in low intensity (moderate stress) leads to transpiration reduction, disruption of water translocation from roots to shoots, reduction of photosynthetic pigments, reduction of the photosynthetic products which ultimately lowers crop and morphological traits (Hosseinzadeh *et al*. 2016). The effect of water stress on the CO_2 assimilation rate, internal CO_2 concentration, photosystem II (PSII) photochemical efficiency and water use efficiency has been reported for many plants, including canola (Kauser *et al*. 2006), cotton (Mssacci *et al*. 2008), tomato (Bender-Özenc 2008), lentil (Ahmadpour *et al*. 2016), and chickpea (Rahbarian *et al*. 2011). 2) Water shortage in high intensity (severe stress) that produces reactive oxygen species (ROS) in plants; it has a detrimental effect on D1 protein of PSII, chlorophyll content, electron transport and production of high-energy molecules such as adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH) (Pagter *et al*. 2005). Severe water stress by reducing the absorption of water from the soil by the roots undermines the sap translocation in the phloem which ultimately leads to a significant decline in morphological features, nutrient uptake, and antioxidant enzymes activity (Armand *et al*. 2016). Osmotic regulation is active accumulation of soluble substances in soil by plants in response to water deficit stress. Previous studies showed that there is a significant correlation in plants, between growth and the ability to osmoregulation (Hu and Schmidhalter 2005). Of many plants, use of organic solute such as proline, soluble protein, mineral ions particularly Ca and K for osmotic regulation (Cakmak 2005). As mentioned above, severe water deficit stress is accompanied with oxidative stress; therefore, stress relief mechanisms play an important role in reducing oxidative stress and increased resistance to water stress (Ahmed *et al*. 2002). Catalase (CAT), peroxidase (POX), and superoxide dismutase (SOD) are the most important antioxidant defense system and play a key role in removal of free radicals (Armand *et al*. 2016).

Vermicompost production is a method of converting organic waste into usable material in which species of earthworm (*Eisenia fetida*) are employed to stabilize organic waste (Atiyeh *et al*. 2002). Some species of earthworms consume decaying organic material and are able to convert this material to nutrient-rich compounds that form an environment in the soil that supports plant growth (Huerta *et al*. 2010). Vermicompost has a high porosity, high capacity of ventilation, good drainage and high water holding capacity (Atiyeh *et al*. 2002). In comparison with other organic fertilizers, vermicompost consists of high levels of nutrients such as nitrogen, phosphorus, potassium, calcium, and magnesium, as well as micronutrients such as iron, zinc, copper, and manganese (Hosseinzadeh *et al*. 2016). Vermicompost has humic substances, which comes out from worm excrement and researchers believe that the hormone-like activities of humic substances play a role in increasing the yield and growth of plants (Bowden *et al*. 2010). Several studies have shown that soil amended with organic matter lead to an increase in agricultural production, for the reason that increases absorption of nutrients, total enzymatic activity, cation exchange capacity and water hold (Lakhdar *et al*. 2009; Huerta *et al*. 2010).

There are numerous studies about the positive effects of vermicompost fertilizer on growth and yield characteristics of legumes whereas very few studies are available about the interaction of vermicompost fertilizer and water stress in Iran. Agricultural land in Iran is facing water stress and chickpea is an economically valuable crop that has a significant role in the human diet. Therefore, the present study aimed to investigate the interaction of vermicompost fertilizer and water stress on photosynthesis, physiological, and biochemical responses of chickpea.

2. Materials and methods

2.1. Experimental details

The experimental was conducted to determine the effects of vermicompost and water stress on some photosynthesis, physiological, and biochemical traits of chickpea in 2015 at the University of Khatam Alanbia under the greenhouse conditions. The experimental design was randomized plot design with four replications. Each experimental unit was a pot (18 cm×12 cm) with 2.5 kg capacity and was planted seeds of chickpeaafter preparation treatments. The first treatment prepared four ratios of vermicompost (V) and soil (S) as follows: control (100 wt% (weight percentage) S); 10 wt% V+90 wt% soil; 20 wt% V+80 wt% soil; 30 wt% V+70 wt% soil, equivalent to: 0 (V)+2 500 g (S); 250 g (V)+2 250 g (S), 500 g (V)+2 000 g (S) and 750 g (V)+1 750 g (S), respectively. Table 1 summarizes the chemical properties of the soil and vermicompost used in the experiments.

Table 1 Soil and vermicompost fertilizer characteristics used in the experiment[1)]

Sample	EC (dS m^{-1})	pH	C/N	P (%)	Ca (%)	K (%)	Fe (%)	Total N (%)	Mg (%)
Vermicompost	1.5	7.1	17.5	0.9	4.5	1.2	0.5	3.0	0.50
Soil	0.4	7.8	14.9	0.03	1	0.4	0.004	1.5	0.01

[1)] EC, electrical conductivity.

The second factor was water stress and treatments included the following; a control (non-stress, 75% of field capacity), moderate water stress (50% of field capacity) and severe water stress (25% of field capacity). Water stress levels were selected based on preliminary tests and the results of other researchers (Ganjeali *et al*. 2011; Rahbarian *et al*. 2011; Hosseinzadeh *et al*. 2016). Chickpea seeds (Pirouz cultivar) were soaked in water for 24 h, until be performed early germination and then transplanted in pots. The pots were maintained in growth chamber under standard conditions (25–30°C; 50–60% relative humidity; 14 h light/10 h dark photoperiod at 25°C light/20°C dark) that are similar to normal field conditions in the chickpea growing region of Iran. Sampling was conducted at the end of the growing season (about 45 days after planting) and shoot was separated of root.

2.2. Physiological parameters measurements

In order to measure the photosynthetic pigments (Chl *a*, Chl *b*, Chl (*a*+*b*), and cartenoied), the method of Lichtenthaler (1987) was used. A total of 0.2 g of chickpea plant leaves were mixed with 4 mL of 80% acetone and centrifuged for 10 min with 3000 r min^{-1}. Absorbances of the resultant supernatant were applied to determine the photosynthetic pigments. Samples absorbance was read at a wavelength of 647, 664, and 470 nm using a spectrophotometer (SPEKOL2000; Analytik Jena, Germany). The photosynthetic pigments were calculated using the following eqs.:

$$\text{Chl } a=12.21OD_{664}-2.79OD_{647} \quad (1)$$

$$\text{Chl } b=21.21OD_{647}-5.1OD_{664} \quad (2)$$

$$\text{Carotenoid}=(1\,000OD_{470}-1.8\text{Chl } a-85.02\text{Chl } b)/198 \quad (3)$$

$$\text{Chl } (a+b)=\text{Chl } a+\text{Chl } b \quad (4)$$

Measurement for levels of elements in root and leaf tissue in chickpea plant was taken by a flamephotometer device (Sherwood Scientific, Cambridge, United Kingdom) at the podding stage (Chapman and Patt 1982). According to this method, 0.05 g of powder obtained from the dried leaf and root of per treatment were mixed with 3 mL of concentrated nitric acid in a 100-mL Erlenmeyer. Then Erlenmeyers for 48–72 h were exposed *in vitro*. Finally, Erlenmeyers were then gently heated under a hood and on a thermal grill. Emission of white smokes and decolonization of the acidic solution signals the end of the digestion. Volume of the residual solution was increased to 50 mL with distilled water. Concentration of cations using a standard curve was determined and its value measured in terms of g/100 g of tissue dry weight (DW).

2.3. Photosynthetic parameters measurements

The internal leaf CO_2 concentration (ppm), CO_2 assimilation rate (μmol m^{-2} s^{-1}), and transpiration (mmol H_2O m^{-2} s^{-1}) were measured on the central sector of the youngest fully-expanded leaf and non-detached young. Measurements were performed using a portable infrared gas analyzer (KR8700 system; Korea Tech Inc., Suwon., Korea). Conditions of the leaf chamber were adjusted with growth chamber (such as relative humidity, CO_2 concentration and leaf temperature). Measurement was carried out on the second and third leaves of the chickpea under uniform conditions for all plants.

The chlorophyll content in the leaves was determined by a portable leaf chlorophyll-meter (CCM-200 Plus, Opti-Sciences Inc., NH, USA). Chl was determined from averages taken from 10 readings per sample. Comply with equal conditions were used from the second uppermost leaf of per seedling.

The maximal quantum yield of PSII photochemistry (F_v/F_m) was conducted by chlorophyll fluorometer (Pocket PEA, Hansatech Instruments Ltd., King's Lynn, and Norfolk, England). This instrument determined F_v/F_m automatically, by equation of $F_v/F_m=(F_m-F_o)/F_m$. F_m and F_o were the maximum and initial fluorescence yields of dark-adapted leaves, respectively. The difference between the maximum fluorescence and initial fluorescence is F_v, or variable fluorescence (Hosseinzadeh *et al*. 2016). Measurements were taken using the same leaf used for the chlorophyll content determination after 15 min of dark adaptation. F_v/F_m ratio was calculated from averages taken from 10 readings per sample.

2.4. Biochemical parameters measurements

Proline assay was determined according to the method of Bates *et al*. (1973). For this purpose, root tissue (0.2 g) was homogenized with 4 mL of 3% of sulphosalicylic acid solution. Then, 2 mL of the above-mentioned solution was added to a mixture of reactive ninhydrin (2 mL) in acetic acid (2 mL), and incubated in boiling water for 1 h. After that

duration, samples were immediately transferred into an ice container. After that duration, samples were immediately transferred into an ice container. After completion of the reaction, samples were allowed to settle until room temperature was reached. Samples were then added to toluene (4 mL) and the obtained solution was shaken strongly. The colored complex was applied to measure absorbance at 520 nm by spectrophotometer (Model SPEKOL 2000, Analyticjena, Germany). Finally, the proline content was calculated based on μmol per g of fresh weight (FW) according to the following eq.:

$$\text{Proline } (\mu\text{mol g}^{-1}\text{ FW}) = \text{Prolin } (\mu\text{g mL}^{-1}) \times \frac{\text{Toloen (mL)}}{115.5\ (\mu\text{g } \mu\text{mol}^{-1})} / \frac{\text{Sample (g)}}{5}$$

Measurement of protein content in root tissue was conducted by Lowry (1951). This experiment is based on proteins hydrolysis and amino acids release that create colored complexes with Fulvin reagent. Finally, absorption of the samples at the 660 nm wavelength was recorded by spectrophotometer (Model SPEKOL 2000, Analyticjena, Germany). The protein concentration in each sample was determined using the standard curve of bovine serum albumin and protein calculated according to the mg g^{-1} DW.

Peroxidase (EC 1.11.1.7, POX) activity was assessed through Holy (1972) method. The 0.2 mol L^{-1} acetate buffer (pH=5) with 3% H_2O_2, benzidin 0.2 mmol L^{-1} in methanol (50%) and 0.1 mL enzyme extract were mixed in an ice buckets. Photo absorption curve of the samples was plotted using spectrophotometer per 30 s for 3 min at a wavelength of 530 nm. The enzyme unit was defined using the standard curve of protein, for μmol mL^{-1} H_2O_2 decomposed per min at 25°C.

Method of Beauchamp and Fridovich (1971) was used for measuring superoxide dismutase (EC 1.15.1.1, SOD) activity. For this purpose, 50 mmol L^{-1} of phosphate buffer solution was prepared and then the compositions of 0.1 mmol L^{-1} of EDTA, 75 mmol L^{-1} of nitroblue tetrazolium (NBT), 13 mmol L^{-1} of methionine, and 4 mmol L^{-1} of riboflavin were added respectively. Finally, the reaction started with addition of 0.1 mL of the extract under the fluorescent lamp light. After 15 min, the absorption of the samples at a wavelength of 560 nm was read by spectrophotometer (Model SPEKOL 2000, Analyticjena, Germany). A unit of the SOD activity is equal to 50% inhibition of the NBT color change in the light. The enzymatic activity was defined according to the enzyme unit in total amount of protein (mg) per 100 mL of the extract.

To measure activity of catalase enzyme (EC 1.11.1.6, CAT), the method of Candlee and Scandalios (1984) was used. First, 2.5 mL of potassium phosphate 50 mmol L^{-1} and 0.3 mL of 3% H_2O_2 were mixed together in an ice container. Then, 2.0 mL of enzyme extract was added immediately. Absorbance variation curve at 240 nm was determined for 3–4 min. Enzyme activity was calculated from the change in absorbance and expressed as mg $protein^{-1}$ min^{-1}.

2.5. Statistical analysis

All data were statistically analyzed using MSTAT-C version 4 (1987). Duncan's multiple range test by using MSTAT-C software was performed to confirm the variability of results and for the determination of significant ($P \leq 0.05$) difference between treatment groups.

3. Results and discussion

3.1. Photosynthetic pigments

The comparison of means showed that under non-water stress conditions, levels of vermicompost resulted in a significant increase in Chl *a* compared with the control but under severe water stress, there was no significant difference between control and vermicompost treatments. Under moderate stress, 30% vermicompost treatment compared with the control treatment resulted in a significant increase. Results for the interaction of vermicompost fertilizer and water stress on the Chl *b* showed that vermicompost levels in all stress treatment had no significant difference compared to those in control levels but Chl *b* content decreased under severe stress compared to that under non-stress conditions. The review of the data associated with the totall chlorophyll content shows that under non-stress conditions, total chlorophyll content had a significant increase by using vermicompost fertilizer (10, 20, and 30 wt% of vermicompost). Under moderate and severe water stresses, there was no statistically significant difference in comparison between control and vermicompost treatments (Table 2). Means comparison on carotenoeid content showed that under non-stress and moderate stress, vermicompost treatments resulted in a significant increase in content of carotenoeid in comparison with control. Under severe water stress, the highest and lowest concentrations of carotenoeid were observed for the treatment at 30 wt% and control, respectively (Table 2).

Chlorophyll content is known as the tolerance index to water stress in plants (Hosseinzadeh *et al*. 2016). In a study on the effects of water stress in chickpea genotype, Rahbarian *et al*. (2011) reported that by reducing the amount of available water, chlorophyll contents (Chl *a*, Chl *b*, carotenoied, and Chl (*a*+*b*)) decreased in leaf tissues. It is proved that under water conditions, the uptake of Mg and Fe from soil is reduced (Armand *et al*. 2016). Mg participates in the chlorophyll synthesis directly. For the reason that, reducing the uptake of Mg ion is associated

Table 2 Means comparison of different vermicompost ratios on physiological features of chickpea under water stress[1)]

Treatment and vermicompost (V)[2)]	Chlorophyll *a* (mg g^{-1} FW)	Chlorophyll *b* (mg g^{-1} FW)	Carotenoid (mg g^{-1} FW)	Chlorophyll (*a*+*b*) (mg g^{-1} FW)
Non-water stress (75% field capacity)				
Control	4.19 cdef	1.90 a	2.80 b	6.10 bcd
10 wt%	5.93 a	1.84 ab	3.59 a	7.78 a
20 wt%	5.77 ab	1.89 a	3.82 a	7.66 a
30 wt%	5.96 a	1.92 a	3.87 a	7.88 a
Moderate water stress (50% field capacity)				
Control	3.75 def	1.73 bc	1.95 de	5.48 bcde
10 wt%	4.80 bcde	1.71 c	2.60 bc	6.51 b
20 wt%	4.82 bcd	1.73 bc	2.65 b	6.22 bc
30 wt%	4.86 bc	1.69 c	2.68 b	6.55 b
Severe water stress (25% field capacity)				
Control	3.43 f	1.24 d	1.54 e	4.68 e
10 wt%	4.03 cdef	1.21 d	2.03 de	5.25 cde
20 wt%	3.73 ef	1.23 d	2.00 de	4.96 de
30 wt%	3.41 f	1.21 d	2.11 cd	4.63 e

[1)] FW, fresh weight.
[2)] Ratios of V to soil: control (100 wt% (weight percentage) soil); 10 wt% V+90 wt% soil; 20 wt% V+80 wt% soil; 30 wt% V+70 wt% soil.
Difference between the data of each column followed by the same letter was not statistically significant (P<0.05).

with reduction of chlorophyll synthesis (Hosseinzadeh *et al*. 2016). The most important effects of water stress are increase in free radicals that it makes peroxidation and decomposition of chlorophyll, especially Chl *a* (Flexas and Medrano 2008). Vermicompost organic fertilizer provides some microelements (such as Fe, Cu, Mn, and Zn) that as prosthetic groups are for antioxidant enzymes. Antioxidant enzymes including CAT, POX, and SOD play an important role in the destruction of free radicals (Ahmed *et al*. 2002). A survey of the literature reveals that vermicompost fertilizer contains higher levels of nutrients such as nitrogen, phosphorus, potassium, calcium, and magnesium, and micronutrients such as iron, zinc, copper, and manganese than other organic fertilizers (Tognetti *et al*. 2005; Suthar 2009). A decrease in the chlorophyll *a* and *b* contents in response to water stress may influence the nitrogen metabolism for biosynthesis of nitrogenous compounds such as proline during osmotic regulation (Flexas and Medrano 2008). An increase in the proline content inhibits glutamate and glutamate is a major participant in the biosynthesis of Chl *a* (Redy *et al*. 2003). It appears that vermicompost and compost organic fertilizers maintain the availability of water and nutrients such as K and N that are involved in the regulation of osmotic pressure. Therefore, glutamate is less in the synthesis of proline and is maintained leaf chlorophyll content. Chlorophylls are sensitive to oxidation and carotenoids play a role in the protection of chlorophylls (Loggini *et al*. 1999). It is recognized that carotenoids are more stable than chlorophylls but under water stress reduced (Loggini *et al*. 1999). Reduction of photosynthetic pigments at the flowering and poding stages of chickpea under water stress conditions, the reasons are more allocation products produced by photosynthesis to flowering and podding stages, reduction the transfer of necessary products to leaves and reduction of leaf growth (Ganjeali and Kafi 2007; Guerfel *et al*. 2008). Studies of olea (*Olea paniculata* L.) and bean (*Phaseolus vulgaris* L.) reported that water stress has a detrimental effect on Chl *a*, Chl *b* and carotenoeid (Redy *et al*. 2003; Zlatev and Yordanov 2004; Guerfel *et al*. 2008). Carotenoid content is proportional to the content of chlorophyll that protects it (Redy *et al*. 2003). Therefore, increased carotenoid contents at vermicompost treatments (10, 20, and 30 wt%) are associated with increased chlorophyll contents.

3.2. Concenteration of Na, K, and Ca in leaf

Comparison of means results on K concenteration showed that at all levels of water stress (non-stress, moderate and severe stress), the use of vermicompost at 20 and 30 wt% treatments significantly increased control levels (Table 3). Results on leaf Ca concentrations showed that under all levels of water stress, Ca concenteration was significantly affected by vermicompost treatments (10, 20, and 30 wt%) compared with the control treatments (Table 3). For leaf Na concenteration, no significant difference was observed between comparison of means of the interactions of vermicompost and water stress (Table 3). One sign of water stress resulting from water deficit or salt stress in plants is wilting due to lack of potassium, and this wilting is known to be variable according to genotype (Cakmak 2005). Water stress decreases nutrient uptake by roots and affects the transfer of materials to stems; this is caused by limited rate of transpiration, active transport and reduced

Table 3 Means comparison of different vermicompost ratios on mineral nutrient content of chickpea under water stress[1)]

Treatment and vermicompost (V)[2)]	Leaf Ca (g 100 g^{-1} leaf DW)	Leaf K (g 100 g^{-1} leaf DW)	Leaf Na (g 100 g^{-1} leaf DW)	Root Ca (g 100 g^{-1} leaf DW)	Root K (g 100 g^{-1} leaf DW)	Root Na (g 100 g^{-1} leaf DW)
Non water stress (75% field capacity)						
Control	0.633 g	2.89 cde	0.829 a	2.40 b	1.13 ef	2.66 e
10 wt%	1.193 ef	4.13 b	0.890 a	3.11 a	2.75 b	2.41 e
20 wt%	1.213 ef	4.29 ab	0.885 a	3.07 a	3.50 a	2.59 e
30 wt%	1.400 cde	4.61 a	1.182 a	3.33 a	3.23 a	2.51 e
Moderate water stress (50% field capacity)						
Control	0.690 g	2.50 de	0.835 a	2.24 b	1.14 ef	5.17 bcd
10 wt%	1.283 def	4.05 bc	0.842 a	3.21 a	2.06 c	4.66 cd
20 wt%	1.537 cde	4.12 b	0.901 a	3.23 a	1.82 cd	4.74 cd
30 wt%	2.117 ab	4.15 b	0.883 a	3.38 a	2.10 c	3.57 d
Severe water stress (25% field capacity)						
Control	0.813 g	2.04 e	0.868 a	2.23 b	1.01 f	6.17 a
10 wt%	1.717 bcd	3.49 bcde	0.915 a	3.24 a	1.52 de	5.54 abc
20 wt%	1.787 abc	3. 44 bcd	0.931 a	3.14 a	1.60 d	5.68 ab
30 wt%	2.240 a	3.76 bc	0.884 a	3.36 a	1.58 d	5.69 ab

[1)] DW, dry weight.
[2)] Ratios of V to soil: control (100 wt% (weight percentage) soil); 10 wt% V+90 wt% soil; 20 wt% V+80 wt% soil; 30 wt% V+70 wt% soil.
Difference between the data of each column followed by the same letter was not statistically significant (P<0.05).

damage to membrane permeability. Absorption of nutrients from the soil is directly related to water status of the soil, in that the flow of nutrients from the soil to the root decreases under conditions of moisture deficit (Arndt *et al*. 2001). K^+ ion is a major and necessary element for plant growth. It plays different roles, particularly in plants that require increasing resistance to environmental stress and pests, protein synthesis, photosynthesis, osmotic adjustment, transpiration control by opening and closing the stomata, and anionic and cationic exchange (Hafsi *et al*. 2007). A study on the effects of water stress and salinity on plants reported that mobility of K is reduced under conditions of water deficit (Hu and Schmidhalter 2005; Hafsi *et al*. 2007). Osuagwu *et al*. (2010) reported that water stress decreased levels of K and Ca in leaves of the *Ocimum gratissimum* plant, which may have been caused by movement of these elements from leaves to roots as these two elements act as osmotic protectors. An important role of Ca ions in plants is functioning as a secondary messenger and as such, these ions are involved in a wide variety of signal transduction pathways, so it may be an important component of plant response to water deficit stress (Hafsi *et al*. 2007). It seems that negative effects of water deficit stress in sensitive plants are from disrupted signal transduction pathways, in which Ca ions have a role. Bender Özenc (2008) reported that farmlands are facing with water stress, have shortages of N, P, Ca, and K. Adding vermicompost to dry farmland can enrich the rhizosphere with macro and micro nutrients that will compensate for any lack. Studies have shown that the cytokinin hormone increases potassium uptake in *Ziziphus rotundifolia* (Arndt *et al*. 2001). Vermicompost contains plant growth hormones such as cytokinin and research has shown that the cytokinin hormone increases K uptake (Ilan 1971). Therefore, vermicompost containing high levels of nutrients, plant hormones, and with good water storage capacity leads to improved uptake of nutrients that serves to reduce the detrimental effects of drought stress.

3.3. Concenteration of Na, K, and Ca in root

Comparison of means on K and Ca consentration in root tissue showed that vermicompost at all treatments has a positive effect on this trait so that under stress treatments (moderate, severe and non-stress), vermicompost levels significantly increased (Table 3). Vermicompost treatments had no significant effect on root Na concenteration but moderate and severe water stress led to a significant increase this trait compred with non-stress (Table 3). In this study, the shortage of water in the soil reduced root K^+ concenteration. The cause of decreased K under water stress, is decreased its solubility that subsequently reduced its absorption by plant roots. However, colloids in the soil strongly absorb K and prevent its absorption by the root (Osuagwu *et al*. 2010). Due to the suitable physical and chemical properties of vermicompost fertilizer, the use of this fertilizer led to an increase in K and Ca absorption by the roots. Jat and Ahlawt (2006) reported that use of vermicompost fertilizer resulted in increased Ca uptake by chickpea root and this result is in accordance results of the present study. Na is among soluble cations in soil of arid and semi-arid areas. Most plants are sensitive to a high concentration of Na, because it undermines the stability of ions within plant cells and leads to poor membrane function and disrupted metabolic reactions (Tester and Davenport

2003). High levels of accumulations of Na, cause the possibility of toxicity in the root, so the rate of photosynthesis is significantly reduced and that has the effect reducing productivity causing low yield (Song and Fujyiama 1996). Studies have reported that Na content in the root increases under water stress (Ahmadpour *et al*. 2016). A plant under water stress will attempt to expel sodium through the vacuole in order to avoid toxicity (Armand *et al*. 2016). Generally, it is found that Na content in roots will increase under conditions of moderate and severe water stress.

3.4. Photosynthetic parameters

Comparison of data related to the F_v/F_m ratio showed that under non-stress conditions, 20 and 30 wt% vermicompost levels significantly increased this ratio compared with control but under moderate and severe water conditions, levels of vermicompost had no significant differences (Table 4). The F_v/F_m ratio is a suitable index for evaluating PSII in plants exposed to environmental stresses such as water and heat stress (Hosseinzadeh *et al*. 2016). The F_v/F_m test is designed to allow the maximum amount of the light energy to take the fluorescence pathway. It compares the dark-adapted leaf pre-photosynthetic fluorescent state, called the minimum fluorescence or F_o, to the maximum fluorescence called F_m. In the maximum fluorescence, the maximum number of reaction centers have been reduced or closed by a saturating light source (Baker 2008). In general, the greater the plant stress, the fewer open reaction centers available, and the F_v/F_m ratio is lowered. The difference between the maximum fluorescence and the minimum fluorescence is F_v, or variable fluorescence. F_v/F_m is a normalized ratio created by dividing variable fluorescence by the maximum fluorescence (Baker 2008). Lu *et al*. (2002) and Rahbarian *et al*. (2011) reported that corn and chickpeas under water stress had a decrease in F_v/F_m over the treatments under non-water stress. Under water stress conditions, D_1 protein of PSII, reaction centers of PSII, and oxygen-evolving complex are destroyed (Lu *et al*. 2002). Vermicompost features desirable properties such as a high capacity for holding water and cation exchange, increased absorption of nutrients and other beneficial physical, chemical, and biological properties, which increase the stability of the photosynthetic system of plants (Hosseinzadeh *et al*. 2016). The use of vermicompost, led to a decrease reactive oxygen species (ROS) production, increased accessibility to nutrients and the required elements for biochemical activity (Lakhdar *et al*. 2009).

Table 4 shows that vermicompost treatments under non-stress and severe stress conditions had no significant difference in leaf CO_2 concenteration but under moderate stress, 10 wt% level of vermicompost increased significantly over that for the control. Numerous studies on legumes such as chickpea, bean, and lentil have shown that stomatal closure affected by water stress decreases leaf CO_2 concenteration (Parsa and Bagheri 2008; Armand *et al*. 2016). Stomatal closure during the water stress occurs, in order to reduce water loss but due to decreased of leaf CO_2 concenteration leads to a significant decrease in photosynthesis (Flexas and Medrano 2008). The researchers reported that the use of vermicompost fertilizer lead to increased presence of microbial populations, such as mycorrhizal symbiosis, actinoiomycetes and fungal spores for plant roots (Bender Özenç 2008; Hosseinzadeh *et al*.

Table 4 Means comparison of different vermicompost ratios on photosynthetic features of chickpea under water stress

Treatment and vermicompost (V)[1)]	F_v/F_m [2)]	Leaf CO_2 concentration (ppm)	CO_2 assimilation (μmol m^{-2} s^{-1})	Transpiration rate (mg dm^{-2} h^{-1})
Non-water stress (75% field capacity)				
Control	0.793 bc	522.2 a	7.13 bc	87.60 bc
10 wt%	0.816 ab	524.9 a	8.64 a	122.10 ab
20 wt%	0.839 a	520.3 a	8.96 a	139.90 a
30 wt%	0.825 a	526.5 a	8.95 a	149.60 a
Moderate water stress (50% field capacity)				
Control	0.780 c	442.8 c	5.03 c	74.04 c
10 wt%	0.789 c	467.7 b	7.53 b	115.30 ab
20 wt%	0.788 c	452.5 bc	7.84 b	118.80 ab
30 wt%	0.789 c	445.6 c	7.56 b	120.20 ab
Severe water stress (25% field capacity)				
Control	0.706 d	413.3 d	5.16 c	72.04 c
10 wt%	0.703 d	412 d	6. 54 bc	84.97 c
20 wt%	0.709 d	416.8 d	6.17 bc	83.44 c
30 wt%	0.703 d	415.8 d	7.29 b	89.43 bc

[1)] Ratios of V to soil: control (100 wt% (weight percentage) soil); 10 wt% V+90 wt% soil; 20 wt% V+80 wt% soil; 30 wt% V+70 wt% soil.
[2)] F_v/F_m, the maximal quantum yield of PSII photochemistry.
Difference between the data of each column followed by the same letter was not statistically significant (P<0.05).

2017). Microbial populations such as mycorrhizal fungi require plant carbohydrates for metabolism and probably accumulate sugars from the roots, decreasing the sugar content of the plant. Because sugars are compatible osmolytes and regulate osmotic pressure in the roots that improves tolerance to water stress (Huerta *et al*. 2010). In an experiment was investigated, application of vermicompost fertilizer increases CO_2 production in the soil surrounding a plant and increases microbial activity by contributing organic material to the soil (Marinari *et al*. 2000). Increased production of CO_2 in the root environment plays an important role in providing CO_2 photosynthesis (Bender Özenç 2008).

Comparison of means for the interaction of vermicompost and water stress on the CO_2 assimilation indicated that under non-water stress and moderate stress, all vermicompost treatments (10, 20, and 30 wt%) significantly increased CO_2 assimilation over the control levels but under severe water conditions, 30 wt% of vermicompost significantly enhanced CO_2 assimilation compared with control treatment (Table 4). Several studies have shown that water stress lowers CO_2 assimilation (Pagter *et al*. 2005; Flexas and Medrano 2008; Armand *et al*. 2016). Vermicompost with high porosity and high water maintain plays a key role in reducing the negative effects of water stress such as closure of the stomata and leaf CO_2 concenteration (Huerta *et al*. 2010; Hosseinzadeh *et al*. 2017). Decrease closure of the stomata and increases the supply of CO_2 necessary for Rubisco enzyme leads to increase in CO_2 assimilation (Flexas and Medrano 2008). Humic acid is a structural component of vermicompost fertilizer and researchers reported that these compounds have a high absorption capacity of metals and essential elements such as Zn, K, Fe, N, Mg, and Ca (Matos and Arruda 2003). Negatively charged groups in humic compounds are responsible for the uptake of positively charged elements (Matos and Arruda 2003). Some of these elements have an important role in the activation of photosynthesis enzymes or antioxidant enzymes (Bender Özenç 2008). In general, the results of this study showed that the use of vermicompost fertilizer increased net-photosynthesis (CO_2 assimilation) by uptake absorption of nutrients and providing of available water.

Results showed that transpiration in response to 20 and 30 wt% vermicompost treatments showed a significant increase compared with control treatment under non-water conditions. In moderate stress conditions, all levels of vermicompost resulted in a significant increase in transpiration, but in severe stress conditions, there was no significant difference (Table 4). Reduction of transpiration under water stress is a mechanism for the preventation of water loss but the hurts to the transport of active and passive in phloem and xylem vessels, respectively (Bender Özenç 2008). Reducing transpiration resulting in a decrease in water absorption in the xylem vessel (Armand *et al*. 2016). With impaired leaf transpiration, the uptake of water and nutrients from the roots is stopped. There is a direct relation between active and passive transport. Therefore, translocation of phloem is impaired (Rahbarian *et al*. 2011). Several studies have reported that an efficient mechanism for adjusting the transpiration better tolerated conditions of water stress (Yordanov *et al*. 2003; Rahbarian *et al*. 2011). Such plants have the more tolerant and better growth because they are able to preserve active and passive transport (Ganjeali *et al*. 2011). Microorganisms on the vermicompost such as mycorrhizal fungi lead to increase in root water uptake (Atik 2013). Therefore, vermicompost with more water preserve and absorption in roots leads to less stomatal closure and increased transpiration.

3.5. Proline and protein contents

Results showed that under moderate stress and non-water stress, 20 and 30 wt% levels of vermicompost treatments led to a significant increase in the root proline content in comparison with the control but there was no significant difference between treatments under severe water stress conditions (Table 3). Increased proline under environmental stresses (such as water shortage, salt stress, heat stress and heavy metals stress) is indicative of a plant response to water deficit stress (Yordanov *et al*. 2003). High accumulation of proline in cells under stress conditions protects cells subjected to stress conditions and prevents toxicity from affecting plant cells (Rahbarian *et al*. 2012). Proline also affects on osmoregulation, membrane stability, detoxification, pH adjustment of the cytosol, and maintaining the structure of enzymes in a cell (Bian and Jiang 2008). Plants with more tolerance to water stress have more ability in the synthesis of proline (Bian and Jiang 2008). Atik (2013) reported that an advantage of using vermicompost in soil is that the microorganisms in vermicompost increase nitrogen availability to plants. Similar results were also reported in other research citing that using vermicompost would increase organic matter and total N content in the soil (Atiyeh *et al*. 2002; Huerta *et al*. 2010). Proline is a nitrogen-containing compound; therefore, increased N uptake by plants leads to raised proline biosynthesis, which results in increased proline content in plants (Armand *et al*. 2016).

Comparison of mean data on protein content of root demonstrated that under non-stress and moderate water conditions, the vermicompost treatments had significant effects on protein content but under severe water conditions, vermicompost treatments had no significant effect on this trait (Table 3). Water stress changed some metabolism pathways in addition to reducing plant growth

and development (Demirevska *et al*. 2008; Ahmadpour *et al*. 2016). These changes can make plants resistant to stress (Bian and Jiang 2008). During dehydration, intense and prolonged stress caused by reduced water availability increases the compatible osmolites inside the root cells, which increases osmotic potential (Bian and Jiang 2008). Expression of a series of proteins increases under abiotic stress conditions such as drought, salinity, heat, and cold. These proteins play a role in plant adjustment to stress conditions. Moreover, they have an important role in osmotic adjustment by plants under conditions of drought and salinity (Armand *et al*. 2016). The soluble proteins in roots are as follows: dehydrins, synthesis enzymes of metabolites and heat-shock proteins (Hsps) (Demirevska *et al*. 2008). Accumulation of solutes such as proline and soluble protein, leads to reduction of root cells osmotic potential therefore, plays an important role in increasing the uptake of water from the soil and maintain turger pressure (Rahbarian *et al*. 2012). The important feature of vermicompost fertilizer is an increase of some soil microbial populations such as nitrogen stabilizers, actinomycetes, fungi spores, and mycorrhizal symbiosis with the roots of plants (Hosseinzadeh *et al*. 2017). Nitrogenous heterocycles are the raw materials required for protein biosynthesis so an increase in protein content in leaves is directly related to an increase of nitrogen uptake by the plants (Atik 2013; Hosseinzadeh *et al*. 2016).

3.6. Antioxidant enzymes activity

Results of Table 5 showed that under non-stress, vermicompost levels resulted in a significant decrease in CAT enzyme compared with the control. Under moderate and severe water conditions, CAT enzyme activity showed no significant difference compared to the controls (Table 5). Comparison of data means (Table 5) for the combined effects of vermicompost fertilizer and water stress showed that severe water stress led to a significant increase in SOD activity compared to non-stress treatment but there was no significant difference in comparison between, levels of vermicompost and control under all stress treatments. Comparison of data means show that under stress treatments (non-stress, moderate and severe water stress), all levels of vermicompost had significantly decreased POX activity compared to the controls (Table 5). ROS are produced in plants under water stress conditions and at low concentrations as a secondary messenger have a role in signal transduction pathways (Eraslan *et al*. 2007) but in high concentrations will result in the destruction of the membrane, destruction of photosystems (PSI and PSII) and reduction of photosynthetic pigments, deactivation of enzymes (Rahbarian *et al*. 2011). Chloroplasts and mitochondria are two major sites for electron transfer cycles in plant cells and are always at risk of the production of ROS (Armand *et al*. 2016). ROS production is one of consequences of stomatal closure under water stress. As a result, there is a decrease in CO_2 concentration in the leaf mesophyll and accumulation of NADPH, in this state, O_2 acts as an alternative receiver of electrons and superoxide radicals are formed (Hosseinzadeh *et al*. 2017). Antioxidant enzymes play considerable roles in helping plant cells to eliminate ROS (Armand *et al*. 2016). The most important challenge to SOD enzyme activity is the difference in activity in different species of plants (Rahbarian *et al*. 2012). Researchers have declared that increased SOD

Table 5 Means comparison of different vermicompost ratios on biochemical features of chickpea under water stress[1)]

Treatment and vermicompost (V)[2)]	Proline (mg g^{-1} dry DW)	Content soluble protein (mg g^{-1} DW)	POX activity (U μg^{-1} protein)	CAT activity (U μg^{-1} protein)	SOD activity (U μg^{-1} protein)
Non-water stress (75% field capacity)					
Control	1.08 e	56.23 c	0.373 e	0.308 bc	1.03 e
10 wt%	1.28 bcde	80.96 ab	0.226 f	0.244 d	1.11 de
20 wt%	1.52 ab	81.74 ab	0.208 f	0.249 d	1.10 de
30 wt%	1.65 a	88.39 a	0.197 f	0.241 d	0.97 e
Moderate water stress (50% field capacity)					
Control	1.09 e	71.25 b	0.692 ab	0.338 abc	1.64 c
10 wt%	1.24 de	85.81 a	0.492 cde	0.281 cd	1.76 bc
20 wt%	1.43 abcd	84.69 a	0.471 cde	0.294 bcd	1.39 cd
30 wt%	1.49 abc	86.88 a	0.436 de	0.286 cd	1.62 c
Severe water stress (25% field capacity)					
Control	1.27 cde	81.05 ab	0.745 a	0.370 a	2.31 a
10 wt%	1.40 bcd	86.87 a	0.587 bc	0.348 ab	2.33 a
20 wt%	1.51 abc	89.94 a	0.535 cd	0.351 ab	2.01 ab
30 wt%	1.45 abcd	94.35 a	0.488 cde	0.352 ab	2.10 ab

[1)] DW, dry weight; POX, peroxidase enzyme; CAT, catalase enzyme; SOD, superoxide dismutase enzyme.
[2)] Ratios of V to soil: control (100 wt% (weight percentage) soil); 10 wt% V+90 wt% soil; 20 wt% V+80 wt% soil; 30 wt% V+70 wt% soil.
Difference between the data of each column followed by the same letter was not statistically significant (P<0.05).

activity under condition of water stress demonstrates a plant's capacity to remove ROS in cells (Terzi and Kadioglu 2006). SOD is a plant's first stage of defense against ROS and is one of the most important refiners of superoxide. As a result of this enzyme activity, superoxide converts to H_2O_2 and O_2. The hydrogen peroxide produced by POX is then refined (Armand *et al*. 2016). This study has demonstrated that SOD activity under conditions of severe and moderate water stress, regardless of vermicompost application, showed a significant increase compared with non-stress conditions. CAT acts as an antioxidant enzyme and plays an important role in removing and sweeping of produced H_2O_2 in peroxisomes and reduces the damaging effects of ROS (Rahbarian *et al*. 2012). The trend of variation in CAT activity under stress conditions such as drought and salinity is species dependent so that in some species an increase in tension may lead to an increase of CAT enzyme activity. However, in other species there may be a decrease (Misra and Gupta 2006; Rahbarian *et al*. 2012). POX is often considered as an antioxidant enzyme that protects cells against H_2O_2 penetration and ROS. CAT and POX activities under conditions of water stress represent formation of a large amount of H_2O_2 during water stress (Misra and Gupta 2006). Previous studies have shown that water stress increases POX and CAT enzymes activities because ROS could be eliminated in cells. The researchers also stated that under these circumstances, increased POX and CAT enzymes activities are directly related to the increase of the production of enzymes and reduction of enzyme degradation (Misra and Gupta 2006; Armand *et al*. 2016). This decrease of POX and CAT activities using vermicompost in water deficit conditions was probably due to the physical and biological structure of vermicompost. The porous structure of vermicompost has good water retention properties and is able to hold a lot of water (Hosseinzadeh *et al*. 2017). By increasing the microbial population, nitrogen stabilizers and the availability of the required mineral nutrients are decreased in conditions of water stress (Huerta *et al*. 2010).

4. Conclusion

We demonstrated that under non-stress condition, different ratios of vermicompost fertilizer (20 and 30 wt%) could improve photosynthetic and physiological parameters. Under moderate stress conditions, 30 wt% of vermicompost fertilizer significantly increased photosynthetic pigments, CO_2 assimilation rate, internal leaf CO_2 concentration, transpiration, the maximal quantum yield of PSII photochemistry (F_v/F_m), concentrations of Ca and K in root and leaf tissues, proline and soluble protein contents in root tissues. Vermicompost fertilizer treatments significantly decreased values of POX and CAT enzyme activity compared with the control, but the activity of superoxide dismutase was not significantly different. This study confirmed that water stress significantly lowered all studied traits. Nevertheless, the application of vermicompost fertilizer did improve photosynthetic and physiological response under non-stress and moderate stress conditions, but no positive effect was determined under severe water stress.

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