

Effects of Vermicompost on Some Morphological, Physiological and Biochemical Parameters of Lettuce (*Lactuca sativa* var. *crispa*) under Drought Stress

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Abstract

Vermicompost can play an effective and important role in plant growth and development and also in reducing harmful effects of various environmental stresses on plants. The vermicompost fertilizer application was evaluated for the growth, physiological and biochemical parameters of lettuce (*Lactuca sativa* var. *crispa*) plants under drought stress conditions. Tests were carried out at different levels of vermicompost (0, 2.5 and 5%) and drought stress [no stress, moderate drought, and severe drought at 100, 50 and 25% of field capacity]. In comparison to control (vermicompost at 0%), lettuce plants treated with vermicompost at 2.5 or 5% had higher shoot height, shoot fresh weight, relative water content, stomatal conductance, chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid contents under moderate and severe drought stress conditions. Malondialdehyde (MDA) content and superoxide dismutase (SOD) and catalase (CAT) activities increased while plants under drought stress conditions. Application of vermicompost caused higher SOD and CAT enzyme activities and lower MDA content under drought stress. Enhancement in antioxidant enzyme activities as a result of vermicompost destroyed reactive oxygen species. Therefore, application of vermicompost under moderate and severe drought stress decreased MDA content in lettuce plant cells. Data indicated a positive effect of the vermicompost on the growth of lettuce under drought stress conditions.

Keywords: antioxidant enzymes; drought; lettuce; pigment; vermicompost

Abbreviations: CAT: Catalase; chl_a: Chlorophyll *a*; chl_b: Chlorophyll *b*; chl_t: Chlorophyll total; DS: Drought stress; EL: Electrolyte leakage; g: Stomatal conductance; MDA: Malondialdehyde, RWC: Relative water content; SOD: Superoxide dismutase; VC: Vermicompost

Introduction

Environmental stresses (drought, salinity, high and low temperatures) adversely affect plants growth and productivity of crops. Abiotic factors affect plant evolution and water availability (Bowden *et al.*, 2010). Morphological, physiological, biochemical and molecular changes occur in plants under abiotic stress conditions (Wang *et al.*, 2001). An increase of solute concentration in environment under drought stress leads to an osmotic movement of water out of plant cells. High solute concentration inside plant cells causes low water potential and membranes disruption along with adverse effects on photosynthesis (Taheri-Asghari *et al.*, 2009). Water deficit stress was reported to decrease plant height, leaf and root area, root length and biomass (Ramegowda *et al.*, 2014). Increasing duration and severity of drought stress (DS) may decrease RWC of plants (Khan *et al.*, 2017). RWC is a good indicator of leaf water status.

Drought stress increases RWC, resulting in some changes in cellular membrane and an increase in electrolyte leakage in the cells (Fu *et al.*, 2004).

Stomatal activity, which is affected by DS, has an impact on photosynthesis and plant growth due to its effect on CO₂ absorption. In response to a drought stress, ion and water-transport systems across membranes control turgor pressure changes in guard cells and stimulate stomatal closure (Osakabe *et al.*, 2014). In addition, DS can change in photosynthetic pigment content. The chl_a and chl_b contents undergo to changes under DS (Farooq *et al.*, 2009; Mibei *et al.*, 2016). The carotenoids have shown to help plants to resist drought stress (Jaleel *et al.*, 2009). The effects of DS on antioxidative enzymes responses have been studied in a number of plant species (Ansari *et al.*, 2017). The relationship between plant antioxidative response to DS and susceptibility and tolerance to DS is still being investigated in many plant species.

Generally, DS leads to a decrease in growth of susceptible plants. A high leaf water content and shallow root system of leafy vegetables, in particularly lettuce, make these crops vulnerable to DS. DS affected lettuce plant growth and productivity. VC was reported to increase beneficial microbial populations and thus improve soil fertility, which is important for the performance of the field and horticultural crops (Kazeminasab *et al.*, 2016). VC was suggested to increase tolerance of plants to drought as an ecological alternative due its simulative effect on protein synthesis and enzyme synthesis and/or activity in various plant organs (Muscolo *et al.*, 2007; Garcia *et al.*, 2012). For this reason, the use of VC in plants with high water content, like lettuce, can contribute to sustainable agriculture at the same time as it can be grown in drought conditions. In this study, the effects of VC on morphological, physiological and biochemical characteristics of lettuce under drought stress conditions were investigated. Thus, it was tried to determine the potential usage of VC in growth of lettuce under DS conditions.

Materials and Methods

Plant materials and experimental treatments

All plants were grown in Soil, Fertilizer and Water Resources Central Research Institute's greenhouse in Ankara (Turkey) from 24 March to the end of 10 July 2017. In the greenhouse, air temperature was set at 24/20 °C (day/night) and relative humidity, 50-55%. Commercial lettuce seeds were sown into a pot filled with vermiculite and perlite [1:1 (v/v)] until they germinated (24 March). At the 3-4 leaf stage, lettuce seedlings were transplanted into 7 L volume pots (25 cm diameter by 22 cm deep) containing soil and VC at different ratios. One plant was grown per pot during this experiment. Lettuce plants were subjected to four ratios of VC and soil as follows: DS 0%, 2.5% VC, 5% VC (w/w) under different DS conditions. The chemical characteristics of the soil and VC are given in Table 1. DS conditions were as follows DS: control (non-stress; 100% of field capacity-FC); moderate DS (50% of field capacity); severe DS (25% of field capacity). Pots were irrigated based on the FC (soil moisture content). After 48 h of irrigation to the saturation point, and drainage of gravity water, each pot was weighed and considered as FC 100%. All FC levels (every day during the experiment) were separately weighed. The drought stress treatments were imposed 2 weeks after transplanting of seedlings to the pots (24 May). Plants were kept under these conditions for 46 days until harvest. After separating the shoot and root systems of each plant at harvest, they were used for morphological measurements.

The leaf samples were frozen in liquid nitrogen and stored at -80 °C until the physiological and biochemical analysis.

Morphological measurements

60 days after transplanting, shoot height, fresh weight (fr wt), and dry weight (dry wt) of shoots were assessed in plant samples. The shoot samples were dried at 70 °C in an oven for 48 h to determine the dry wt.

Physiological analysis

RWC, g, photosynthetic pigments and electrolyte leakage percent were measured 40 days after transplanting.

RWC is determined in fresh leaf samples. Leaf samples were weighed (fr wt) and immediately floated on deionized distilled water for the next 8 h, in dark. After removing the excess surface water of leaf sample, turgid weight was determined (t wt). Leaf dry weight (dry wt) was recorded after drying leaf samples at 65 °C for 48 h. RWC was calculated using the following formula (Kaya and Higgs, 2003):

$$RWC = 100 \times [(fr\ wt - dry\ wt) / (t\ wt - dry\ wt)]$$

where: fr wt = fresh weight; dry wt = dry weight; t wt = turgid weight; g, was determined by portable gas exchange measuring system leaf porometer (Model SC-1, Decagon). g, was measured in the second youngest leaf of the plants in each treatment every fifteen days. Measurements were performed between 12:00 noon and 1:00 p.m.

To determine electrolyte leakage, 0.1 g of fresh leaf samples were weighted and placed into vials containing 10 ml distilled deionized water. After closing, vials were placed in a water bath and maintained at 32 °C. After 2 hours, the initial electrical conductivity of the medium (EC1) was measured. After autoclaving at 121 °C for 20 min and cooling down to 25 °C, the electrical conductivity of the leaf samples (EC2) was re-measured. The electrolyte leakage (EL) was calculated using the following formula (Dionisio-Sese and Tobita, 1998):

$$EL = EC1 / EC2 \times 100$$

The method proposed by Lichtenthaler (1987), was used to measure chlorophyll (chl) and carotenoid contents. Fresh leaves (0.1 g) were mixed with 4 ml 80% acetone and centrifuged at 4000 rpm for 15 min. The absorbance of the supernatant was measured at 647, 664, and 470 nm using a spectrophotometer (Perkin Elmer Lambda-EZ200). The chl and carotenoid pigments contents (mg g⁻¹) were estimated using the formulas as follows:

$$chl_a = 12.21 (A_{664}) - 2.79(A_{647})$$

$$chl_b = 21.21 (A_{647}) - 5.1(A_{664})$$

$$chl_t = chl_a + chl_b$$

$$Carotenoid = (1000 A_{470} - 1.8 chl_a - 85.02 chl_b) / 198$$

Table 1. Soil and vermicompost (VC) properties

Properties	Soil	VC
Soil texture	Sand clay loam	
pH	7.75	8.1
Electrical conductivity (dS/m)	1.28	6.5
Organic matter (%)	0.54	65.5
Nitrogen (%)	0.18	2.2
Phosphate (%)	3.60	1.7
Potash (%)	0.86	1.5

Biochemical analysis

Enzyme extraction was performed by homogenizing 1 g of fresh leaf samples in 5 mL of 0.1 mol l⁻¹ potassium phosphate buffer (pH 6.8) containing 0.1 mmol l⁻¹ EDTA. After centrifugation at 12,000 rpm for 20 min at 4 °C, the supernatant was recovered and immediately used for the following enzyme assays. SOD activity was assayed according to the method by Beyer and Fridowich (1987), by monitoring the inhibition of photochemical reaction of nitro blue tetrazolium (NBT). The amount of enzyme required for the 50% inhibition of the reduction of NBT at 560 nm was defined as 1 unit of SOD activity. CAT activity was measured using the reaction mixture containing 2.6 ml of 25 mM Na-Phosphate buffer (pH 6.8), 400 µl of 10 mM H₂O₂, and 40 µl of enzyme by the method of Cakmak and Horst (1991). The decomposition of H₂O₂ was followed by the decline of absorbance at 240 nm.

Lipid peroxidation was determined by the MDA method in 200 mg fresh leaves (Lutts *et al.*, 1996). MDA content was determined by measuring absorbance of the supernatant at 532 and 600 nm [Extinction coefficient: 155 mM⁻¹ cm⁻¹] and expressed as µmol g⁻¹ fr wt.

Experiment design and statistical analysis

This research is conducted by applying completely randomized design, which consists of 2 factor and 4 replicates. Analysis of variance (ANOVA) was performed for statistical analysis of the data using MSTAT-C. Means were compared by Duncan's multiple range test (p < 0.05).

Results and Discussion*Morphological parameters*

The results showed a significant effect of VC, DS and their interaction on shoot height (p < 0.05) (Table 2). As the DS level increased, the shoot height showed a decreasing trend. However, VC applications under DS conditions increased the shoot height (Fig. 1A). Shoot height of 5% VC treated plants under moderate and severe drought stress was significantly higher than the respective controls. Studies have shown that the decrease in transpiration rate under water deficit stress significantly decreases plant height and dry matter content (Ramegowda *et al.*, 2014). Bowden *et al.* (2010) found an increase in plant height as a result of the stimulation of auxin-like substances produced during VC consumption.

The results showed a significant effect of VC, DS and DS × VC interaction on shoot fresh weight (Sfr wt) (p < 0.05) (Table 2). The fr wt of shoot decreased with the

increasing raised DS level. The minimum fr wt was determined in the lowest irrigation regime (25% FC). This may be due to the decrease in the water consumption by the plant, thus leading to the decrease the absorption of nutrient elements of fr wt. Drought stress reduces plant growth through various physiological and biochemical processes. However, VC application increased the shoot fr wt (Fig. 1B). In agreement with our findings, Baghbani-Arani *et al.* (2017) reported the improvement of the shoot fr wt of plants by VC application. Bacteria present in VC promote plant growth- due to its positive effects on the nitrogen fixation, solubilization of nutrients, and production of growth hormones (Azarmi *et al.*, 2008). It has also been reported that vermicomposting contains biological compounds such as aqueous extract, auxine and cytokinin (García Martínez *et al.*, 2002).

DS × VC interaction was not significant on shoot dry weight (Sdry wt) (p > 0.05) (Table 2). Compared to 100% FC treatment, the Sdry wt of plants decreased under DS condition. VC application increased the Sdry wt (Table 3) when the plants were irrigated with 100% FC treatment. A decrease in dry wt caused by DS is as a result of the joint effects of the osmotic stress, reduced CO₂ assimilation and a change in nutrient concentration (Junjittakarn *et al.*, 2013). It appears that the use of VC enhances absorption of water and nutrients due to its potential to encourage microbial activity (Amiri *et al.*, 2017).

Physiological parameters

Table 2 presents the response of lettuce plant to DS and the effect of VC on RWC. A considerable difference in RWC was observed between the treatments of DS and VC (p < 0.05). Water-stressed plants showed a significant decrease in RWC in comparison to other treatments. Many studies showed that water stressed plants had lower RWC than non-stressed ones (Vassileva *et al.*, 2011; Cetinkaya *et al.*, 2014; Gulen *et al.*, 2018). The highest reduction was recorded in plants without VC under severe DS (25% FC) (Fig. 1C). To determine the water status of crops under 100% FC or DS conditions, one of the potential indicators is RWC having strong correlation with plant water relation. VC fertilizer improved growth traits such as RWC in lemon balm plants (Kazeminasab *et al.*, 2016). In our study, the rise of the RWC has come about as a result of the VC. This may be due to the balance between water loss and ion uptake. Bio-fertilizers play a significant role in the process of photosynthesis and promotes plant growth by increasing RWC and nitrogen uptake (Han and Lee, 2015). Moreover, VC plays an important role in physical and

Table 2. ANOVA for morphological and some physiological parameters related to the traits of lettuce treated with vermicompost (VC) under drought stress (DS)

Source of variance	df	SH	Sfr wt	Sdry wt	RWC	g _s	EL
DS	2	**	**	**	**	**	**
VC	2	**	**	**	**	**	**
DS x VC	4	*	**	ns	**	**	ns
Error	18	2.278	39.93	2.362	9.981	131.289	9.84
CV(%)		6.62	5.22	8.86	4.49	7.54	13.09

ns: Non significant, ** and *, significant in 1% and 5% area. Shoot height (SH), shoot fresh weight (Sfr wt), shoot dry weight (Sdry wt), relative water content (RWC), stomatal conductance (g_s), electrolyte leakage (EL).

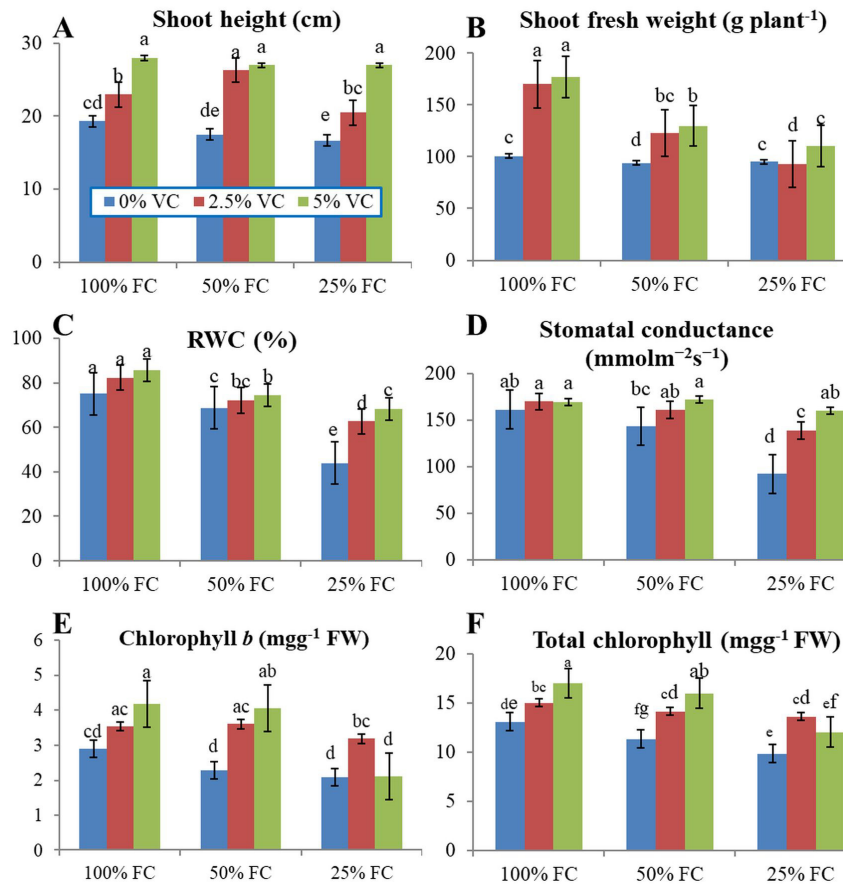


Fig. 1. Effect of drought stress (DS) and vermicompost (VC) interaction on shoot height (A), shoot fresh weight (B), relative water content (RWC) (C), stomatal conductance (D), chlorophyll *b* (E) and total chlorophyll (F) contents. Means followed by the different letter are significantly different ($p < 0.05$). DS conditions are as follows 100% field capacity (FC), 50% FC and 25% FC

hydraulic features of the soil texture, aeration, soil compaction and thus enhances more water and nutrients uptake by plants from their surrounding areas of root zone (Kashem *et al.*, 2015).

DS \times VC interaction was significant on g_s ($p < 0.05$). g_s of lettuce leaves decreased significantly under severe DS as compared to 100% FC conditions (Table 2). g_s plant treated with 5% VC at 50% FC and 25% FC of irrigation regime exhibited higher values than other treatments (Fig. 1D). Important effects of DS on plants includes the decrease in leaf water potential and turgor loss, closure of stomata and decrease in internal CO_2 concentration and CO_2 assimilation (Rahbarian *et al.*, 2011; Cetinkaya *et al.*, 2014). VC limits stomata closure and increases CO_2 supply necessary for photosynthesis due to fact that it has highly porous texture and high capacity for ventilation, proper drainage and water storage (Arancon *et al.*, 2004). Also, as a result of VC application, increase in g_s can depend on increase in leaf relative humidity content.

The results obtained indicated no significant interaction effect between DS and VC treatment on electrolyte leakage ($p > 0.05$) (Table 2). The reduced water availability under moderate and severe DS conditions caused significant increase in electrolyte leakage (Table 3). Application of VC caused a reduction in electrolyte leakage in plants subjected to DS.

DS \times VC interaction was not significant on chl_a content ($p > 0.05$) (Table 4). A reduction in water availability (from 100% FC to 25% FC treatment) led to significant decrease in leaf chl_a content (Table 3). The 5% VC treated plants had the highest leaf chl_a content, whereas the control (VC free) had the lowest leaf chl_a content. Moreover, the interaction effects of DS and VC on the chl_b and chl_t contents were significant ($p < 0.05$) (Table 4). The chl_b and chl_t contents decreased under DS. Under moderate DS (50% FC) plants treated with 2.5 and 5% VC showed a significant increase in chl_b content, compared to control treatment (Fig. 1E). However, 5% VC-treated was not sufficient to increase chl_b content under severe drought (25% FC). The chl_t content demonstrated a downward trend in response to a decrease in DS; however, VC application prevented the decrease in chl_t (Fig. 1F). Thus, the highest chl_t content was observed in the plant treated with 5% VC moderate stress (50% FC). The lowest chl_t content was observed in the control treatment under severe DS (25% FC). Data indicated a decrease in leaf RWC with increasing DS. This may be due to the destruction of chloroplast and chlorophyll loss under DS. Reactive oxygen species (ROS) cause peroxidation and therefore degradation of chl pigments (Ramírez *et al.*, 2014). Obtained results showed that leaf chl_b content decreased with increased DS, but V application increased the leaf chl_b content. The delay

in the destruction of photosynthetic pigments by VC application can be attributed to improved water efficiency. Previous reports have shown that VC application improved chl_i content in different plants such as *Melissa officinalis* L. (Kazeminasab *et al.*, 2016) under DS conditions.

DS × VC treatments interaction was significant on carotenoid content ($p < 0.05$) (Table 4). In this study, carotenoid content decreased under water deficit conditions. Under moderate and severe DS (50% and 25% FC) were at the same level in carotenoid content. ROS activity can cause the loss of pigments such as chlorophyll and carotenoid. A decrease in contents of chl_a, chl_b and carotenoids under DS in chickpea has also been reported (Baghbani-Arani *et al.*, 2017). Obtained results showed that moderate and severe DS increases substantially the carotenoid content for all VC treated over the control (Fig. 2A). VC application may have contributed to the further synthesis of carotenoids in stressed plants. The chl content is generally believed to be proportional to the amount of carotenoids that protect it (Amiri *et al.*, 2017) thus; the increase in the carotenoid content for different VC treated can be attributed to the increase in chl synthesis.

Biochemical parameters

DS, VC treatments and their interaction showed significant effects on MDA ($p < 0.05$) (Table 4). An increase in DS intensity (50 and 25% FC) led to increase leaf MDA content (Fig. 2B). Plants treated with VC under DS conditions exhibited lower values of lipid peroxidation than non-VC treated plants under DS condition. The 2.5 and 5% V applications have similar effect on lipid peroxidation under both DS conditions. The data indicate that VC treatment reduced effects of DS condition on the plants. MDA is a product of membrane lipid peroxidation and, considered a reliable marker of oxidative stress. It is

routinely used to assess the degree of stress induced by oxidative damage in plants (Nayyar and Gupta, 2006). MDA levels increased in response to increasing stress level. The application of VC in rice under DS alleviated some of the deleterious stress-related effects on plant development (Patade *et al.*, 2011; García *et al.*, 2012). Findings obtained indicated a beneficial effect of VC on lettuce plant under DS plants. This may be as a result of a reduction in the ROS and membrane injury in response to DS.

Water shortages can cause oxidative damage. Antioxidative enzymes such as SOD and CAT alleviate the adverse effects of oxidative stress on plants. DS, VC treatments and their interaction significantly affected SOD and CAT contents ($p < 0.05$) (Table 4). Fig. 2C and Fig. 2D show that increase in VC levels under DS led an increase in SOD and CAT contents of leaves, compared to control (100% FC and without VC). The highest SOD enzyme activity was determined in plants treated with 5% VC under DS (50% and 25% FC). CAT enzyme activity was highest in plants treated with 2.5 and 5% VC under DS (25% FC) SOD and CAT enzyme activities were induced when plants supplemented with VC under moderate and severe DS. Enhancement of SOD and CAT activities in VC treated plants may be induced by the drought resistance of lettuce plants. Application of VC can be used as a growth regulator which improves plant growth and enhanced stress tolerance. VC application may increase the uptake of ions such as K and Ca in the plant. Indeed, Andersen *et al.* (1992) reported that Ca acts as an activator for the enzyme and K inhibits plant damage by reducing transpiration. Higher activity of CAT and SOD decreased ROS level in cell, therefore; increased the stability of membranes and reduced lipid peroxidation. VC application increased SOD and CAT enzyme activities in the rice plants under drought stress (García *et al.*, 2014; Wang *et al.*, 2017).

Table 3. Comparison of mean effects of vermicompost (VC) and drought stress (DS)

Traits treatments	Sdry wt, g plant ⁻¹	EL, %	chl, mg g ⁻¹ fr wt
DS			
100% FC	19.05 ^a	18.96 ^b	11.50 ^a
50% FC	17.632 ^a	23.27 ^b	10.51 ^b
25% FC	15.330 ^b	29.70 ^a	9.38 ^c
VC			
0	10.68 ^c	31.98 ^a	9.00 ^b
2.5%	17.02 ^b	22.17 ^b	10.82 ^a
5%	24.32 ^a	17.94 ^b	11.57 ^a

Within each column, means followed by the same letter do not differ significantly at $p < 0.05$. Shoot dry weight (Sdry wt), chlorophyll *a* (chl_a), electrolyte leakage (EL).

Table 4. ANOVA for pigment contents, MDA, SOD and CAT enzymes related to the traits of lettuce treated with vermicompost (VC) under drought stress (DS)

Source of variance	df	chl _a	chl _b	chl _t	Carotenoid	MDA	SOD	CAT
DS	2	**	**	**	**	**	**	**
VC	2	**	**	**	**	**	**	**
DS×VC	4	ns	*	*	*	**	**	**
Error	18	1.00	0.26	0.85	0.09	0.53	124.58	194.23
CV(%)		9.58	16.42	6.81	8.13	7.70	11.25	10.22

ns: Non significant, ** and *, significant in 1% and 5% area. Chlorophyll *a* (chl_a), chlorophyll *b* (chl_b); chlorophyll total (chl_t), malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT).

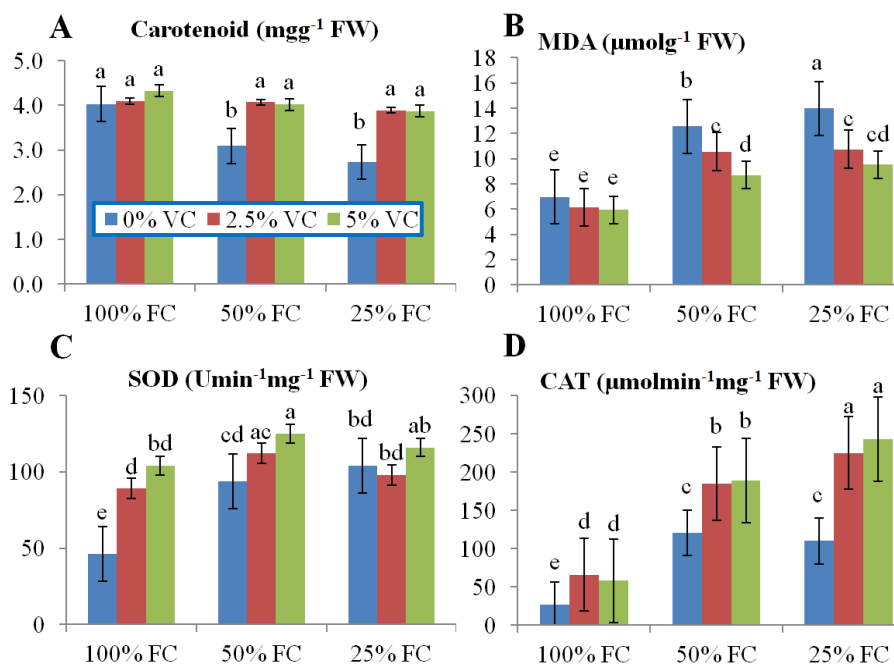


Fig. 2. Effect of drought stress (DS) and vermicompost (VC) interaction on carotenoid (A) and malondialdehyde (MDA) (B) contents, superoxide dismutase-SOD (C) and catalase CAT (D) enzyme activities. Means followed by the different letter are significantly different ($p < 0.05$). DS conditions are as follows 100% field capacity (FC), 50% FC and 25% FC

Conclusions

Lettuce is sensitive to drought, which has adverse effects on morphological and physiological characteristics. VC at different ratios can increase lettuce growth in non-stress environment. In this study, we observed VC applications alleviated the adverse effects of DS on lettuce plant growth and related physiological process. VC improved plant growth under moderate and severe DS conditions. However, at 5% VC severe drought (25% FC), shoot height, Sfr wt, RWC, g_s and SOD enzyme activity were more effective than 2.5% VC and contributed to plant tolerance to drought. The beneficial effect of VC may be due to not only providing nutrient into the soil, but also containing the plant growth regulators or microorganisms which can change the soil structure and have positive effect on plant growth.

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