

Effect of vermicompost on morphological and physiological performances of pot marigold (*Calendula officinalis* L.) under salinity conditions

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

Abstract: A greenhouse study was conducted in order to evaluate the interactions of vermicompost and salinity effects on morphology and physiology of pot marigold. The experiment was conducted with vermicompost treatments at five levels (0%, 5%, 10%, 15% and 20%) and salinity treatments at five levels (0, 50, 100, 150 and 200 mM NaCl) in a completely randomized factorial design arrangement with four replications. Results showed that increasing levels of salinity led to decline in leaf area, fresh and dry weights of flower, shoot, and root, N, P, K, Fe, Mg and Zn concentrations, chlorophyll and carotenoid contents, while proline content increased in the plants. APX, SOD, POD and CTA enzyme activities significantly increased with increasing salinity from 0 to 150 mM NaCl, then declined in 200 mM treatment in the plants. Application of vermicompost increased the morpho-physiological indices and mineral nutrient uptake in the plants and could increase the plant yield by alleviating the harmful effects of salinity.

1. Introduction

Calendula officinalis, known as “pot marigold”, is a plant in *Calendula* genus of Asteraceae family. It is perhaps native and widely naturalized further Northern to Southern Europe and elsewhere in warm temperate regions of the world, and it may possibly be planted widely in gardens and landscapes (Gharineh *et al.*, 2013). Among ornamental bedding plants, pot marigold is known to grow well under saline conditions. In fact, some pot marigold cultivars that are used as cut flowers or as bedding plants in landscaping can be grown by maintaining the quality of plants under saline conditions with an EC_w of $<8 \text{ dS m}^{-1}$ (Koksal *et al.*, 2016).

Plants are exposed to ever-changing and often unfavorable environmental conditions, which cause both biotic and abiotic stresses such as extreme temperatures, flood, drought, and salinity. Overexploitation of

available water resources as well as environmental factors such as low precipitations, high temperatures and contamination from parental rocks are leading to increases in soil salinization (Aroca *et al.*, 2013). Soil salinization is one of the most important agricultural and eco-environmental problems nowadays, which is increasing steadily in many parts of the world. Saline soils have been estimated to occupy more than 7% of the Earth's land surface and it is expected to be increased by up to 50% by the middle of the twenty-first century (Ruiz-Lozano *et al.*, 2012). Salinity stress is one of the major abiotic threats to plant life and agriculture worldwide and significantly reduces crop yield in the affected areas. Excessive salt above what plants need limits plant growth and productivity and can lead to plant death. About 20% of all irrigated land is affected by soil salinity, decreasing crop yields. Plants are affected by salinity stress in two main ways: osmotic stress and ionic toxicity. These stresses affect all major plant processes, including photosynthesis, cellular metabolism, and plant nutrition (Aslamsup *et al.*, 2011). Amelioration of salt-affected soils can be accomplished through many effective methods, such as water leaching, chemical remediation, and phytoremediation (Qadir *et al.*, 2007). The amelioration of salt-affected soils using chemical agents, including gypsum, calcite, calcium chloride and organic matter, is a successful approach that has been implemented worldwide (Sharma and Minhas, 2005; Tejada *et al.*, 2006). According to a study, the application of organic matter conditioners has become a common practice in salt-affected areas in the last several decades and constitutes an important method of soil regeneration and fertility enhancement (Melero *et al.*, 2007). Organic matter is very important for maintaining structural stability in soils as well as improving the physical, chemical and biological properties of soils. Salt-affected soils generally exhibit poor structural stability due to low organic matter. The addition of organic materials (e.g. green, farmyard and poultry manures, compost, food processing wastes, etc.) has been suggested for improving structural stability of soils by many researchers (Tejada *et al.*, 2006). Barzegar *et al.* (1997) found that addition of plant residues improved the water-stable aggregate in soils because of increased organic matter content and decreased soil salinity. The application of organic matter for soil remediation is considered essential for sustainable land use and crop productivity. Given the importance of pot marigold in green space, and their being placed in saline soils in most cultivable regions, so far, enough research has not been conducted to understand morpho-physiolog-

ical properties of pot marigold under salinity stress conditions and determine the status of vermicompost in reducing the devastating effects of salinity stress in pot marigold. For this purpose, an experiment was conducted to determine the effects of vermicompost on some morphological and physiological characteristics of pot marigold under salinity stress conditions.

2. Materials and Methods

To investigate the effects of vermicompost on some physiological characteristics of pot marigold (*Calendula officinalis* L. cv. Candyman Orange) under salinity stress, a greenhouse experiment was conducted in a completely randomized factorial design including vermicompost at five levels (0%, 5%, 10%, 15% and 20%) and salinity stress at five levels (including 0, 50, 100, 150 and 200 mM NaCl) with four replicates. This study was conducted at the Research Greenhouse of the Department of Horticultural Science, College of Agriculture, Shiraz University, Shiraz, Iran (52°32'E and 29°36'N). After disinfection of pots (20 and 30 cm in diameter and length, respectively), 5%, 10%, 15% and 20% (v/v) vermicompost from Kian Pars Shiraz Company, were dried in the shade and were mixed with four kilograms of a native soil. The main physical and chemical characteristics of the vermicompost and soil mixture are shown in Table 1. Then, four marigold seeds were planted in each pot. The pots containing seeds were kept in a greenhouse with 27/18°C (day/night) temperature, 16 h light conditions, and 35% relative humidity. After germination of seeds one plant was selected in each pot and the three other plants were removed. When the plants reached the four-leaf stage, the plants were treated with four levels of salinity stress.

Table 1 - Some physico-chemical properties of vermicompost and soil

| Sample | Vermicompost | Soil |
|------------------|--------------|------------|
| Organic matter % | 33 | 0.41 |
| pH | 8.1 | 7.05 |
| EC (d/Sm) | 1.7 | 0.6 |
| P (%) | 1.8 | |
| PWP (%) | | 5.1 |
| K (%) | 1.2 | |
| FC (%) | | 14 |
| C/N | 13 | |
| Total N (%) | 1.5 | |
| Soil texture | | Sandy-loam |

Salinity stress treatment was applied by adding net quantities of sodium chloride (NaCl) to the irrigation water so that pots were irrigated with 50, 100, 150, and 200 mM NaCl containing water based on field capacity of the soil, and the amount of the decreased water obtaining by the salinity treatment resulted in the average electrical conductivity (EC) of 3.62, 6.27, 9.36 and 12.71 dS/m in each level of the treatment, respectively. The control treatment was applied using distilled water. After 35 days of the treatments, the plants were harvested in order to measure morphological and biochemical traits.

Growth parameters

Growth parameters including, flower diameter (mm), leaf area (cm²) and fresh and dry weights of flower, shoot, and root (g) were measured. For dry weight determination, the shoots, roots, and flowers were dried in an oven at 70°C for 48 h and weighed.

Proline content

The leaf proline content was determined using the method of Bates *et al.* (1973). Proline was extracted from leaf samples of 100 mg weight fresh with 2 ml of 40% methanol. 1 ml of the extract was mixed with 1 ml of a mixture of glacial acetic acid and orthophosphoric acid (6 M) (3:2, v/v) and 25 mg ninhydrin. After 1 h of incubation at 100°C, the tubes were cooled, and 5 ml toluene was added. The absorbance of the upper phase was spectrophotometrically determined at 528 nm. The proline concentration was determined using a standard curve.

Chlorophyll and carotenoid contents

Chlorophyll and carotenoid contents were estimated by the method of Hiscox and Israelstam (1979). Fresh leaf and petal material (1 mg) and 10 ml DMSO were taken in vials and kept in an oven at 65°C for 4 h. Absorbance was read at wavelengths of 665 and 649 for leaves, and 480 nm for petals using a spectrophotometer (Beckman DU 640 B, Fullerton, USA). The following equations were used to calculate each compound.

$$\text{Chl a} = (12.47 \times A_{665}) - (3.62 \times A_{649})$$

$$\text{Chl b} = (25.06 \times A_{649}) - (6.5 \times A_{665})$$

$$\text{Total chlorophyll (mg g}^{-1} \text{ F.W.)} = \text{Chla} + \text{Chlb}$$

$$\text{Carotenoids (mg g}^{-1} \text{ F.W.)} = \frac{(1000 \times A_{480}) - (1.29 \text{Chla} - 53.78 \text{Chlb})}{220}$$

where A stands for the absorbance reading of a sample at 665, 649 and 480 nm of wavelength.

Antioxidant analysis

Fresh leaf samples were homogenized in extraction buffer (0.1 M phosphate buffer pH 6.8) with a mortar and pestle on ice. The homogenate was then

centrifuged at 12,000 g for 15 min at 4°C and the supernatant was used as the crude extract for the superoxide dismutase, guaiacol peroxidase, ascorbate peroxidase and catalase. The superoxide dismutase (SOD), guaiacol peroxidase (POD), ascorbate peroxidase (APX) and catalase (CAT) enzymes were estimated using the methods previously described by Beauchamp and Fridovich (1971), Chance and Maehly (1955), Nakano and Asada (1981), and Dhindsa *et al.* (1981), respectively.

Plant nutrient element analysis

Leaf material was ground to pass 0.5 mm sieve in a cyclone laboratory mill, weighed into ceramic crucibles, ashed overnight at 550°C in a muffle furnace, and the ash was suspended in 2M HCl for determination of mineral nutrients. Then, total nitrogen (N), phosphorus (P) and potassium (K) were determined in the leaf using the Kjeldahal, colorimetrically and ammonium acetate methods respectively., Zinc (Zn), magnesium (Mg) and Iron (Fe) were determined by atomic absorption spectroscopy (Baumard *et al.*, 1998).

Statistical analyses

The data were analyzed using one-way analysis of variance at $P < 0.05$ significance with SAS version 9.4 software (SAS Institute Inc., Cary, NC). Fisher's LSD test was conducted to determine the statistical differences among different treatments.

3. Results and Discussion

Leaf area and fresh and dry weight of shoots

The growth (leaf area and fresh and dry weight of shoots) was significantly affected by the treatments (Table 2). The treatment of plants with NaCl significantly reduced the growth parameters and the decrease was proportional to the concentration of NaCl. The highest concentration (200 mM NaCl) was the most deleterious and decreased the leaf area by 53.32%, fresh weight by 72.76%, and dry weight by 48.74% as compared to those of the control plants. However, all the above parameters were significantly enhanced by the vermicompost treatments. The highest and the lowest leaf area and fresh and dry weight of shoots were obtained in the 20% vermicompost and the control treatment, respectively (Table 2). Leaf area and fresh and dry weight of shoots increased (12.03%, 39.52% and 38.96%, respectively) at the 20% vermicompost treatment compared to the control plants. Researches showed

that soil salinity reduces the growth of plant shoots. This water potential reduction in the soil or osmotic effect is due to the presence of salt in the soil which limits the root water absorption (Nguyen *et al.*, 2015). In the salt stress, the plant's hormonal system which synthesizes and transmits a number of hormones such as cytokinins is impaired and their transport from roots to the upper parts of the plant is limited. The termination of the hormone transfer from roots to branches and the consequent reduced water absorption capacity can lead to a decline in plant growth. Reduction of leaf area can be explained by decreased cell growth or a reduced cell division rate due to decreased cellular turgor. Decrease in leaf area also reduces the rate of photosynthesis, resulting in decreased fresh and dry weight of plants (Zarei *et al.*, 2016). In addition, Alshammary *et al.* (2004) showed that decrease in growth was due to reduction of flexibility, development of cells, and reduction of auxins. Vermicompost, due to its high microbial activity resulting from the presence of fungi, bacteria,

yeasts, actinomycetes, and algae, can produce different growth regulators such as auxins, gibberellins, and cytokinins all of which may have positive effects on plant growth and development (Xu *et al.*, 2016; Ullah *et al.*, 2018). Thus, the cause of rises in height and leaf area of the plants treated with vermicompost is probably from the stimulation of production of growth regulators including auxins and gibberellins. Furthermore, vermicompost contains humic substances that increase the availability of plant N, P, K, and in particular Zn for the synthesis of tryptophan, a precursor to auxins that are used for rooting and plant growth (Sharifianpour *et al.*, 2015; Scaglia *et al.*, 2016).

Fresh and dry weight of roots

The fresh and dry weight of roots of the pot marigold plants was lower with the salinity stress treatments as compared to that of the plants under non-saline conditions (Table 3). Fresh and dry weight of roots decreased (51.91% and 77.98%, respectively)

Table 2 - Effect of salinity and vermicompost and their interaction on leaf area and shoot fresh and dry weight

| Vermicompost (%) | Salinity (mM) | | | | | Mean vermicompost |
|-------------------------------|---------------|----------------|----------------|---------------|--------------|-------------------|
| | 0 | 50 | 100 | 150 | 200 | |
| <i>Leaf area</i> | | | | | | |
| 0 | 36.10±1.33 gh | 36.10±0.62 gh | 35.12±0.62 i | 27.15±0.62 m | 15.91±0.62 q | 30.05±8.10 E |
| 5 | 37.05±0.62 f | 36.71±0.62 fg | 35.01±0.62 gh | 27.85±0.62 m | 16.61±0.62 q | 30.03±8.08 D |
| 10 | 38.39±0.62 de | 38.05±0.62 e | 37.16±0.62 f | 29.19±0.62 l | 17.95±0.62 p | 32.14±8.08 C |
| 15 | 39.28±0.62 bc | 38.94±0.62 cd | 38.05±0.62 e | 30.08±0.62 k | 18.84±0.62 o | 33.03±8.08 B |
| 20 | 40.40±1.18 a | 40.06±1.18 ab | 39.17±1.18 cd | 31.20±1.18 j | 19.96±1.18 n | 34.16±8.08 A |
| Mean salinity | 38.24±1.73 A | 37.95±1.65 A | 37.06±1.65 B | 29.09±1.65 C | 17.85±1.65 D | |
| <i>Shoot fresh weight (g)</i> | | | | | | |
| 0 | 59.92±1.18 h | 57.79±1.18 i | 54.37±1.18 k | 27.19±1.18 n | 9.79±1.18 p | 44.05±1.92 E |
| 5 | 60.62±0.62 h | 58.49±0.62 i | 55.07±0.62 kj | 39.11±0.62 m | 10.49±0.62 p | 44.75±1.92 D |
| 10 | 70.35±0.82 e | 68.22±0.82 f | 64.22±0.82 f | 48.84±0.82 l | 20.22±0.82 o | 54.49±1.92 C |
| 15 | 76.27±0.91 ab | 74.14±0.91 c | 74.14±0.91 c | 54.76±0.91 kj | 26.14±0.91 n | 60.41±1.92 B |
| 20 | 77.32±0.75 a | 75.19±0.75 bc | 75.19±0.75 bc | 55.8±0.75 j | 16.22±0.75 n | 61.46±1.92 A |
| Mean salinity | 68.89±7.67 A | 66.76±7.67 B | 63.34±7.67 C | 47.38±7.67 D | 18.76±7.67 E | |
| <i>Shoot dry weight (g)</i> | | | | | | |
| 0 | 13.18±1.18 gh | 12.84±1.18 hi | 11.38±1.18 jk | 8.92±1.18 mn | 5.8±1.18 1p | 10.42±3.01 E |
| 5 | 13.88±0.62 fg | 13.54±0.62 fgh | 12.08±0.62 ij | 9.62±0.62 lm | 6.5±0.62 1p | 11.12±2.87 D |
| 10 | 15.22±0.62 cd | 14.88±0.62 de | 13.42±0.62 gh | 10.96±0.62 k | 7.85±0.62 o | 12.46±2.87 C |
| 15 | 16.11±0.62 b | 15.77±0.62 bc | 14.31±0.62 ef | 11.85±0.62 j | 8.74±0.62 n | 13.35±2.87 B |
| 20 | 17.23±0.62 a | 16.89±0.62 a | 15.43±0.62 bcd | 12.97±0.62 h | 9.62±0.62 lm | 14.48±2.87 A |
| Mean salinity | 15.12±1.65 A | 14.78±1.65 A | 13.32±1.65 B | 10.86±1.65 C | 7.75±1.65 D | |

* In each variable, data followed by the same letters (small letters for interactions and capital letters for means) are not significantly different using LSD at 5% level. Data represent the mean value ± S.D. the mean of four replicates.

at salinity level of 200 mM NaCl compared to the control treatment. Ionic toxicity, nutritional imbalance, and osmotic osmolality adjustment are the negative effects of salinity stress on plant metabolism. Roots are the organ responsible for absorbing water and minerals, and salinity stress affects plant shoots more than the root system; however, the root system is the first organ that is exposed to salinity stress (Demiral and Türkan, 2005). One of the most effective indices in salinity tolerance is the maintenance of cellular turgor and osmotic regulation due to salt absorption and organic matter production. Plants, for production of organic materials such as glycine betaine, sorbitol, mannitol, and proline, spend a large amount of energy to regulate osmotic resistance in response to salinity stress. As a consequence, plants under salinity stress conditions reduce root efficacy in supplying nutrients and water to other organs. This reduces growth of shoots and dry matter production, and eventually reduces the transfer of nutrients from roots to shoots, and thus leads to a reduction in dry weight of roots and stems of plants (Claussen, 2005; Butt et al., 2016; Sattar et al., 2016). The vermicompost treatments of the plants grown under stress-free conditions significantly ele-

vated the amount of biomass; the total fresh and dry weight were 31.37% and 79.04% higher than those of the control plants, respectively. The vermicompost treatments also improved the amount of biomass in the plants that were subjected to the salinity stress treatments (Table 3). Edwards and Burrows (1988) reported that increase in fresh and dry weight of roots depends on increase in the activity of hormonal substances such as auxins, cytokinins, and gibberellins as well as vitamin B12.

Flower diameter and fresh and dry weight of flower

Flower diameter and fresh and dry weight of flower of the pot marigold plants were found to significantly decrease as the salt concentration was raised. These parameters decreased (33.58%, 67.94% and 27.48%, respectively) at the salinity level with 200 mM NaCl compared to the control treatment (Table 3 and 4). Several reports have shown that salinity decreases the flower diameter and fresh and dry weight of flower in (*Zinnia elegans*) (Carter and Grieve, 2010), Madagascar periwinkle (*Catharanthus roseus*) (Jaleel et al., 2008) and garden mum (*Chrysanthemum × morifolium*) (Lee and Van Iersel, 2008). The vermicompost applications significantly

Table 3 - Effect of salinity and vermicompost and their interaction on root fresh and dry weight and flower diameter

| Vermicompost (%) | Salinity (mM) | | | | | Mean vermicompost |
|------------------------------|----------------|----------------|----------------|---------------|---------------|-------------------|
| | 0 | 50 | 100 | 150 | 200 | |
| <i>Root fresh weight (g)</i> | | | | | | |
| 0 | 13.33±0.54 bcd | 13.25±0.54 bcd | 13.18±0.54 cde | 9.28±0.54 fg | 6.26±0.54 i | 11.06±2.96 D |
| 5 | 13.33±1.32 bcd | 13.46±1.32 bcd | 12.08±1.32 ij | 9.49±1.32 fg | 6.30±1.32 i | 11.24±3.18 D |
| 10 | 14.46±0.73 b | 14.38±0.73 bc | 14.31±0.73 bc | 10.41±0.73 f | 6.47±0.73 i | 12.00±3.34 C |
| 15 | 16.01±1.24 a | 15.93±1.24 a | 15.86±1.24 a | 11.96±1.24 e | 7.85±1.24 h | 13.52±3.48 B |
| 20 | 17.02±0.66 a | 16.94±0.66 a | 16.87±0.66 a | 12.97±0.66 de | 8.86±0.66 gh | 14.53±3.36 A |
| Mean salinity | 14.87±1.69 A | 14.79±1.69 A | 14.72±1.69 A | 10.82±1.69 B | 7.15±1.36 C | |
| <i>Root dry weight (g)</i> | | | | | | |
| 0 | 6.50±0.54 d-g | 6.38±0.54 efg | 6.07±0.54 fg | 2.17±0.54 jk | 0.83±0.54 l | 4.39±2.51 D |
| 5 | 6.71±1.32 e-g | 6.59±1.32 e-g | 6.28±1.32 fg | 2.38±1.32 ijk | 0.89±1.32 l | 4.57±2.73 D |
| 10 | 7.63±0.73 cd | 7.51±0.73 de | 7.20±0.73 def | 3.30±0.73 ij | 1.30±0.73 ik | 5.38±2.76 C |
| 15 | 9.18±1.24 ab | 9.06±1.24 ab | 8.75±1.24 bc | 4.85±1.24 h | 3.43±1.24 i | 6.85±3.02 B |
| 20 | 10.19±0.66 a | 10.07±0.66 a | 9.76±0.66 ab | 5.86±0.66 gh | 7.85±0.66 h | 7.86±2.87 A |
| Mean salinity | 8.04±1.69 A | 7.92±1.69 A | 7.61±1.69 A | 3.71±1.69 B | 1.77±1.28 C | |
| <i>Flower diameter (mm)</i> | | | | | | |
| 0 | 65.50±1.91 cde | 61.50±1.91 def | 59.50±1.91 fg | 52.50±1.91 i | 41.50±1.91 k | 55.50±8.20 C |
| 5 | 62.50±2.08 cde | 61.50±2.08 def | 59.50±2.08 fg | 52.50±2.08 i | 41.50±2.08 k | 55.50±8.23 C |
| 10 | 63.25±2.98 bcd | 62.25±2.98 c-f | 60.25±2.98 ef | 53.25±2.98 i | 42.25±2.98 k | 56.25±8.45 C |
| 15 | 65.00±3.55 abc | 64.00±3.55 bcd | 62.00±3.55 def | 55.00±3.55 hi | 44.00±3.55 kj | 58.00±8.62 B |
| 20 | 67.00±1.41 a | 66.00±1.41 ab | 64.00±1.41 bcd | 57.00±1.41 gh | 46.75±1.41 j | 60.15±7.85 A |
| Mean salinity | 65.05±2.85 A | 63.05±2.85 A | 61.05±2.85 B | 54.05±2.85 C | 43.20±3.03 D | |

* In each variable, data followed by the same letters (small letters for interactions and capital letters for means) are not significantly different using LSD at 5% level. Data represent the mean value ± S.D. the mean of four replicates.

increased these parameters compared to the control under both absence and presence of the salinity stress treatments. The 20% vermicompost treatment under salinity stress conditions gave the higher values for these parameters than those with the other treatments (Table 3 and 4). The extent of the increase in the values mentioned above was by 8.37%, 56.53% and 6.19 in the 20% vermicompost treatment as compared with those of the control, respectively. Hidalgo *et al.* (2006) stated that using vermicompost fertilizer increased flower diameter and fresh and dry weight of flower in marigold which is consistent with the findings of this study.

Proline content

As shown in Table 4, proline content of the pot marigold plants was markedly increased by the salinity stress treatments. The maximum and the minimum proline content were observed in the 200 mM NaCl and the control treatments, respectively. Proline content increased 447.87% at 200 mM NaCl compared to the control treatment (Table 4). Proline content increase is one of the protective mechanisms of this plant against salinity stress. It has been reported when plants are exposed to salinity stress, the

breakdown of proteins and thus the increase of amino acids and amides accelerates, one of which is proline (Iqbal *et al.*, 2015). Protein degradation, decrease in proline oxidase enzyme activity, and exacerbation of *P5CS* gene expression are the most important factors affecting proline concentration under stress conditions. Increase in *P5CS* gene expression is one of the most important factors affecting proline concentration under stress conditions (Kubala *et al.*, 2015). The proline content was clearly affected by the vermicompost treatments. As compared to the control, the increase was 1.28% at 20% vermicompost. Interaction between levels of salinity and vermicompost resulted in the highest and the lowest proline content in the plants with 200 mM NaCl and 20% vermicompost treatment and with 0 mM NaCl and 0% vermicompost treatment, respectively (Table 4). In this study, the application of vermicompost increased proline content and reduced the damaging effects of salinity stress. Vermicompost increases the amount of N available in plants due to the presence of N in the proline structure, which leads to increased proline synthesis under salinity conditions and increased plant resistance to salinity stress (Rafiee *et al.*, 2017).

Table 4 - Effect of salinity and vermicompost and their interaction on flower fresh and dry weight and proline content

| Vermicompost (%) | Salinity (mM) | | | | | Mean vermicompost |
|---|---------------|---------------|---------------|---------------|--------------|-------------------|
| | 0 | 50 | 100 | 150 | 200 | |
| <i>Flower fresh weight (g)</i> | | | | | | |
| 0 | 4.35±0.57 ef | 4.28±0.57 ef | 3.88±0.57 fg | 2.89±0.57 ij | 1.04±0.57 m | 3.29±1.37 D |
| 5 | 4.71±0.65 de | 4.64±0.65 de | 4.24±0.65 ef | 3.25±0.65 ih | 1.56±0.65 lm | 3.68±1.33 C |
| 10 | 5.07±0.65 d | 5.00±0.65 d | 4.60±0.65 de | 2.37±0.65 jk | 1.92±0.65 kl | 3.79±1.51 C |
| 15 | 6.25±0.47 bc | 6.18±0.47 bc | 5.78±0.47 c | 3.55±0.47 gh | 2.03±0.47 kl | 4.76±1.77 B |
| 20 | 6.93±0.82 a | 6.86±0.82 a | 6.46±0.82 ab | 3.27±0.82 ih | 2.22±0.82 kl | 5.15±2.16 A |
| Mean salinity | 5.46±1.14 A | 5.39±1.14 A | 4.99±1.14 B | 3.07±0.74 C | 1.75±0.65 D | |
| <i>Flower dry weight (g)</i> | | | | | | |
| 0 | 1.26±0.07 e-h | 1.25±0.07 e-h | 1.20±0.07 hi | 1.11±0.07 jkl | 0.84±0.07 p | 1.13±0.07 C |
| 5 | 1.27±0.06 e-g | 1.26±0.06 e-g | 1.21±0.06 gh | 1.12±0.06 jk | 1.05±0.06 lm | 1.16±0.07 B |
| 10 | 1.30±0.05 cde | 1.30±0.05 cde | 1.23±0.05 gh | 1.14±0.05 ji | 0.88±0.05 op | 1.18±0.07 AB |
| 15 | 1.30±0.01 cde | 1.30±0.01 cde | 1.28±0.01 e-g | 1.07±0.01 klm | 1.04±0.01 m | 1.20±0.08 A |
| 20 | 1.40±0.05 a | 1.38±0.05 ab | 1.36±0.05 bcd | 0.96±0.05 n | 0.92±0.05 no | 1.20±0.09 A |
| Mean salinity | 1.31±0.22 A | 1.29±0.13 A | 1.25±0.16 B | 1.08±0.10 C | 0.95±0.16 D | |
| <i>Proline content (µmol g⁻¹ F.W.)</i> | | | | | | |
| 0 | 4.19±0.12 k | 4.31±0.12 ij | 4.76±0.12 f | 10.32±0.12 d | 23.18±0.12 b | 9.35±7.47 D |
| 5 | 4.20±0.11 k | 4.32±0.11 hi | 4.77±0.11 f | 10.33±0.11 d | 23.19±0.11 b | 9.36±7.47 D |
| 10 | 4.22±0.10 k | 4.35±0.10 h | 4.80±0.10 f | 10.35±0.10 d | 23.22±0.10 b | 9.39±7.47 C |
| 15 | 4.28±0.08 j | 2.40±0.08 g | 4.85±0.08 e | 10.41±0.08 c | 23.27±0.08 a | 9.44±7.47 B |
| 20 | 4.30±0.07 ij | 4.42±0.07 g | 4.87±0.07 e | 10.43±0.07 c | 23.30±0.07 a | 9.47±7.47 A |
| Mean salinity | 4.24±0.10 E | 4.36±0.10 D | 4.81±0.10 C | 10.37±0.10 B | 23.23±0.10 A | |

* In each variable, data followed by the same letters (small letters for interactions and capital letters for means) are not significantly different using LSD at 5% level. Data represent the mean value ± S.D. the mean of four replicates.

Chlorophyll content

The plants treated with NaCl exhibited a significant decrease in chlorophyll content and the greatest damage was caused by 200 mM NaCl treatment. In comparison with the control, chlorophyll content decreased by 28.12% with 200 mM NaCl treatment (Table 5). Whereas, the vermicompost treatments reversed the adverse effects of NaCl and caused a significant increase in chlorophyll content in the salt-treated plants (Table 5). The highest value of chlorophyll content in leaves (18.71%) was recorded in the vermicompost-treated plants over the control plants. Usually, decreasing chlorophyll when plants face stress conditions may be due to an alternation in N metabolisms in relation to the production of compositional compounds such as proline, which are used in osmosis regulation, because an increase in proline production causes glutamate to less involve in the chlorophyll biosynthesis pathways (Jaleel *et al.*, 2008; Håkanson and Eklund, 2010). In addition, increased oxidative stress that is caused by reactive oxygen species damage to the chloroplast structure reduces the concentration of chlorophyll. Reduction of chlorophyll content has been reported in plants under salinity stress conditions due to the activity of

chlorophyllase enzyme. Furthermore, some growth regulating agents such as abscisic acids and ethylene stimulate the activity of this enzyme (Ali *et al.*, 2004; Zhao *et al.*, 2007). The application of vermicompost significantly increased chlorophyll content in the leaves in the present study. Vermicompost increases the synthesis of chlorophyll under salinity stress conditions by providing nutritious elements such as Fe, Zn, Mg, and N directly and indirectly (Nadi *et al.*, 2011; Narkhede *et al.*, 2011).

Total carotenoid content

The content of carotenoid decreased in the plants that received 50, 100, 150 and 200 mM NaCl concentrations. The content of carotenoid was the lowest (37.16% over control) for the plants that received the highest level of the salinity treatment (200 mM NaCl) (Table 5). The vermicompost treatments not only improved the production of total carotenoid under saline-free conditions, but also successfully ameliorated the adverse effects caused by the salinity stress treatments on the plants (Table 5). The content of carotenoid increased in the vermicompost-treated plants grown with the 20% level by 1.30% compared to that in the control plants. Carotenoids act as

Table 5 - Effect of salinity and vermicompost and their interaction on chlorophyll content, total carotenoid content and superoxide dismutase

| Vermicompost (%) | Salinity (mM) | | | | | Mean vermicompost |
|--|-----------------|-----------------|-----------------|----------------|---------------|-------------------|
| | 0 | 50 | 100 | 150 | 200 | |
| <i>Chlorophyll content (mg g⁻¹ F.W.)</i> | | | | | | |
| 0 | 2.09±0.11 k | 2.06±0.11 l | 1.97±0.11 m | 1.77±0.11 r | 1.46±0.11 v | 1.87±0.26 E |
| 5 | 2.17±0.11 g | 2.14±0.11 i | 2.05±0.11 l | 1.85±0.11 p | 1.54±0.11 u | 1.95±0.26 D |
| 10 | 2.24±0.11 e | 2.21±0.11 f | 2.12±0.11 j | 1.92±0.11 o | 1.60±0.11 t | 2.02±0.26 C |
| 15 | 2.27±0.11 d | 2.24±0.11 e | 2.15±0.11 h | 1.95±0.11 n | 1.64±0.11 s | 2.05±0.26 B |
| 20 | 2.44±0.11 a | 2.41±0.11 b | 2.32±0.11 c | 2.12±0.11 j | 1.81±0.11 q | 2.22±0.26 A |
| Mean salinity | 2.24±0.15 A | 2.21±0.15 B | 2.13±0.15 C | 1.92±0.15 D | 1.61±0.15 E | |
| <i>Total carotenoid content (µg·g⁻¹ F.W.)</i> | | | | | | |
| 0 | 12.48±0.92 k | 12.46±0.92 l | 12.43±0.92 o | 12.35±0.92 r | 7.82±0.92 w | 11.51±2.06 E |
| 5 | 12.50±0.92 i | 12.49±0.92 j | 12.45±0.92 m | 12.37±0.92 q | 7.84±0.92 v | 11.53±2.06 D |
| 10 | 12.52±0.92 f | 12.51±0.92 h | 12.48±0.92 k | 12.40±0.9 2p | 7.87±0.92 u | 11.55±2.06 C |
| 15 | 12.57±0.92 d | 12.55±0.92 e | 12.52±0.92 g | 12.44±0.92 n | 7.91±0.92 t | 11.60±2.06 B |
| 20 | 12.63±0.92 a | 12.61±0.92 b | 12.58±0.92 c | 12.50±0.92 i | 7.97±0.92 s | 11.66±2.06 A |
| Mean salinity | 12.54±0.82A | 12.52±0.82 B | 12.49±0.82 C | 12.41±0.82 D | 7.88±0.82 E | |
| <i>Superoxide dismutase (Ug⁻¹ F.W.)</i> | | | | | | |
| 0 | 146.00±1.92 m | 152.00±1.92 m | 156.00±1.92 kl | 440.00±1.92 d | 104.00±1.92 q | 199.60±1.25 C |
| 5 | 155.50±0.80 kl | 161.50±0.80 ijk | 165.50±0.80 ghi | 449.50±0.80 c | 113.50±0.80 p | 209.10±1.24 B |
| 10 | 15.50±0.80 jkl | 163.50±0.80 hij | 167.50±0.80 f-i | 451.50±0.80 bc | 115.50±0.80 o | 211.10±1.24 B |
| 15 | 163.50±0.80 hij | 169.50±0.80 e-h | 173.50±0.80 ef | 457.50±0.80 ab | 121.50±0.80 n | 217.10±1.24 A |
| 20 | 165.50±0.80 ghi | 171.50±0.80 efg | 175.50±0.80 e | 459.50±0.80 a | 123.50±0.80 n | 219.10±1.24 A |
| Mean salinity | 157.60±1.22 D | 163.60±1.22 C | 167.60±1.22 B | 451.60±1.22 A | 115.60±1.22 E | |

* In each variable, data followed by the same letters (small letters for interactions and capital letters for means) are not significantly different using LSD at 5% level. Data represent the mean value ± S.D. the mean of four replicates.

helper pigments in chloroplasts, but their most important role is antioxidant properties. Because of the oxidative stress caused by salinity stress in plant tissues, carotenoid activity in both antioxidant enzymatic and non-enzymatic systems decrease (Pant *et al.*, 2009). In similar studies in summer savory (*Satureja hortensis* L.) (Najafi and Khavari-Nejad, 2010), wheat (*Triticum vulgare* L.) (Reddy and Vora, 2005), and marjoram (*Origanum majorana*) (Baatour *et al.*, 2010), reduction of carotenoids under salinity conditions has been reported. In a study consistent with the findings of the present study, Ayyobi *et al.* (2014) stated that vermicompost increased carotenoid content in peppermint (*Mentha piperita* L.) leaves.

Enzyme activities

The activities of APX, POD, CAT and SOD in pot marigold plants were significantly affected by the salinity stress and vermicompost treatments. With the increasing extent of salinity stress, the activities of APX, POD, CAT and SOD increased markedly and then significantly decreased (Table 5 and 6). APX, POD, CAT and SOD enzyme activities increased (12.08%, 13.67%, 36.08% and 186.54%, respectively)

in the plants at salinity level of 150 mM NaCl compared to those in the control plants. Usually one of the biochemical changes that occur in plants under stress conditions is the accumulation of reactive oxygen species such as superoxide, hydrogen peroxide, and radical hydroxyl, all of which are highly toxic and reactive, and disrupt the normal metabolism of the cells. These radicals create secondary oxidative stress, through peroxidation of lipids, resulting in membrane degradation, protein degradation, deactivation of enzymes, elimination of pigments, and disruption of DNA, leading to serious damage to the structure of cells and eventually to the whole plant. One strategy of plants to counteract this stress is the accumulation of antioxidant enzymes (Kang *et al.*, 2014). Similar results have been reported in pot marigold (*Calendula officinalis* L.) (Hemmati *et al.*, 2018) and chickpea (*Cicer arietinum* L.) (Sadak *et al.*, 2017). The vermicompost treatments significantly enhanced the activities of APX, POD, CAT and SOD under salinity stress conditions, and the increases in the activities of APX, POD, CAT and SOD were 2.90%, 21.08%, 2.41% and 9.76% in the plants under the 20% vermicompost treatment compared with those in the control plants (Table 5 and 6). Similar results

Table 6 - Effect of salinity and vermicompost and their interaction on catalase, peroxidase and ascorbate peroxidase

| Salinity (mM) | Sanility | | | | | Mean vermicompost |
|---------------|--|---------------|---------------|---------------|---------------|-------------------|
| | 0 | 50 | 100 | 150 | 200 | |
| | <i>Catalase (Ug⁻¹ F.W.)</i> | | | | | |
| 0 | 31.65±1.58 r | 31.85±1.58 p | 32.16±1.58 m | 43.18±1.58 e | 20.78±1.58 w | 31.92±7.4 E |
| 5 | 31.80±1.58 q | 32.00±1.58 o | 32.31±1.58 k | 43.33±1.58 d | 20.93±1.58 v | 32.07±7.4 D |
| 10 | 31.85±1.58 p | 32.05±1.58 n | 32.36±1.58 j | 43.38±1.5 8 c | 20.98±1.58 u | 32.13±7.4 C |
| 15 | 32.05±1.58 n | 32.26±1.58 l | 32.56±1.58 h | 43.58±1.58 b | 21.19±1.58 t | 32.33±7.4 B |
| 20 | 32.41±1.58 i | 32.61±1.58 g | 32.92±1.58 f | 43.94±1.58 a | 21.54±1.58 s | 32.69±7.4 A |
| Mean salinity | 31.95±1.43 D | 32.16±1.43 C | 32.46±1.43 B | 43.48±1.43 A | 21.08±1.43 E | |
| | <i>Peroxidase (Ug⁻¹ F.W.)</i> | | | | | |
| 0 | 68.40±0.93 o | 69.07±0.93 no | 70.80±0.93 mn | 78.62±0.93 gh | 41.48±0.93 t | 65.68±12.99 E |
| 5 | 70.75±3.98 nm | 71.42±3.98 lm | 73.15±3.98 kl | 80.97±3.98 ef | 43.83±3.98 s | 68.03±13.43 D |
| 10 | 75.03±3.98 jk | 75.71±3.98 ij | 77.44±3.98 hi | 85.26±3.98 c | 48.12±3.98 r | 72.31±13.43 C |
| 15 | 77.59±3.98 hi | 78.27±3.98 gh | 80.00±3.98 fg | 87.82±3.98 b | 50.67±3.98 q | 74.87±13.43 B |
| 20 | 82.25±3.98 ef | 82.93±3.98 de | 84.66±3.98 cd | 92.48±3.98 a | 55.33±3.98 p | 79.53±13.43 A |
| Mean salinity | 74.80±5.96 C | 75.48±5.96 C | 77.21±5.96 B | 85.03±5.96 A | 47.89±5.96 D | |
| | <i>Ascorbate peroxidase (Ug⁻¹ F.W.)</i> | | | | | |
| 0 | 869.60±1.51 s | 875.40±1.51 q | 880.40±1.51 n | 976.10±1.51 e | 459.60±1.51 x | 812.20±1.85 E |
| 5 | 873.20±1.51 r | 878.90±1.51 p | 883.90±1.51 m | 979.60±1.51 d | 463.20±1.51 w | 815.80±1.85 D |
| 10 | 879.60±1.51 o | 885.40±1.51 l | 890.40±1.51 k | 986.10±1.51 c | 469.60±1.51 v | 822.20±1.85 C |
| 15 | 885.40±1.51 l | 891.10±1.51 j | 896.10±1.51 h | 991.80±1.51 b | 475.40±1.51 u | 827.90±1.85 B |
| 20 | 893.20±1.51 i | 898.90±1.51 g | 903.90±1.51 f | 999.60±1.51 a | 483.20±1.51 t | 835.80±1.85 A |
| Mean salinity | 880.20±1.6 D | 885.90±1.6 C | 890.90±1.6 B | 986.60±1.6 A | 470.20±1.6 E | |

* In each variable, data followed by the same letters (small letters for interactions and capital letters for means) are not significantly different using LSD at 5%level. Data represent the mean value ± S.D. the mean of four replicates.

have been reported in cowpea (*Vigna unguiculata*), rice (*Oryza sativa* L.), and tall fescue (*Festuca arundinacea* Schreb.) (Cavalcanti et al., 2004; García et al., 2014; Adamipour et al., 2016).

Nutrients in leaf tissue

N, P, K, Fe, Mg and Zn concentrations significantly declined by the increasing salinity stress level from 0 to 200 mM NaCl (Table 7 and 8). Their concentrations

Table 7 - Effect of salinity and vermicompost and their interaction on nitrogen, phosphorus and potassium

| Vermicompost (%) | Salinity | | | | | Mean vermicompost |
|------------------|-----------------------|----------------|----------------|---------------|---------------|-------------------|
| | 0 | 50 | 100 | 150 | 200 | |
| | <i>Nitrogen (%)</i> | | | | | |
| 0 | 2.01±0.04 ghi | 1.99±0.01 hi | 1.90±0.01 j | 1.01±0.01 op | 0.97±0.01 q | 1.58±0.49 E |
| 5 | 2.03±0.02 g | 2.02±0.009 gh | 1.93±0.009 j | 1.04±0.009 o | 1.00±0.009 pq | 1.60±0.49 D |
| 10 | 2.15±0.04 e | 2.10±0.01 f | 2.01±0.01 ghi | 1.12±0.01 m | 1.08±0.01 n | 1.69±0.49 C |
| 15 | 2.40±0.08 c | 2.38±0.02 c | 2.29±0.02 d | 1.40±0.02 k | 1.36±0.02 l | 1.97±0.49 B |
| 20 | 3.01±0.02 a | 2.99±0.01 a | 2.90±0.01 b | 2.01±0.01 gh | 1.97±0.01 i | 2.58±0.49 A |
| Mean salinity | 2.32±0.38 A | 2.30±0.38 B | 2.21±0.38 C | 1.32±0.38 D | 1.28±0.38 E | |
| | <i>Phosphorus (%)</i> | | | | | |
| 0 | 0.15±0.005 ijk | 0.14±0.005 lm | 0.12±0.005 no | 0.11±0.005 p | 0.08±0.005 r | 0.12±0.02 E |
| 5 | 0.15±0.006 hi | 0.15±0.006 jkl | 0.13±0.006 mn | 0.11±0.006 op | 0.09±0.006 qr | 0.13±0.02 D |
| 10 | 0.16±0.009 gh | 0.15±0.009 ij | 0.14±0.009 klm | 0.12±0.009 no | 0.10±0.009 q | 0.13±0.02 C |
| 15 | 0.19±0.006 e | 0.18±0.006 f | 0.16±0.006 g | 0.15±0.006 ij | 0.12±0.006 no | 0.16±0.02 B |
| 20 | 0.24±0.005 a | 0.23±0.005 b | 0.22±0.005 c | 0.20±0.005 d | 0.18±0.005 f | 0.22±0.02 A |
| Mean salinity | 0.18±0.03 A | 0.17±0.03 B | 0.15±0.03 C | 0.14±0.03 D | 0.11±0.03 E | |
| | <i>Potassium (%)</i> | | | | | |
| 0 | 3.93±0.14 fgh | 3.89±0.14 ghi | 3.84±0.14 hi | 2.93±0.14 mn | 2.11±0.14 p | 3.34±0.74 D |
| 5 | 4.11±0.18 d-h | 4.07±0.18 d-h | 4.02±0.18 e-h | 3.11±0.18 lm | 2.29±0.18 op | 3.52±0.75 C |
| 10 | 4.22±0.12 de | 4.18±0.12 def | 4.13±0.12 d-g | 3.22±0.12 kl | 2.40±0.12 o | 3.63±0.74 C |
| 15 | 4.63±0.22 b | 4.59±0.22 bc | 4.54±0.22 bc | 3.63±0.22 ij | 2.81±0.22 n | 4.04±0.76 B |
| 20 | 5.32±0.31 a | 5.28±0.31 a | 5.23±0.31 a | 4.32±0.31 cd | 3.50±0.31 jk | 4.73±0.78 A |
| Mean salinity | 4.44±0.54 A | 4.40±0.54 A | 4.35±0.54 A | 3.44±0.54 B | 2.62±0.54 C | |

* In each variable, data followed by the same letters (small letters for interactions and capital letters for means) are not significantly different using LSD at 5% level. Data represent the mean value ± S.D. the mean of four replicates.

Table 8 - Effect of salinity and vermicompost and their interaction on zinc, magnesium and iron

| Vermicompost (%) | Salinity | | | | | Mean vermicompost |
|------------------|-------------------|----------------|----------------|----------------|----------------|-------------------|
| | 0 | 50 | 100 | 150 | 200 | |
| | <i>Zn (mg/kg)</i> | | | | | |
| 0 | 91.47±0.47 efg | 91.42±0.47 fg | 91.34±0.47 fg | 90.35±0.47 i | 88.88±0.47 k | 90.69±1. 10E |
| 5 | 91.78±0.51 def | 91.73±0.51 def | 91.65±0.51 def | 90.66±0.51 hi | 89.19±0.51 jk | 91.00±1. 10D |
| 10 | 92.14±0.32 d | 92.09±0.32 d | 92.01±0.32 de | 91.02±0.32 gh | 89.55±0.32 j | 91.36±1. 06C |
| 15 | 93.25±0.86 c | 93.20±0.86 c | 93.12±0.86 c | 92.13±0.86 d | 90.66±0.86 hi | 92.47±1. 28B |
| 20 | 95.44±0.51 a | 95.39±0.51 a | 95.31±0.51 a | 94.32±0.51 b | 92.85±0.51 c | 94.66±1. 11A |
| Mean salinity | 92.82±1. 56 A | 92.77±1. 56 A | 92.69±1. 56 A | 91.70±1. 56 B | 90.23±1. 56 C | |
| | <i>Mg (mg/kg)</i> | | | | | |
| 0 | 1.69±0.09 efg | 1.64±0.09 e-h | 1.60±0.09 e-h | 1.46±0.09 ghi | 1.23±0.09 i | 1.52±0.18 D |
| 5 | 1.72±0.06 efg | 1.69±0.06 efg | 1.65±0.06 e-h | 1.51±0.06 f-i | 1.28±0.06 i | 1.57±0.17 CD |
| 10 | 1.82±0.12 cde | 1.79±0.12 c-f | 1.75±0.12 d-g | 1.61±0.12 e-h | 1.38±0.12 i | 1.67±0.20 C |
| 15 | 2.10±0.20 c | 2.07±0.20 c | 2.03±0.20 cd | 1.89±0.20 cde | 1.66±0.20 e-h | 1.95±0.24 B |
| 20 | 3.38±0.58 a | 3.35±0.58 a | 3.31±0.58 a | 3.17±0.58 ab | 2.94±0.58 b | 3.23±0.54 A |
| Mean salinity | 2.14±0.70 A | 2.11±0.70 A | 2.07±0.70 A | 1.93±0.70 B | 1.70±0.70 C | |
| | <i>Fe (mg/kg)</i> | | | | | |
| 0 | 122.05±1.68 ij | 121.92±1.68 ij | 120.69±1.68 j | 115.50±1.68 k | 100.27±1.68 m | 116.09±8.60 E |
| 5 | 124.19±1.96 hi | 124.06±1.96 i | 122.83±1.96 ij | 117.64±1.96 k | 102.41±1.96 m | 118.23±8.65 D |
| 10 | 128.42±2.33 g | 128.29±2.33 g | 127.06±2.33 gh | 121.87±2.33 ij | 106.64±2.33 l | 122.45±8.72 C |
| 15 | 154.19±2.69 d | 154.06±2.69 d | 152.83±2.69 d | 147.64±2.69 e | 132.41±2.69 f | 148.23±8.80 B |
| 20 | 196.53±4.15 a | 196.40±4.15 a | 195.17±4.15 a | 189.98±4.15 b | 174.75±4.15 c | 190.56±9.24 A |
| Mean salinity | 145.07±29.02 A | 144.94±29.02 B | 143.71±29.02 B | 138.52±29.02 C | 123.29±29.02 D | |

* In each variable, data followed by the same letters (small letters for interactions and capital letters for means) are not significantly different using LSD at 5% level. Data represent the mean value ± S.D. the mean of four replicates.

decreased (44.82%, 36.06%, 40.99%, 15.01%, 20.56% and 2.79%, respectively) in the plants at 200 mM NaCl compared to those in the control plants. N, P, K, Fe, Mg and Zn concentrations in the leaf tissues of pot marigold increased significantly with increasing vermicompost composition in the media compared to those in the control plants. The highest and the lowest concentrations of the elements were observed in the 20% and the 0% vermicompost treatments, respectively (Table 7 and 8). Furthermore, N, P, K, Fe, Mg and Zn concentrations in leaves increased 46.20%, 80.32%, 4.61%, 64.14%, 112.50%, and 4.37% in the plants with the 20% vermicompost treatment compared to those in plants with the control treatment, respectively. Interaction between the levels of salinity and vermicompost resulted in the highest and the lowest nutrient concentrations in the plants with the 0 mM NaCl and 20% vermicompost and with the 200 mM NaCl and 0% vermicompost treatments, respectively (Table 7 and 8). Plant growth is reduced in salt-affected soil because of the excess uptake of potentially toxic ions. Soil salinity is characterized by high amounts of Na, Mg, Ca, Cl, HCO₃⁻ and B ions in soil which have negative effects on plant growth. Eventually, high salt concentrations in soil reduce the absorption of nutrients of plants which negatively affects the fertility of the soil (Zhao and Ren, 2007). In this study, the increase of vermicompost levels increased the mineral nutrition. Vermicompost contains humic substances that have multiple effects in the soil. It may improve soil properties such as micronutrient transport and availability (Aşık *et al.*, 2009). Chen and Aviad (1990) summa-

ri- zed the effects of humic substances on plant growth and mineral nutrition, pointing out the positive effects on the uptake of macro- (such as N, P, and K) and micro-elements. Vermicompost may enhance the uptake of nutrients and reduce the uptake of some toxic elements of plants under salinity stress conditions. Chamani *et al.* (2008) reported that addition of vermicompost to soils resulted in increased mineral contents in the substrate and higher concentrations P, Ca, Mg, Cu, Mn and Zn in shoot tissues of red clover and cucumber.

Correlation analysis

The results of the Pearson correlation analysis between vermicompost abundance and the measured morphological traits and elements showed a positive and significant correlation. Therefore, a positive relation between the increases of the amount of vermicompost and improvement in the morphological traits and measured elements can be inferred. According to the results, shoot fresh weight compared to shoot dry weight (r=0.89**) and flower diameter (r=0.57**) showed the highest and the lowest correlations among the morphological traits, respectively (Table 9). In the measured elements under the vermicompost treatments, the highest correlation was observed between shoot fresh weight and K content (r=0.81**) while the lowest correlation was seen in Mg content (r=0.67**) (Table 9). This improvement can be attributed to the presence of macro-micro elements, the release of elements in the soil and soil amendment of physical and biological conditions which have been previously reported in other plants

Table 9 - Correlation coefficients for evaluated traits on pot marigold in vermicompost treatment

| Traits | Shoot FW | Shoot DW | Leaf area | Root FW | Root DW | Flower diameter | Fruit FW | Fruit DW | Zn | Fe | Mg | Ca | K | P | N |
|-----------------|----------|----------|-----------|---------|---------|-----------------|----------|----------|---------|---------|---------|---------|---------|---------|---|
| Shoot FW | 1 | | | | | | | | | | | | | | |
| Shoot DW | 0.891** | 1 | | | | | | | | | | | | | |
| Leaf area | 0.886** | 0.992** | 1 | | | | | | | | | | | | |
| Root FW | 0.798** | 0.799** | 0.777** | 1 | | | | | | | | | | | |
| Root DW | 0.798** | 0.799** | 0.777** | 1.00** | 1 | | | | | | | | | | |
| Flower diameter | 0.574** | 0.603** | 0.586** | 0.519* | 0.519* | 1 | | | | | | | | | |
| Fruit FW | 0.811** | 0.795** | 0.801** | 0.618** | 0.618** | 0.334 NS | 1 | | | | | | | | |
| Fruit DW | 0.618** | 0.728** | 0.695** | 0.734** | 0.734** | 0.536* | 0.417 NS | 1 | | | | | | | |
| Zn | 0.789** | 0.883** | 0.875** | 0.864** | 0.864** | 0.623** | 0.778** | 0.722** | 1 | | | | | | |
| Fe | 0.809** | 0.836** | 0.822** | 0.825** | 0.825** | 0.624** | 0.818** | 0.686** | 0.951** | 1 | | | | | |
| Mg | 0.679** | 0.797** | 0.781** | 0.764** | 0.764** | 0.538* | 0.733** | 0.749** | 0.927** | 0.907** | 1 | | | | |
| Ca | 0.789** | 0.883** | 0.875** | 0.864** | 0.864** | 0.623** | 0.778** | 0.722** | 1.00** | 0.951** | 0.927** | 1 | | | |
| K | 0.818** | 0.851** | 0.842** | 0.799** | 0.799** | 0.561** | 0.778** | 0.765** | 0.911** | 0.929** | 0.884** | 0.911** | 1 | | |
| P | 0.815** | 0.847** | 0.835** | 0.815** | 0.815** | 0.554* | 0.831** | 0.698** | 0.937** | 0.981** | 0.921** | 0.937** | 0.938** | 1 | |
| N | 0.805** | 0.834** | 0.814** | 0.831** | 0.831** | 0.604** | 0.810** | 0.704** | 0.956** | 0.989** | 0.933** | 0.956** | 0.916** | 0.981** | 1 |

FW = fresh weight; DW= dry weight.
NS= Not significant, * and **: Significant at 5% and 1% respectively.

(Bachman and Metzger, 2008). In this correlation analysis, peroxidase activity ($r=0.79^{**}$) and proline content ($r=0.44^{**}$) had the highest and the lowest correlations with shoot fresh weight. Further, insignificant correlations were observed in carotenoid content and catalase enzyme activity (Table 10). It seemed the positive correlations in activities of antioxidant enzymes, chlorophyll and proline content in the plant was due to increased concentrations of the elements which could be provided by vermicompost. Because in the structure of the mentioned traits, there are a variety of macro- and micro- elements that vermicompost provides directly and indirectly to plants (Hidalgo *et al.*, 2006). The results of a simple correlation analysis between the morphological traits and the studied elements under salt stress conditions showed that shoot fresh weight had a positive and

significant correlation with all the morphological indices and concentration of elements. In this analysis, the highest and the lowest correlations were seen in leaf area ($r=0.99^{**}$) and fresh flower weight ($r=0.91^{**}$), respectively (Table 11). Also, the highest correlation of shoot fresh weight was observed in Fe content ($r=0.98^{**}$) and the lowest correlation was found in N content ($r=0.88^{**}$) (Table 11). There are many reports of positive correlations between plant yield and concentration of elements under salinity stress conditions. Increase in soil pH, reduction in the amount of absorbent elements in the soil, increase in toxic elements of soil such as Cl and Na, and osmotic stress in the soil that prevents water absorption are the most critical factors for this phenomenon in plants (Edwards *et al.*, 2010). Analysis of correlations of shoot fresh weight with chlorophyll and proline con-

Table 10 - Correlation coefficients for evaluated traits on pot marigold under salinity stress conditions (above diameter) and vermicompost treatment (below diameter)

| Traits | Shoot fresh weight | Chlorophyll | Proline | Carotenoid | Ascorbate peroxidase | Peroxidase | Catalase | Superoxide dismutase |
|----------------------|--------------------|-------------|-----------|------------|----------------------|------------|----------|----------------------|
| Shoot fresh weight | 1 | 0.913 ** | -0.904 ** | -0.874 ** | 0.872 ** | 0.990 ** | 0.905 ** | 0.861** |
| Chlorophyll | 0.664 ** | 1 | -0.992 ** | 0.857 ** | -0.856 ** | 0.888 ** | 0.888 ** | 0.626** |
| Proline | 0.444 ** | 0.176 NS | 1 | 0.854 ** | 0.680 ** | -0.816 ** | 0.714 ** | 0.571** |
| Carotenoid | 0.059 NS | 0.488 * | 0.454 * | 1 | 0.801 ** | 0.651 ** | -0.544 * | -0.346 NS |
| Ascorbate peroxidase | 0.490* | 0.064 NS | 0.038 NS | -0.435 NS | 1 | 0.760 ** | 0.407 NS | -0.085 NS |
| Peroxidase | 0.794 ** | 0.480 * | 0.540 * | 0.093 NS | 0.746 ** | 1 | 0.458* | -0.020 NS |
| Catalase | 0.131 NS | 0.444 * | -0.341 NS | 0.252 NS | 0.430 NS | 0.317 NS | 1 | -0.006 NS |
| Superoxide dismutase | 0.514 * | 0.793 ** | -0.210 NS | 0.131 NS | -0.002 NS | 0.241 NS | 0.355 NS | 1 |

NS= Not significant, * and **: Significant at 5% and 1% respectively.

Table 11 - Correlation coefficients for evaluated traits on pot marigold in salinity stress conditions

| Traits | Shoot FW | Shoot DW | Leaf area | Root FW | Root DW | Flower diameter | Fruit FW | Fruit DW | Zn | Fe | Mg | Ca | K | P | N |
|-----------------|----------|----------|-----------|---------|---------|-----------------|----------|----------|---------|---------|---------|---------|---------|---------|---|
| Shoot FW | 1 | | | | | | | | | | | | | | |
| Shoot dw | 0.939** | 1 | | | | | | | | | | | | | |
| Leaf area | 0.995** | 0.956** | 1 | | | | | | | | | | | | |
| Root fw | 0.966** | 0.886** | 0.961** | 1 | | | | | | | | | | | |
| Root dw | 0.923** | 0.868** | 0.921** | 0.986** | 1 | | | | | | | | | | |
| Flower diameter | 0.986** | 0.977** | 0.991* | 0.952** | 0.919** | 1 | | | | | | | | | |
| Fruit fw | 0.918** | 0.830** | 0.906** | 0.844** | 0.798** | 0.865** | 1 | | | | | | | | |
| Fruit dw | 0.942** | 0.947** | 0.948** | 0.901** | 0.855** | 0.972** | 0.779** | 1 | | | | | | | |
| Zn | 0.938** | 0.947** | 0.956** | 0.850** | 0.802** | 0.941** | 0.923** | 0.892** | 1 | | | | | | |
| Fe | 0.985** | 0.940** | 0.987** | 0.914** | 0.851** | 0.977** | 0.909** | 0.948** | 0.963** | 1 | | | | | |
| Mg | 0.895** | 0.910** | 0.803** | 0.762** | 0.897** | 0.919** | 0.856** | 0.969** | 0.912** | 0.857** | 1 | | | | |
| Ca | 0.938** | 0.947** | 0.956** | 0.850* | 0.802** | 0.941** | 0.923** | 0.892** | 1.00** | 0.963** | 0.969** | 1 | | | |
| K | 0.981** | 0.961** | 0.991* | 0.954** | 0.928** | 0.982** | 0.915** | 0.927** | 0.966** | 0.967** | 0.932** | 0.966** | 1 | | |
| P | 0.936** | 0.877** | 0.911** | 0.933** | 0.926** | 0.919** | 0.843** | 0.858** | 0.804** | 0.879** | 0.756** | 0.804** | 0.894** | 1 | |
| N | 0.885** | 0.870** | 0.892** | 0.941** | 0.976** | 0.886** | 0.809** | 0.796** | 0.831** | 0.811** | 0.781** | 0.923** | 0.817** | 0.891** | 1 |

FW = fresh weight; DW= dry weight.

NS= Not significant, * and **: Significant at 5% and 1% respectively.

tent, and antioxidant enzyme activity showed significant positive and negative correlations. From the viewpoint of biochemistry, most of significant correlations were found in content of chlorophyll ($r=0.91^{**}$), proline ($r= -0.90^{**}$), carotenoid ($r= -0.87^{**}$), in activity of ascorbate peroxidase ($r=0.87^{**}$), peroxidase ($r=0.99^{**}$), catalase ($r=0.90^{**}$), and superoxide dismutase ($r=0.86^{**}$) compared to the shoot fresh weight (Table 10). There were also few negative correlations in which proline and carotenoid content can be attributed to their function in dealing with osmotic stress in the plants due to increased salinity in the soil. In similar studies, positive correlation of antioxidant enzyme activity under salinity stress conditions in German chamomile (*Matricaria recutita* L.), sunflowers (*Helianthus annuus* L.) and basil (*Ocimum basilicum* L.) have been reported (Baghalian *et al.*, 2008; Heidari *et al.*, 2011; Heidari, 2012).

4. Conclusions

Salinization of soil is a serious land degradation problem and is increasing steadily in many parts of the world, particularly in arid and semiarid areas. Soil salinity affects the establishment, growth, and development of plants leading to huge losses in productivity. Vermicompost is one of the major organic fertilizer which can improve growth and salinity tolerance with containing plenty nutrition elements, hormones, and organic materials. In this study, increase in salinity stress significantly led to decline in morphological and physiological indices of pot marigold. Application of vermicompost under salinity conditions increased morpho-physiological indices in this plant. Vermicompost increased the activity of the antioxidant system, the content of proline and chlorophyll in the plant by increasing the nutrients in the soil environment that could increase the plant yield and alleviating the harmful effects of salinity.

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