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Scientific Article

Morphological and physiological responses of *Calliandra haematocephala* to water salinity stress and vermicompost

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Abstract

Salinity is one of abiotic stress that affects plant growth and production. The objective of this study was to investigate the effect of salinity and vermicompost on morphology and physiology parameters of *Calliandra haematocephala*. The experiment was carried out with salinity treatments at 1000, 2000 3000 ppm in addition to the control (tap water, 280 ppm), vermicompost treatments at 5%, 10%, 15%, and 20% in addition to the control. The results indicated that increasing level of salinity resulted in reduction in vegetative growth and flowering parameters (plant height, number of branches/plant, stem diameter, root length, fresh and dry weights of leaves and roots/plant, number of flowers /plant, as well as fresh and dry weights of flowers /plant), total chlorophylls, total carbohydrates, K^+%, Ca^{2+}%, and K^+/Na^+ ratio, while increased proline, Na^+ and Cl^-% in the plants. Catalase (CAT), superoxide dismutase (SOD), Ascorbate peroxidase (APX) enzyme activities significantly increased with elevating salinity level from 0 to 3000 ppm. Application of vermicompost with any concentration had a positive effect on vegetative growth and flowering parameters, total chlorophylls, total carbohydrates, nutrient uptake, K^+/Na^+ ratio, proline content**,** enzyme activities and reducing accumulation of Na^+ and Cl^-% toxic ions in leaves. Based on the results, application of vermicompost at 20% recommended for alleviating the harmful efects of salinity on *Calliandra haematocephala* plants irrigated with saline water at concentration up to 3000 ppm.
Key words: antioxidative enzymes, abiotic stress, salt concentrations.

Resumo

Respostas morfológicas e fisiológicas de *Calliandra haematocephala* ao estresse de salinidade da água e vermicomposto

A salinidade é um estresse abiótico que afeta o crescimento e a produção das plantas. O objetivo deste estudo foi investigar o efeito do vermicomposto e dos efeitos da salinidade sobre os parâmetros morfológicos e fisiológicos de *Calliandra haematocephala*. O experimento foi realizado com tratamentos de vermicompostagem a 5%, 10%, 15% e 20% além do controle, tratamentos de salinidade a 1000, 2000 3000 ppm além do controle (água da torneira, 280 ppm). Os resultados indicaram que o aumento do nível de salinidade resultou em redução no crescimento vegetativo e nos parâmetros de floração (altura da planta, número de ramos/planta, diâmetro do caule, comprimento da raiz, massa fresca e seca das folhas, raízes/planta, número de flores/planta, bem como pesos frescos e secos de flores/planta), clorofilas totais, carboidratos totais, K^+%, Ca2$^+$% % e relação K^+/Na^+, enquanto aumentou prolina, Na^+ e Cl^-% nas plantas. As atividades das enzimas catalase (CAT), superóxido dismutase (SOD), ascorbato peroxidase (APX) aumentaram significativamente com a elevação do nível de salinidade de 0 a 3000 ppm. A aplicação de vermicomposto em qualquer concentração teve efeito positivo sobre o crescimento vegetativo e parâmetros de floração, clorofilas totais, carboidratos totais, absorção de nutrientes, relação K^+/Na^+, teor de prolina, atividades enzimáticas e redução do acúmulo de íons tóxicos Na^+ e Cl^-% nas folhas. Com base nos resultados, a aplicação de vermicomposto a 20% é recomendada para aliviar os efeitos nocivos da salinidade em plantas de *Calliandra haematocephala* irrigadas com água salina em concentração de até 3000 ppm.
Palavras-chave: enzimas antioxidantes, salinidade, stress abiótico.

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Introduction

Calliandra haematocephala Hassk., is an evergreen shrub or small trees belonging to the Family Leguminosae (Fabaceaae), commonly known as powderpuff. It is native to tropical America, Bolivia. It grows to a height of 3.5 to 4.5 m tall and 3 to 4.5 m spread. The leaves are 2 to 4 inches long, oblong, entire, pinnate, parallel, bowed with leaflets numerous. The flowers are showy, pink, and red in color, flowers during the period from autumn to spring. The fruits are brown dry or hard pods 3 to 6 inches in length (Fox et al., 2005). Moreover, the use of the plant for landscape activates as an ornamental shrubs, leaves and barks are used in folk medicine as anti-tumor, antioxidant, anti-cholinergic, insecticide, anti-malarial, and astringent (El-Ghaly, 2014).

Plants are subjected to constantly changing and often injurious environmental conditions, which resulted in biotic and abiotic stresses such as temperatures, drought, salinity, etc. Salinity is one of the major abiotic stresses decrease growth and production of plants causing significant problem in landscape activities. The adverse effect of salinity stress on plant growth is attributed to its effect on osmotic effect, toxicity of ions, nutritional imbalance and reactive oxygen species (ROS) production which affect the necessary plant processes, including photosynthesis, cellular metabolism, and plant nutrition (Ruiz-Lau et al., 2020). Under salt stress, excessive energy headed for molecular oxygen stimulates the oxygen poisoning through over-production of singlet oxygen, superoxide ion, hydrogen peroxide and different free oxygen radicals. Plant uses an efficient antioxidant system to overcome slat induced oxidative stress, this involves antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) (Azeem et al., 2023). Previous studies on some ornamental plants exposed to salinity stress showed reduction in vegetative growth or flowering parameters, total chlorophylls, total carbohydrates, K^+, Ca^{2+} % and K^+/Na^+ ratio, increase in contents of proline, Na^+, Cl^- % in leaves (Abdel-Mola and Ayyat, 2020; Ashour et al., 2021; Azeem et al., 2023), increase in antioxidant enzymes catalase, superoxide dismutase and peroxidase (Adamipour et al., 2019; Azeem et al., 2023).

Vermicompost is the non-thermophilic biodegradation of organic wastes such as vegetables, animals, industrial, and urban via the interaction of earthworms and microorganisms. It is an organic product has high microbial, enzymatic activity and contains useful elements, vitamins, humic substances and plant growth regulators such as auxins, gibberellins cytokinins. In recent years the use of vermicompost as an organic fertilizer has increased as it is a friendly way to fertilize plants moreover; it has also ability to tolerate salinity (Ruiz-Lau et al., 2020).

Recently, the positive effects of vermicompost on salt resistance is related to improve plant growth, promote of Rubisco content, enhance regulation of mineral nutrition maybe related to Na^+ absorption for sustaining K^+/Na^+ homeostasis, safety of the integrity of thylakoid membranes, and improving transcription and translation of proteins. Vermicompost normalizes the regulation and function of photosynthetic machinery, which may assist to protect thylakoid integrity, photosynthesis, ROS scavenging, metabolism, and stress-responsive and molecular adaptation under salt stress (Tammam et al., 2023). In this regard, previous studies have been developed in order to evaluate vermicompost effect on some species subjected to salinity including *Origanum majorana* (Mohammed et al., 2018), *Cotinus coggygria* (Moghimi et al., 2019) and *Sedum album* (Cicek et al., 2022) and the findings showed the useful role of vermicompost on alleviating the adverse effect of salinity and attributed positively responses to reduction in accumulation of Na^+ and Cl^- toxic ions in plant organs.

Calliandra haematocephala is one of the common shrubs used in landscape activates of coastal resorts in Egypt where relatively saline water is used. However, so far, enough research has not been performed to understand morpho-physiological characteristics of powderpuff under salinity stress and/or evaluate the effect of vermicompost in decreasing the harmful effects of salinity stress on the plants. Therefore, this work aimed to investigate the response of the pants irrigated with different levels of saline water to vermicompost treatments.

Material and Methods

To investigate response of *Calliandra haematocephala* irrigated with various levels of saline water to vermicompost treatments, this study was conducted at the experimental nursery of the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, Giza, Egypt during the two successive seasons of 2020 and 2021.

Plant material

On 15^{th} March, in both seasons, uniform seedlings of *Calliandra haematocephala* (one-year old), having 2 branches and 20-25cm height were obtained from a private nursery and repotted (one seedling/pot) in plastic pots (30 cm in length and 30 cm inner diameter) filled with the mixture of clay + sand (2:1: v/v). The physical and chemical properties of soil mixture used in the study were performed at Soil Water and Environment Research Institute, Agriculture Research Centre (A.R.C) according to (Jackson, 1973) as shown in Table 1.

Experimental procedures

Vermicompost treatments

Vermicompost prepared from rice straw and tree leaves with the earthworm (*Eisenia foetida* L.) was obtained from the Agricultural Research Center, Giza, Egypt and intermingled thoroughly with the prepared soil mixture at concentrations of 5, 10, 15 and 20% (w w^{-1}), in addition to the control. Some physical and chemical properties of vermicompost are shown in Table 1.

Table 1. Some physical and chemical properties of experimental soil and vermicompost (mean of two seasons).

Parameter	Soil mixture	Vermicompost
Organic matter (%)	1.36	35.5
EC(dS/m)	1.25	0.84
pH	7.35	6.56
Field capacity (% V)	57.33	--
N %	74.59	6.55
P %	21.29	1.13
K %	69.64	4.39
Soil texture	Clay loam	--

EC: Electrical conductivity, pH: soil acidity, N: Nitrogen; P: phosphorous; K: potassium

Salinity treatments

On 15th April the plants were irrigated twice/week with saline water at concentration of 1000, 2000 and 3000 ppm, in addition to the control (tap water, 280 ppm). The concentrations of saline water were prepared (completely in one day) by mixing salts of NaCl and $CaCl_2$ at the ratio of 1:1 (w w^{-1}) and applied at one liter/ pot.

All the plants were fertilized monthly with kristalon (NPK 19:19:19) at a rate of 2.5 g pot^{-1}, hand picking of weeds, resistance of diseases, pests were also performed.

Experimental layout

The layout of the experiment was factorial in randomized complete blocks design with 20 treatments [4 salt concentrations (including the control) × 5 vermicompost (including the control)] each treatment consisting of 9 pots arranged in 3 replicates, each replicate containing 60 pots (3 pots from each treatment).

The data recorded

On 15th December, in two seasons (after 8 months), the experiment was terminated and morphological, physiological characteristics were registered.

Morphological parameter

Vegetative growth parameters including plant height (cm), number of branches $plant^{-1}$, stem diameter (mm, at 5 cm above soil surface), root length (cm), fresh and dry weights of leaves, and roots $plant^{-1}$, additionally flowering parameters including number of flowers $plant^{-1}$, as well as fresh and dry weights of flowers (g $plant^{-1}$) were also determined. Dry weight $plant^{-1}$ was estimated by drying at 70 °C until constant weight.

Chemical constituents

Total chlorophylls were determined in fresh leaf samples by using chlorophyll meter Model SPAD 502 (Netto et al., 2005). The total carbohydrates concentration (% of dry matter) was estimated in dried leaves samples as described by Dubois et al. (1956). The proline content in fresh leaves (µ moles/g fresh matter of leaves) was determined using the method of Bates et al., (1973). Mineral constituents: 100 mg of dried leaves samples were digested and the content of K^+, Ca^{2+} and Na^+ were determined according to Karla (1998). Chloride content was determined using the method described by Gavlak et al. (1994). Preparation of enzyme extract and measurement of total protein: Fresh leaves (0.05 g) were weighed and completely powdered with liquid nitrogen to make the enzyme extract. Then 1.0 mL of the phosphate-buffered saline (PBS) (50 mM, pH = 7.8) and ethylene diamine tetra acetic acid (EDTA) with a concentration of 0.1 mM were added, and the mixture was thoroughly homogenized. The product was centrifuged at 12,000 g and 4 °C for 15 min. The supernatant was separated and stored in a freezer at − 80 °C to measure protein and enzyme activity. To determine the amount of protein in the extract, 2.5 mL of Bradford solution was added to 50 µL of the separated supernatant, and the absorbance was read at 595 nm after 5 min to determine the amount of protein in the extract. Specific concentrations of standard bovine serum albumin (BSA) were prepared in the extraction buffer to create a protein calibration curve (Bradford, 1976). Antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), Ascorbate peroxidase (APX) were assayed as described by Haida and Hakiman (2019). Enzymes activities were expressed as units/min/mg protein.

Statistical analysis

The obtained results were subjected to one-ways analysis of variance (ANOVA) in randomized complete blocks design. Combined analysis of the two growing seasons was carried out and the means were compared using Duncan's multiple range test or "Least Significant Difference (LSD)" test at the 0.05 level (Steel and Torrie, 1997).

Results and Discussion

Growth and flowering parameters

Effect of salinity stress

The data in Figure 1, Figure 2 exhibited that, salinity stress had a deleterious effect on the various growth and flowering traits of *Calliandra haematocephala* plants. In most cases, raising salinity levels from 1000 to 2000 or 3000 ppm lead to significant decrease in all of tested growth and flowering attributes compared to the control plants.

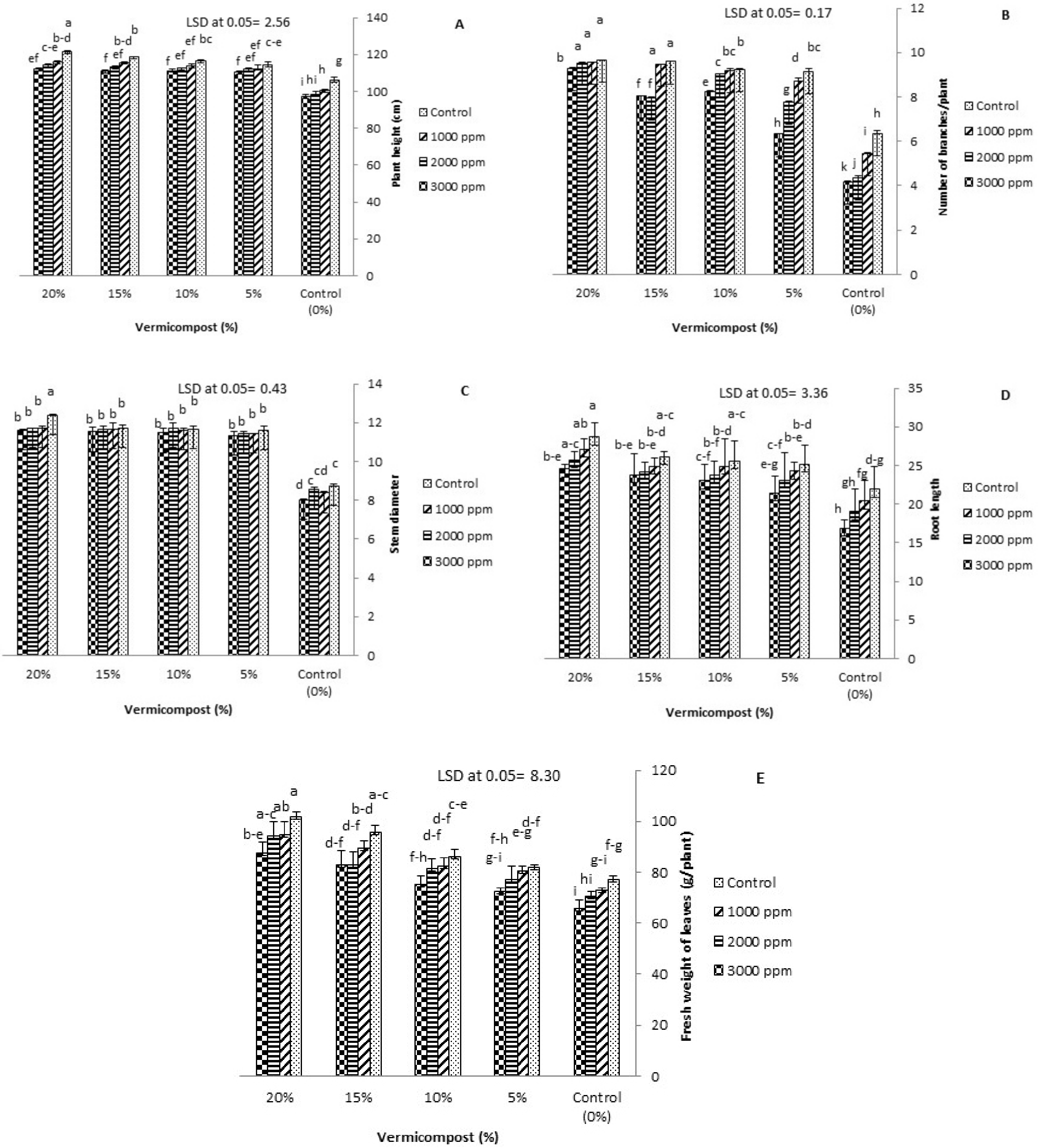

Figure 1. Plant height (a), number of branches/plant (b), stem diameter (c), root length (d) and fresh weight of leaves(e) as affected by the interaction between water salinity and vermicompost treatments (mean of two seasons). Column with different letters indicate a significant difference at 5% level. Vertical bars indicate to standard error (SE) of three replicates

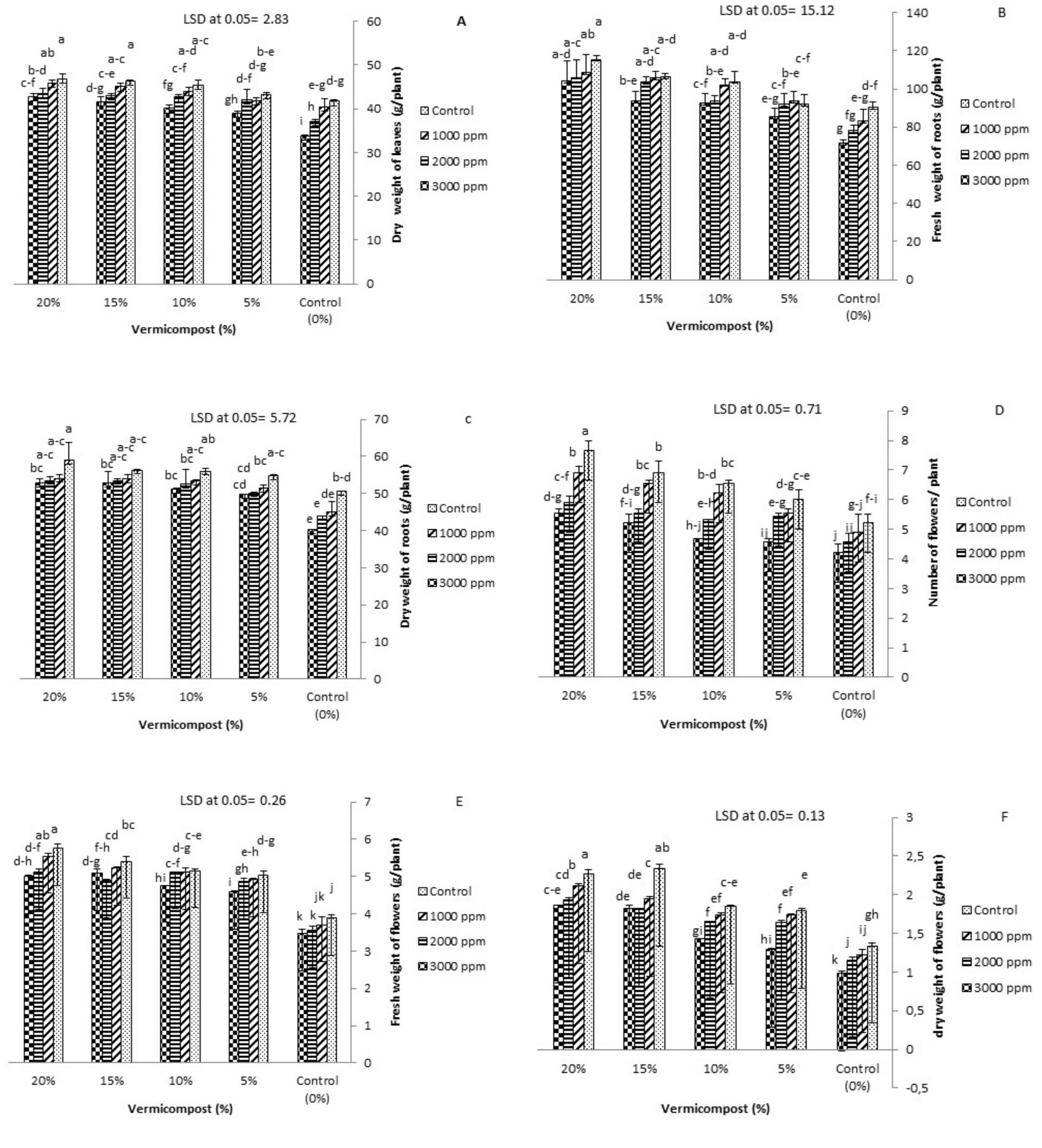

Figure 2. Dry weight of leaves (a), fresh weight of roots (b), dry weight of roots (c), number of flowers/ plant (d), fresh weight of flowers(e) and dry weight of flowers (f) as affected by the interaction between water salinity and vermicompost treatments (mean of two seasons). Column with different letters indicate a significant difference at 5% level. Vertical bars indicate to standard error (SE) of three replicates.

The lowest salinity levels (1000 ppm) was the only one exception since resulted in insignificant reduction in root length, dry weight of leaves and fresh weight of roots compared to control. Our findings are in accordance with those observed by several workers (García-Caparrós and Lao, 2018; Abd-El-Hady et al., 2019; Abdel-Mola and Ayyat 2020; Ashour et al., 2021; Bayat et al., 2022; Azeem et al., 2023) who reported decrease in growth or flowering characteristics of ornamental plants due to adverse effect of salt stress. The reductions in the tested characteristics at high salinity levels may be related to the adverse effect of salinity on ionic toxicity, nutritional imbalance and oxidative damages in cellular compounds such as proteins, lipids and DNA (Ma *et al*., 2020), which resulting reduction in vegetative biomass and followed by decrease in flowering parameters.

Effect of vermicompost treatments

The Data in Figure 1, Figure 2 also indicated that growth and flowering parameter of *Calliandra haematocephala* plants were markedly affected by vermicompost treatments. The plants treated with any concentration of vermicompost had significantly higher values for growth and flowering traits compared to control plants. These findings are similar to those obtained by earlier researches on ornamental plants including *Gladiolus grandiflorus* (Cruz et al., 2018; Karagöz et al., 2019), *Tagetes erecta* (Kumar et al., 2019), *Gerbera jamesonii* (Arunesh et al., 2020) and *Chrysanthemum morfolium* (Padamanabhan, 2021; Yadav and Chandla, 2021).

The Superior effect of vermicompost may attributed to its high microbial activity ensuing from the existence of fungi, bacteria yeasts, actinomycetes, and algae that produce various growth regulators like auxins, gibberellins, and cytokinins which may have favourable impacts on plant growth and development. Moreover, vermicompost includes humic substances which enhance the availability N, P, K, and specially Zn for the synthesis of tryptophan, a precursor to auxins that are utilized for rooting and plant growth (Adamipour et al., 2019).

The Interaction effects between of salinity levels and vermicompost treatments the data in Figure 1, Figure 2 elucidated that, within each level of salinity, in most cases the plants treated with any concentration of vermicompost had significantly higher values for the studied parameters than those recorded with the control plants (plants irrigated with salinity level and not received any vermicompost treatments). The highest values of the tested growth and flowering characteristics were resulted from plants irrigated with tap water and treated with the highest concentration of vermicompost (20%).

On other hand, the lowest values were obtained from plants irrigated with the highest level of salinity (3000 ppm) and not received any vermicompost treatments. These results were confirmed by previous studies (Mohammed et al., 2018; Adamipour et al., 2019; Moghimi et al., 2019; Cicek et al., 2022) who reported increases in growth or flowering characters of plants subjected to salt stress as result of vermicompost treatment.

Chemical constituents

Contents of chlorophylls and total carbohydrates

It is obvious from data in in Figure 3 (a and b) that contents of total chlorophylls and total carbohydrates were negatively affected by salinity stress, the tested parameters were decreased significantly with raising salinity levels from 1000 to 3000 ppm compared to the control.

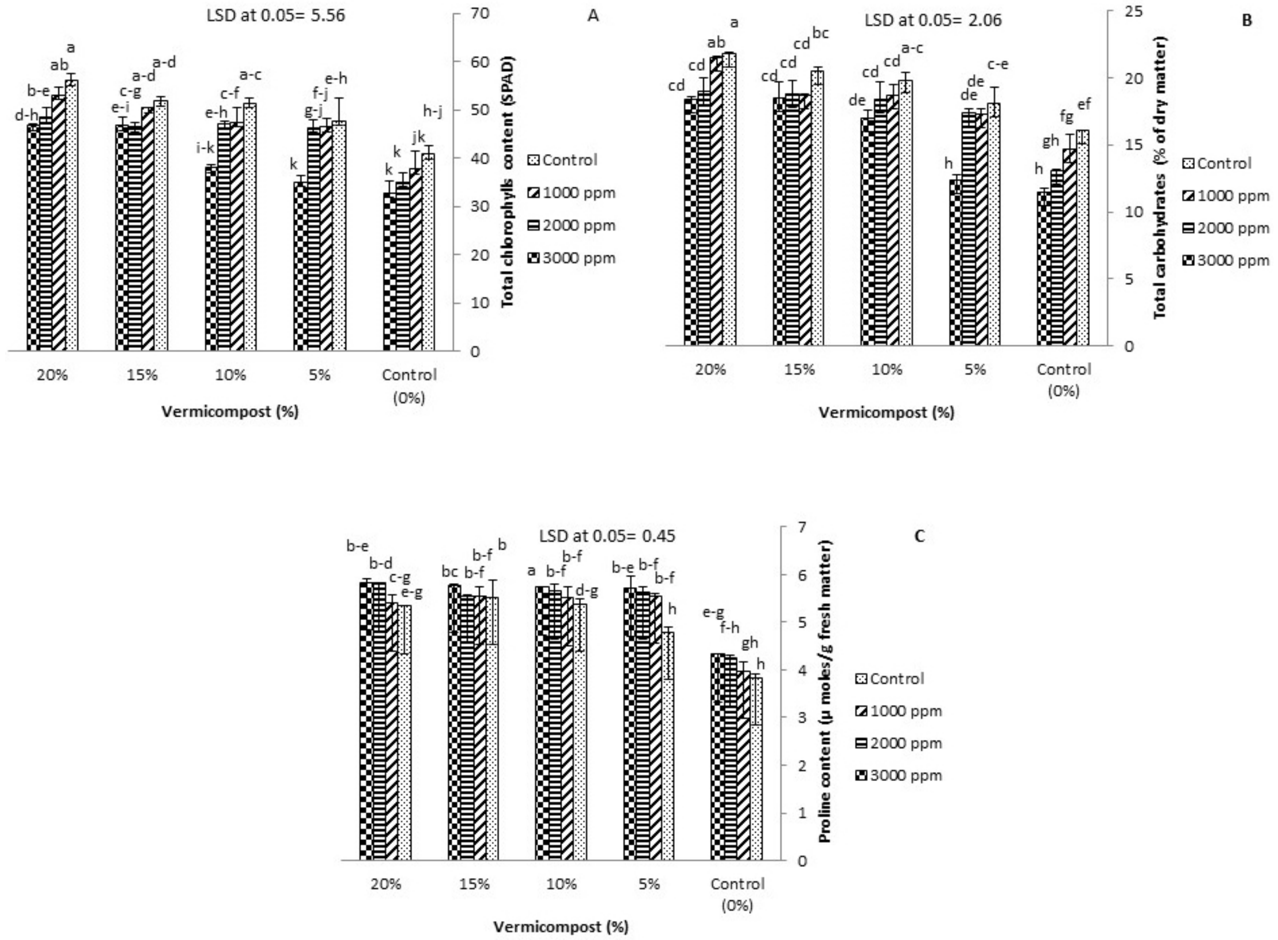

Figure 3. Total chlorophylls (a), total carbohydrates (b) and proline content (c) as affected by the interaction between water salinity and vermicompost treatments (mean of two seasons). Column with different letters indicate a significant difference at 5% level. Vertical bars indicate to standard error (SE) of three replicates.

The present diminution due to salinity stress is supported by the results of various workers (García-Caparrós and Lao, 2018; Abd-El-Hady et al., 2019; Abdel-Mola and Ayyat 2020; Hamayl et al., 2020; Ashour et al., 2021; Bayat et al., 2022). Salinity induced reduction in chlorophyll content is contributed to excessive chloroplast swelling, loss of chloroplast membranes, the development of intracellular lipid droplet, and distortion of lamellae vesiculation. Low photosynthetic pigment content may inhibit photosynthetic potential (Ma et al., 2020). The reduction in the carbohydrates may be indirectly attributed to the reduction in chlorophylls synthesis and photosynthesis as salinity levels increased.

Application of vermicompost concentrations caused significant increase in the recorded mean values compared to control. Such increase may be due to vermicompost providing nutritious elements such as Fe, Zn, Mg, and N directly and indirectly which lead to increase the synthesis of chlorophyll and consequently increase in chlorophylls synthesis (Adamipour et al., 2019). These results confirmed the reports of previous authors that indicated to increase in total chlorophylls (Afkari, 2018; Qafari Rahbar et al., 2020; Jokar and Hassanpour Asil, 2021), total carbohydrates (Helaly and El-Dakak, 2021) due to vermicompost treatment.

It is also clear from data in in Figure 3 (a and b) that, within each level of salinity, in most cases the plants treated with any concentration of vermicompost had significantly higher values for the studied parameters than those recorded with the control plants. The highest values were gained from plants irrigated with tap water and treated with the highest concentration of vermicompost (20%). On contrary, the lowest values were scored from plants irrigated with the highest level of salinity (3000 ppm) and not received any vermicompost treatments. In this concern previous studies stated that application of vermicompost caused increase in chlorophyll content (Adamipour et al., 2019), carbohydrate percentage (Mohammed et al., 2018) in plants exposed to salt stress conditions. Moreover, Tammam et al. (2023) stated that vermicompost controls protein expression at the level of transcriptional and translational which may protect photosynthetic components and prevent salt-induced harmful effects.

Proline content

As shown in Figure 3 (c) the data revealed that increasing salinity stress resulted in significantly increase in proline content compared to the non-saline treatment (control). The obtained increase in proline content due to salinity stress confirmed the reports of several studies (García-Caparrós and Lao, 2018; Abdel-Mola and Ayyat 2020; Hamayl et al., 2020; Ashour et al., 2021; Azeem et al., 2023). They attributed such increase to the role of proline as osmotic adjustment, acting as a reservoir of energy and nitrogen for utilization, protection of enzymes and membranes. Moreover, proline accumulation under salt stress has been shown to maintain plants against free radical-induced damage (Ma et al., 2020).

Application of any concentration of vermicompost resulted in significant increase in proline content compared to control. These results are concordant with those obtained by Ardebiliand and Sharifi (2018).

It is also clear from data in in Figure 3 (c) that, within each level of salinity, the plants treated with any concentration of vermicompost had significantly higher values of proline content than those recorded with the control plants (plants irrigated with salinity level and not received any concentration of vermicompost). The highest value (5.84 µ moles g fresh matter^{-1}) was resulted from plants irrigated with the highest level of salinity (3000 ppm) and treated with the highest concentration of vermicompost (20%). On contrary, the lowest value (3.84 µ moles g fresh matter^{-1}) were obtained from plants irrigated with tap water and not received any vermicompost treatments (control). Previous study (Mohammed et al. 2018) stated that application of vermicompost reduced proline concentration in shoot of salt stressed plants**.**

However, earlier work (Adamipour et al., 2019) reported increase in accumulation of proline in salt stressed plants treated with vermicompost which confirmed the results of the present study. Increasing proline content as a result of vermicompost application may be due to vermicompost increases the amount of N available in plants, as N contributed in the proline structure that leads to elevate proline synthesis under salinity stress which increases plant resistance to salinity conditions (Adamipour et al., 2019).

K^+% and Ca^{2+}%

Evidently data in Table 2 showed that accumulation of K^+ and Ca^{2+} % in leaves were reduced significantly in response to raising salinity levels from 2000 to 3000 ppm, while the lowest salinity level (1000 ppm) caused insignificant reduction compared to control plants.

The reduction in K^+ or Ca^{2+}% a result of raising salinity stress is similar to findings of previous studies (García-Caparrós and Lao, 2018; Abd-El-Hady et al., 2019; Hamayl et al., 2020; Ashour et al., 2021). This reduction of K^+ and $Ca+^2$% in leaves due to salt stress may be related to physical and chemical similarities between K^+ and Na^+ and the tendency of Na^+ to compete with K^+ for major binding sites, including control of enzymatic activity which occurs at unfavorable cytosolic K^+/Na^+ ratios. The inhibition in $Ca+^2$ uptake is due to the opposite effect between Ca^{2+} and Na^+ ions, that affects membrane properties due to displacement of membrane-associated Ca^{2+} by Na^+ which leading to degradation of membrane integrity and selectivity (García-Caparrós and Lao, 2018).

The data in Table 2 also indicated that, the plants treated with any concentration of vermicompost had significantly higher values of K^+ and Ca^{2+} % compared to control. The obtained increase in tested components due to vermicompost treatments are in conformity with prior studies (Ruiz-Lau et al., 2020; Helaly and El-Dakak, 2021; Mahboub Khomami et al., 2021). Increasing K^+ and Ca^{2+}% may be due to vermicompost includes high amount of nutrients as well as humic substances which have different effects in the soil. It may enhance soil properties such as micronutrient transport and availability (Gashaw, 2019).

Table 2. K, Ca, Na, Cl (% dry matter) and K^+/Na^+ ratio in leaves of *Calliandra haematocephala* as affected by water salinity, vermicompost treatments and their interactions (mean of two seasons).

Vermicompost (%)	Salinity treatments,(ppm)				Mean (V)
	control	1000	2000	3000	
			K^+%		
0	1.96	1.86	1.69	1.67	1.80
5	2.20	2.18	2.07	1.71	2.04
10	2.32	2.28	2.20	2.14	2.24
15	2.33	2.30	2.24	2.23	2.28
20	2.35	2.33	2.27	2.25	2.30
Mean (S)	2.23	2.19	2.10	2.00	
L.S.D. (0.05)	S = 0.13		V = 0.15		S X V = 0.29
			Ca^{2+}%		
0	0.97	0.94	0.84	0.81	0.89
5	1.05	1.02	0.99	0.97	1.01
10	1.07	1.03	1.00	0.98	1.02
15	1.17	1.09	1.07	1.05	1.09
20	1.22	1.18	1.10	1.12	1.16
Mean (S)	1.09	1.05	1.00	0.99	
L.S.D. (0.05)	S = 0.08		V = 0.09		S X V = 0.17
			Na^+%		
0	0.99	1.16	1.19	1.22	1.14
5	0.66	0.76	0.77	0.78	0.74
10	0.62	0.66	0.73	0.75	0.69
15	0.56	0.64	0.74	0.72	0.67
20	0.55	0.62	0.69	0.72	0.64
Mean (S)	0.67	0.77	0.83	0.84	
L.S.D. (0.05)	S = 0.15		V = 0.16		S X V = 0.23
			Cl^-%		
0	0.63	0.84	0.85	0.86	0.79
5	0.55	0.61	0.62	0.64	0.60
10	0.47	0.52	0.59	0.61	0.55
15	0.43	0.51	0.56	0.59	0.52
20	0.41	0.49	0.53	0.56	0.50
Mean (S)	0.50	0.59	0.63	0.65	
L.S.D. (0.05)	S = 0.11		V = 0.12		S X V = 0.24
			K^+/Na^+ ratio		
0	2.02	1.74	1.61	1.43	1.70
5	3.37	2.89	2.68	2.19	2.78
10	3.78	3.48	3.06	2.90	3.31
15	4.21	3.70	3.01	3.12	3.51
20	4.40	3.80	3.31	3.28	3.70
Mean (S)	3.56	3.12	2.74	2.58	
L.S.D. (0.05)	S = 0.51		V = 0.57		S X V = 1.14

As for the interaction affects between two studied factors, the data in Table 2 showed that, the lowest values of the tested components were resulted from plants irrigated with the highest level of salinity (3000 ppm) and not received any vermicompost treatments. On the other hand, the highest values were gained from plants irrigated with tap water and treated with the highest concentration of vermicompost (20%). The data also indicated that, within each level of salinity, the plants treated with vermicompost especially the highest concentration (20%) had significantly higher values of K^+ and Ca^{2+} % than those registered with the control plants (those irrigated with salinity level and not received any concentration of vermicompost). In this regard prior studies (Mohammed et al., 2018; Adamipour et al., 2019; Moghimi et al., 2019; Cicek et al., 2022) showed that application of vermicompost enhanced the uptake of mineral nutrient in plants subjected to salt stress.

Na^+ and Cl^- %

As shown in Table 2 the data exhibited that increasing salinity levels from 2000 to 3000 ppm resulted in significant increase in Na^+ and Cl^- % in leaves of *Calliandra haematocephala* plants, while the lowest level (1000 ppm) caused insignificant increase compared to control plants. Increasing Na^+ or Cl^- % in leaves of salt stressed plants is quite in line with those reported by Abd-El-Hady et al. (2019); Abdel-Mola and Ayyat (2020); Hamayl et al. (2020); Ashour et al. (2021); Azeem et al. (2023). The accumulation of Na^+ and Cl^-% in the leaves may cause some toxic effects that may be responsible for decreasing vegetative and flowering traits. Accumulation of Cl^- decreases the photosynthetic capacity due to chlorophyll loss which may product from a structural effect of high Cl^- concentration on PSII. High Na^+ interferes with K^+ and Ca2+ nutrition and confuses efficient stomatal regulation which causes a depression of photosynthesis and growth (Ruiz-Lau et al., 2020).

The data in Table 2 also pointed out that, application of vermicompost concentrations resulted in significantly lower values of Na^+ and Cl^-in leaves compared to control. The reduction in Na^+ and Cl^- due to application of vermicompost were affirmed by earlier study (Ruiz-Lau et al., 2020).

Regarding the interactions effect between salinity levels and vermicompost treatments, the data in Table 2 showed that, within each level of salinity, the plants treated with any concentration of vermicompost had significantly lower values of Na^+ and Cl^- in leaves than those obtained with the control plants (plants irrigated with salinity level and not received any concentration of vermicompost). The highest values were obtained from plants irrigated with the highest level of salinity (3000 ppm) and not received any concentration of vermicompost (control). On other hand, the lowest values were obtained from plants irrigated with tap water and received the highest concentration of vermicompost (20%). These results agree with those reported by Mohammed et al., 2018 who showed that application of vermicompost reduced Na+ concentration in shoot of salt stressed plants. In another study (Moghimi et al., 2019) showed that vermicompost reduced Na+ and Cl in leaf of salt stressed plants.

K^+/Na^+ ratio

The data in Table 2 manifestly that K^+/Na^+ ratio in leaves was declined gradually with raising salinity levels from 1000-3000 ppm compared to control. This reduction was insignificant with the lowest level of salinity (1000 ppm), while the higher levels (2000-3000 ppm) caused significant reduction compared with the non-saline treatment (control). The results of decreasing K^+/Na^+ ratio in leaves of salt stressed plants are in good accordance with those elicited by Ashour et al. (2021) and Azeem et al. (2023). Under salt stress conditions excessive absorption of Na^+ and Cl^- by roots inhibited the availability of K^+ and other essential minerals which causing reduction in K^+/Na^+ ratio (Azeem et al., 2023).

The data in Table 2 also illustrated that vermicompost treatments had pronounced effect on K^+/Na^+ ratio in leaves, application any concentration of vermicompost resulted in significant increase in the recorded mean values compared to control. The increases in K^+/Na^+ ratio due to application of vermicompost treatments have been reported by previous work (Ruiz-Lau et al., 2020).

The data in the same table also indicated that within each level of salinity, the plants received any concentration of vermicompost had significantly higher values of K^+/Na^+ ratio than those registered with the control plants (plants irrigated with salinity level and not received any concentration of vermicompost). The highest values were obtained from plants irrigated with tap water and received the highest concentration of vermicompost (20%), while the lowest values were obtained from plants irrigated with the highest level of salinity (3000 ppm) and not received any concentration of vermicompost.

Enzyme activity

The data in in Figure 4 (a, b and c) disclosed that increasing salinity levels resulted in significantly increase the activity of CAT, SOD and APX compared to the non-saline treatment (control).

The obtained results were affirmed by the findings of Azeem et al. (2023) on *Moringa oleifera*. Excessive sodium content in the plant activates the accumulation of reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, and radical hydroxyl. Such free radicals are hurtful for proteins, lipids, nucleic acid and other macromolecules leading to serious damage to the structure of cells and eventually to the whole plan. One strategy of plants to overcome this stress is the accumulation of antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX). SOD is considered as a first line of defense, which converts superoxide into H_2O_2. Furthermore, CAT and APX enzymes convert H_2O_2 into H_2O (Dumanović et al., 2021; Azeem et al., 2023).

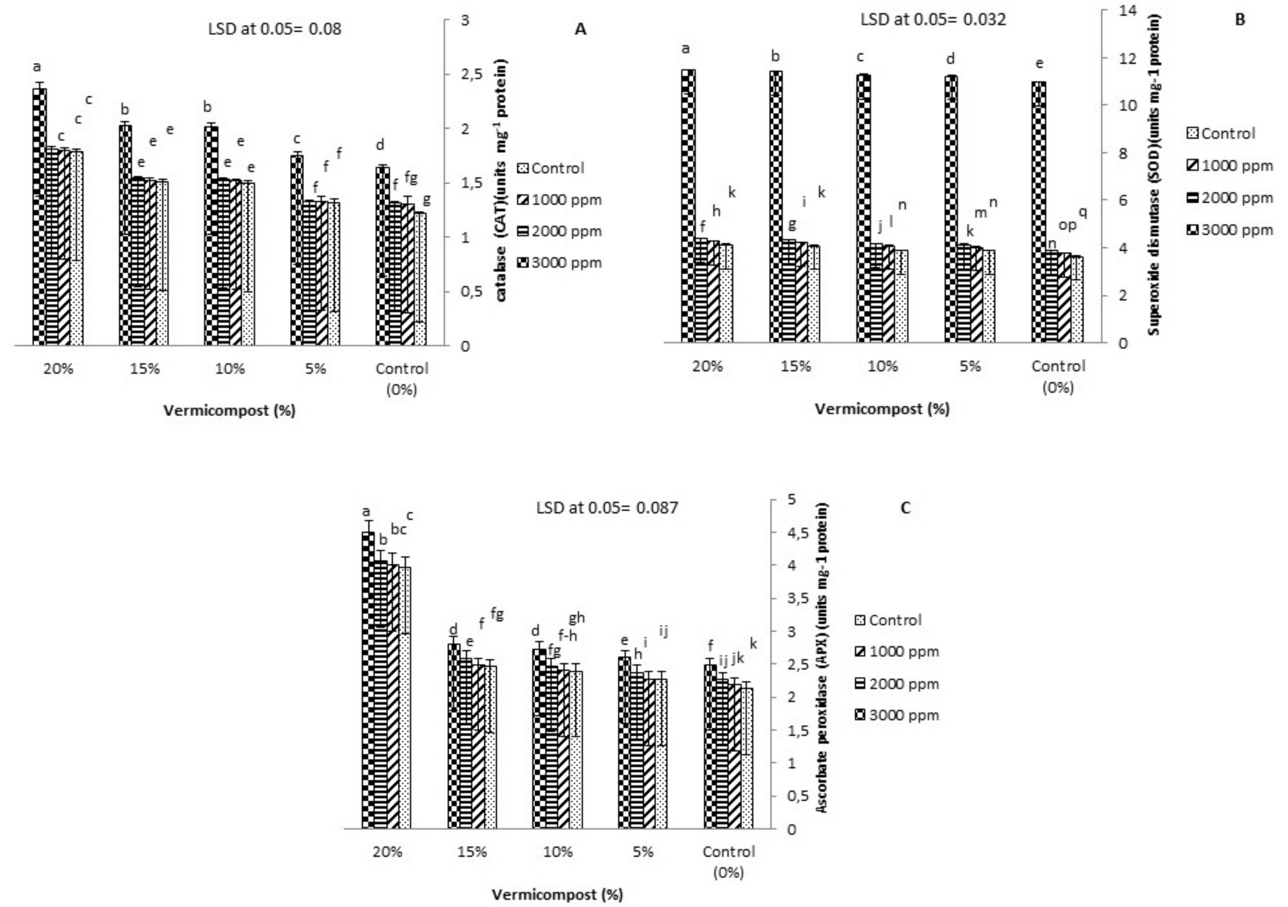

Figure 4. Catalase (a), superoxide dismutase (b) and ascorbate peroxidase (c) as affected by the interaction between water salinity and vermicompost treatments (mean of two seasons). Column with different letters indicate a significant difference at 5% level. Vertical bars indicate to standard error (SE) of three replicates.

The data also showed that, vermicompost treatments had superior effect on increasing antioxidative enzymes (CAT, SOD and, APX) compared to control. These results are similar to findings of Afkari (2018) on *Borago officinalis*. Within each level of salinity, the plants received the higher concentration of vermicompost (15%-20%) had significantly higher values of antioxidative enzymes (CAT, SOD and, APX) than those obtained from the control plants. The highest values (2.37, 11.47 and 4.49-units mg^{-1} protein for CAT, SOD and, APX respectively) were obtained from plants irrigated the highest level of salinity and received the highest concentration of vermicompost (20%), whereas the lowest values were obtained from plants irrigated with tap water and not received any concentration of vermicompost (control). These results agree with the findings of Adamipour et al. (2019) who reported increase in CAT, SOD and, APX in salt stressed plants.

Conclusions

Salinity stress had a harmful impact on morphological and physiological traits of plants and the plants could be considered sensitive to salinity. Application of vermicompost under salinity stress enhanced morphological and physiological parameters, promoted total chlorophylls total carbohydrates, nutrient uptake, K^+/Na^+ ratio, enzyme activities and reducing accumulation of Na^+ and C^-l% toxic ions in leaves. Based on the results, application of vermicompost at 20% recommended for alleviating the harmful efects of salinity on *Calliandra haematocephala* plants irrigated with saline water at concentration up to 3000 ppm.

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Author Contribution

HAA: Designed and performed the experiment, data analysis, preparation and writing the manuscript. **SMH**: Assistance in designing of the experiment, chemical analysis, statistical analysis, preparation and writing of the manuscript. **MMS**: Assistance in data collection, chemical analysis, statistical analysis, manuscript preparation

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