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RESEARCH ARTICLE

Enhancing salt tolerance in maize using co-application of exogenously applied lipoic acid or silicon with soil-based vermicompost-tea by reinforcing antioxidant defense mechanisms and regulating plant performance

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ABSTRACT

Maize (*Zea mays* L.) is highly sensitive to salt stress, which poses a threat to its production, especially with predicted increases in salinity due to climate change. Enhancing salt tolerance in maize is critical to mitigating these adverse effects, sustaining its production, and ensuring global food security. This study aimed to evaluate the effects of exogenously applied lipoic acid or Si, alone or in integration with soil application of vermicompost-tea on physio-biochemical components, antioxidant defense systems, growth, and yield of maize plants exposed to salt stress. The application of vermicompost-tea was carried out as a soil treatment incorporated into irrigation water at a concentration of 200 L ha^{-1}, and administered in three equal doses in irrigation water within the last 10 min of drip irrigation. Silicon was applied at a rate of 6 mM using potassium silicate and lipoic acid was applied at 0.1 mM. Silicon and lipoic acid were applied three times at 25, 40, and 55 d after planting. The results indicated that the integrative application of soil-based vermicompost-tea with exogenously applied Si or lipoic acid significantly enhanced all studied parameters. Specifically, the combination of Si with vermicompost-tea exhibited the highest improvement percentage compared to untreated control in chlorophyll *a* (60.3%), chlorophyll *b* (72.7%), carotenoids (69.9%), net photosynthetic rate (111%), transpiration rate (134%), stomatal conductance (129.5%), relative water content (28.4%), membrane stability index (44.3%), N (33.4%), P (71.9%), K (39.7%), Ca (89.9%), K/Na (81.2%), total soluble sugars (63.7%), proline (60.1%), ascorbate (44.4%), glutathione (83.5%), and α-tocopherol (72.1%). These positive effects were reflected in enhancing yield traits under salt-affected soil conditions by enhancing plant height (25.1%), number of grains per row (43.3%), number of rows per ear (26.7%), 1000-grain weight (25.9%), grain yield (45.4%), and biological yield (42.8%) compared to untreated control.

Key words: Agronomic performance, heatmap and hierarchical clustering, Mediterranean environment, physio-biochemical parameters, *Zea mays*.

INTRODUCTION

Maize (*Zea mays* L.) is a widely planted cereal crop for various purposes (Rizwan et al., 2017). It is used as a staple food for humans, and fodder for animals and poultry, and shows potential as a source for biofuels and industrial products. Globally, maize is grown across an expansive area, covering approximately 205.9 million hectares and yielding around 1210 million tons annually (FAOSTAT, 2024). Maize production should be improved to ensure worldwide food security considering the challenges posed by population growth and abrupt climate fluctuations.

Salinity affects large agricultural areas in the Mediterranean region. It presents a widespread environmental challenge impacting crop production, particularly in arid environments (El Sabagh et al., 2020). Irrigation with poor-quality water remains a primary factor leading to soil salinization. Furthermore, recent climate change has been marked by reduced precipitation and rising temperatures, increasing aridity. These conditions contribute to soil salinity, as higher evaporation rates result in salt accumulation in surface layers. Many plants exhibit sensitivity to salt stress and struggle to endure even low salinity levels. Salinity adversely affects the plant ability to access water, alters the nutrient balance, and reduces the absorption of certain essential elements and the toxic impact of Na and Cl ions. Salt stress significantly reduces chlorophyll content, photosynthesis, plant metabolism, water relations, and stomatal closure, decreasing CO_2 levels (Hniličková et al., 2017). Moreover, the plants respond to salinity-induced changes through osmotic stress and ion toxicity with salinity triggering the generation of reactive oxygen species (ROS) like O_2^-, OH^-, and H_2O_2 (Hasanuzzaman et al., 2021). These adverse impacts cause metabolic inhibition and hinder physiological processes and plant growth. Besides, salt accumulation in leaf tissues up to toxic levels results in the loss of turgor, dehydration, and tissue damage, ultimately leading to tissue and cell death.

Maize is highly sensitive to salt stress, posing a threat to its production, especially with predicted increases in salinity due to climate change. Enhancing salt tolerance in maize is critical to mitigate these effects and maintain crop productivity. Various approaches have been employed to alleviate salinity detrimental effects, such as applying osmoprotectant materials and plant growth regulators (Quamruzzaman et al., 2021). Among these materials, Si and lipoic acid have shown the potential to improve plant salinity tolerance. Lipoic acid plays a role in energy metabolism and detoxifying ROS. In addition, it plays a crucial function in recycling oxidized antioxidants such as ascorbate, glutathione, and tocopherol. Lipoic acid has strong antioxidant capabilities in its reduced oxidized lipoic acid and dihydrolipoic acid forms. Furthermore, Si can enhance salt tolerance by improving physiological pathways in plants. It enhances photosynthesis activity, addresses nutrient imbalances, and diminishes the toxicity caused by certain elements (Zhang et al., 2019). It reduces Na uptake and elevates the K/Na ratio to attenuate plant ion toxicity under salinity stress (Li et al., 2016). Soil application of vermicompost-tea has been observed to alleviate salt stress by enhancing plant growth and enzymatic activities. It is an organic soil amendment derived from decomposing organic matter like animal manure and food wastes by microorganisms and earthworms, forming fine-granulated particles (Yatoo et al., 2021). Vermicompost-tea, a liquid extract of solid vermicompost, contains microbes, soluble nutrients, and beneficial elements. It boosts nutrient uptake, photosynthetic pigments, enzymatic activities, protein synthesis, lateral root development, improves soil and microbial structure, and enhances plant growth and yield (Rehman et al., 2023). This study hypothesized that the integrated applications of Si, lipoic acid, and vermicompost-tea could enhance plant membrane stability index and relative water content by improving nutrient levels, osmoprotectants, and antioxidant activities for alleviating salt stress. Henceforth, this work aimed to explore the impact of exogenously applied Si or lipoic acid, alone or in integration with soil application of vermicompost-tea on physio-biochemical components, antioxidant defense systems, growth, and yield of maize plants under salt stress conditions.

MATERIALS AND METHODS

Experimental site and plant material

Two field trials were performed during the consecutive summer seasons of 2021 and 2022 at Elnoubaria (30°43′ N, 30°33′ E), Egypt. Soil samples were collected from the study site before each agricultural season and analyzed. Based on the soil analysis before planting, the soil electrical conductivity (EC) values were 6.11 and 6.17 dS m^{-1} for the 2021 and 2022 seasons, in the same order. The soil exhibited a loamy texture across its profile (45.53% sand, 29.97% silt, and 24.50% clay). These EC values categorized the soil as saline following the classification by Dahnke and Whitney (1988). The maize (*Zea mays* L.) cultivar used was Giza-167, a high-yielding commercial yellow single-cross hybrid listed in the Egyptian recommendations.

Healthy seeds were obtained from the Agricultural Research Centre. Considering the optimal maize cultivation period in the region, seed sowing took place on the first of May in both seasons. The seeds were planted in plots comprising six rows, each 5.0 m long, with a spacing of 0.70 m between rows and 0.25 m between hills. Before sowing, 75 kg P_2O_5 ha^{-1} was applied as a single superphosphate (15% P_2O_5), and K was added at a rate of 100 kg K_2O ha^{-1} using potassium sulfate (48% K_2O). Nitrogen fertilization was administered at a rate of 295 kg N ha^{-1} as ammonium sulfate (21% N) in four splits, spaced at 10 d intervals after sowing.

Application of vermicompost-tea

The tea was prepared using the Compost Tea System10 (Growing Solutions, Eugene, Oregon, USA), an aerated system with an air pump employing fine bubble diffusion technology. A gallon of vermicompost was positioned in a perforated plastic container suspended from the main container's rim. Nine gallons of tap water filled the brewer and left to brew for 24 h. The physicochemical properties of vermicompost are illustrated in Table 1. The application of vermicompost-tea carried out as a soil treatment, was incorporated into irrigation water at a concentration of 200 L ha^{-1}, and administered in three equal doses during the 2nd, 4th, and 6th irrigations within the last 10 min of drip irrigation.

Table 1. Physicochemical properties of vermicompost.

Characteristic	Value
PH	7.43
Electrical conductivity, dS m^{-1}	0.56
Total organic C, %	27.15
Total organic matter, %	44.60
Total N, %	2.40
N, mg kg^{-1}	101.00
P, mg kg^{-1}	41.00
K, mg kg^{-1}	330.00
Ca, mg kg^{-1}	1710.00
Mg, mg kg^{-1}	250.00
Fe, mg kg^{-1}	22.00
Mn, mg kg^{-1}	808.00
Zn, mg kg^{-1}	0.76
Cu, mg kg^{-1}	0.62
Cd, mg kg^{-1}	0.42
Pb, mg kg^{-1}	Below detection limit

Foliar spray of Si or lipoic acid

Silicon was applied at a rate of 6 mM using potassium silicate (Powder, pH 11.3, stable water-soluble silicic acid potassium salt; 1312-76-1, Henan Daken Chemical, Zhengzhou, China). Lipoic acid was applied at a rate of 0.1 mM LA (pure lipoic acid powder complies with US Pharmacopeia (USP) quality standard, NuSci Brand). The spraying process with Si and lipoic acid was applied three times at 25, 40, and 55 d after planting using a hand atomizer, and a small amount of Tween-20 was added as a surfactant agent to enhance the penetration of the spray solution into the leaf tissue. Spraying was performed in the early morning before sunrise. Untreated control plants were treated with tap water containing Tween-20.

Measured agronomic traits

Ten maize plants were randomly selected from each treatment to measure agronomic traits. Plant height (cm), number of grains per row, number of rows per ear, grain yield (kg ha^{-1}), biomass yield (kg ha^{-1}), and 1000-grain weight were determined.

Determinations of physiological parameters

Total chlorophyll and carotenoids were extracted from fresh leaves using pure acetone following Avron method (Avron, 1960). Leaf net photosynthetic rate, transpiration rate, and stomatal conductance were assessed using a portable photosynthesis system (LF6400XTR, LI-COR, Lincoln, Nebraska, USA) between 09:00 and 11:00 h. Relative water content was calculated as Barrs and Weatherley (1962) technique, and the membrane stability index was determined following Premachandra et al. (1990) protocol. Total ion seepage from leafy tissue was measured as described in Sullivan (1979) method, using electrical conductivities (EC1, EC2, and EC3). Lipid peroxidation was assessed by measuring malondialdehyde content using the method by Heath and Packer (1968) for H_2O_2 extracts. Total soluble sugar content was estimated following Irigoyen et al. (1992) technique. Proline content was assessed by the rapid colorimetric method of Bates et al. (1973). Enzyme extraction and analysis were performed for catalase, ascorbate peroxidase, peroxidase, superoxide dismutase, and glutathione reductase according to Vitória et al. (2001). Ascorbate content was assessed using the Kampfenkel et al. (1995) method. Reduced and total glutathione content was determined using Griffith (1980). The HPLC determined α-tocopherol content following Konings et al. (1996) and Ching and Mohamed (2001) methodologies. Hydrogen peroxide (H_2O_2) levels were estimated according to Velikova et al. (2000). Superoxide ($O_2^{\bullet -}$) content was determined by Kubiś (2008) method. Elemental concentrations (N, P, K, Ca, Na) were determined using different methods: Sulphuric acid digestion for N, P, K, Ca with flame photometer Lachica et al. (1973), microkjeldahl method for N (Chapman and Pratt, 1982), and calorimetric method for P (Watanabe and Olsen, 1965).

Statistical analysis

The R software (version 3.6.2) was used for statistical analyses. The least significant difference (LSD) at $P \leq 0.05$ was calculated to explore the significant differences among studied treatments. Heatmap was performed using RcolorBrewer package implemented in R software. The experimental design was a randomized complete block design (RCBD) with three replicates.

RESULTS

Photosynthetic pigments and photosynthetic efficiency

All treatments involving vermicompost-tea, lipoic acid or Si significantly enhanced photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, and carotenoids) and photosynthetic efficiency (net photosynthetic rate, transpiration rate, and stomatal conductance) compared to the untreated control (sprayed by tap water and untreated by vermicompost-tea) across both the 2021 and 2022 growing seasons (Table 2). The integrative application of soil-based vermicompost-tea with exogenously applied Si or lipoic acid resulted in the highest increases. Remarkably, the combination of Si with vermicompost-tea exhibited the most substantial improvement, increasing chlorophyll *a* by 60.5% and 60.0%, chlorophyll *b* by 69.0% and 76.3%, carotenoids by 70.3% and 69.5%, net photosynthetic rate by 108.0% and 114.0%, transpiration rate by 133.0% and 135.0%, and stomatal conductance by 130.0% and 129.0% during the two seasons, respectively. The aforementioned treatment was followed by the combined application of lipoic acid with vermicompost-tea. The co-application of lipoic acid and vermicompost-tea enhanced chlorophyll *a* by 44.0% and 44.5%, chlorophyll *b* by 62.8% and 69.8%, carotenoids by 65.4% and 64.6%, net photosynthetic rate by 101.0% and 104.0%, transpiration rate by 125.0% and 123.0% and stomatal conductance by 117.0% and 123.0% during two seasons respectively. These improvements were observed under salinity conditions compared to the untreated control (Table 2).

Table 2. Impact of vermicompost-tea application and/or exogenously sprayed lipoic acid or Si on photosynthetic pigments and the efficiency of salt-stressed maize plants across 2021 and 2022 growing seasons. The data presented as mean ± standard error (SE). Same letters within each column for each season reveal nonsignificant variations according to LSD test (P ≤ 0.05).

Foliar spray (FS)	Vermicompost (VC)	Chlorophyll *a*	Chlorophyll *b*	Carotenoids	Net photosynthetic rate	Transpiration rate	Stomatal conductance
		mg g^{-1}	mg g^{-1}	mg g^{-1}	µmol CO_2 m^{-2} s^{-1}	mmol H_2O m^{-2} s^{-1}	mmol H_2O m^{-2} s^{-1}
First season							
Tap water	Without-VC	1.09 ± 0.15^{f}	0.530 ± 0.30^{f}	0.81 ± 0.07^{f}	6.46 ± 0.32^{e}	3.34 ± 0.11^{e}	0.243 ± 0.03^{f}
Tap water	With-VC	1.33 ± 0.09^{e}	0.762 ± 0.40^{e}	1.22 ± 0.10^{de}	10.60 ± 0.78^{d}	5.93 ± 0.15^{d}	0.446 ± 0.02^{e}
Lipoic acid	Without-VC	1.39 ± 0.11^{d}	0.784 ± 0.05^{d}	1.25 ± 0.07^{cd}	11.60 ± 0.89^{c}	6.22 ± 0.34^{d}	0.476 ± 0.04^{d}
Lipoic acid	With-VC	1.56 ± 0.14^{b}	0.862 ± 0.08^{b}	1.33 ± 0.08^{ab}	13.00 ± 0.85^{a}	7.55 ± 0.55^{b}	0.540 ± 0.02^{b}
Si	Without-VC	1.48 ± 0.14^{c}	0.824 ± 0.10^{c}	1.29 ± 0.06^{bc}	12.10 ± 0.84^{b}	7.02 ± 0.25^{c}	0.506 ± 0.05^{c}
Si	With-VC	1.73 ± 0.13^{a}	0.893 ± 0.09^{a}	1.37 ± 0.09^{a}	13.40 ± 0.87^{a}	7.81 ± 0.58^{a}	0.573 ± 0.03^{a}
ANOVA	df				P-value		
FS	2	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
VC	1	<0.001	<0.001	<0.001	<0.001	<0.001	0.007
FS×VC	2	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Second season							
Tap water	Without-VC	1.10 ± 0.07^{e}	0.515 ± 0.03^{e}	0.810 ± 0.07^{e}	6.39 ± 0.41^{e}	3.30 ± 0.11^{f}	0.250 ± 0.02^{d}
Tap water	With-VC	1.34 ± 0.08^{d}	0.771 ± 0.05^{d}	1.23 ± 0.09^{d}	10.70 ± 0.65^{d}	5.87 ± 0.32^{e}	0.460 ± 0.03^{c}
Lipoic acid	Without-VC	1.39 ± 0.11^{d}	0.792 ± 0.05^{d}	1.27 ± 0.06^{cd}	11.60 ± 0.80^{c}	6.27 ± 0.53^{d}	0.502 ± 0.05^{b}
Lipoic acid	With-VC	1.58 ± 0.11^{b}	0.875 ± 0.07^{b}	1.33 ± 0.06^{ab}	13.20 ± 0.95^{a}	7.49 ± 0.64^{b}	0.570 ± 0.05^{a}
Si	Without-VC	1.48 ± 0.10^{c}	0.835 ± 0.08^{c}	1.29 ± 0.07^{bc}	12.30 ± 0.87^{b}	7.09 ± 0.53^{c}	0.509 ± 0.04^{b}
Si	With-VC	1.75 ± 0.09^{a}	0.909 ± 0.09^{a}	1.38 ± 0.08^{a}	13.80 ± 0.78^{a}	8.02 ± 0.38^{a}	0.586 ± 0.06^{a}
ANOVA	df				P-value		
FS	2	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
VC	1	<0.001	<0.001	<0.001	<0.001	<0.001	0.024
FS×VC	2	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Leaf integrity, oxidative stress markers, and oxidative damage

The application of vermicompost-tea and/or lipoic acid or Si significantly affected oxidative biomarkers (hydrogen peroxide and malondialdehyde contents), oxidative damage (superoxide radical level), electrolyte leakage, membrane stability index and relative water content in maize plant leaves under salt stress is illustrated in Table 3. Maize plants subjected to salinity stress exhibited decreased leaf relative water content and membrane stability index but increased leaf electrolyte leakage, malondialdehyde (MDA), hydrogen peroxide, and superoxide radical level. Conversely, the application of vermicompost-tea and/or lipoic acid or Si significantly increased membrane stability index and relative water content while decreased MDA, electrolyte leakage, hydrogen peroxide, and superoxide radical level compared to untreated control treatment (sprayed by tap water and untreated by vermicompost-tea). The integrative treatment of foliar spray and soil-based vermicompost-tea application yielded more favorable results than the sole treatment. Specifically, the combined application of Si with vermicompost-tea displayed the most significant improvements, increasing relative water content by 28.4% and 28.4%, membrane stability index by 44.1% and 44.6%, and decreasing electrolyte leakage by 52.0% and 51.7%, MDA by 63.6% and 63.3%, hydrogen peroxide by 44.7% and 45.2%, and superoxide radical by 45.2% and 36.6% during the two seasons, respectively, followed by the combined application of lipoic acid and vermicompost-tea which boosted relative water content by 25.8% and 25.3%, membrane stability index by 40.0% and 39.7% and decreased electrolyte leakage by 50.3% and 49.5%, MDA by 57.8% and 58.7%, hydrogen peroxide by 38.2% and 38.7% and superoxide radical by 37.0% and 45.1% during two seasons compared to untreated control under salinity stress.

Table 3. Impact of vermicompost-tea application and/or exogenously sprayed lipoic acid or Si on leaf integrity, oxidative stress markers, and oxidative damage of salt-stressed maize plants across 2021 and 2022 growing seasons. The data presented as mean ± standard error (SE). Same letters within each column for each season reveal nonsignificant variations according to LSD test (P ≤ 0.05).

Foliar spray (FS)	Vermicompost (VC)	Relative water content	Membrane stability index	Electrolyte leakage	Malondialdehyde	Hydrogen peroxide	Superoxide radical
		%	%	%	µmol g^{-1}	mol g^{-1}	A580 g^{-1}
First season							
Tap water	Without-VC	64.0 ± 2.6^{e}	54.3 ± 2.5^{f}	14.3 ± 0.96^{a}	2.56 ± 0.11^{a}	2.46 ± 0.11^{a}	0.73 ± 0.06^{a}
Tap water	With-VC	76.9 ± 2.9^{d}	68.3 ± 2.9^{e}	8.77 ± 0.79^{b}	1.79 ± 0.08^{b}	1.88 ± 0.12^{b}	0.54 ± 0.04^{b}
Lipoic acid	Without-VC	78.2 ± 3.9^{c}	71.1 ± 3.8^{d}	7.91 ± 0.65^{c}	1.45 ± 0.14^{c}	1.80 ± 0.13^{bc}	0.52 ± 0.03^{c}
Lipoic acid	With-VC	80.5 ± 4.5^{b}	76.0 ± 3.3^{b}	7.11 ± 0.52^{e}	1.08 ± 0.07^{e}	1.52 ± 0.15^{d}	0.46 ± 0.04^{e}
Si	Without-VC	79.1 ± 3.8^{c}	73.8 ± 3.6^{c}	7.41 ± 0.66^{d}	1.30 ± 0.13^{d}	1.68 ± 0.16^{c}	0.49 ± 0.02^{d}
Si	With-VC	82.2 ± 3.6^{a}	78.3 ± 4.1^{a}	6.85 ± 0.45^{f}	0.95 ± 0.06^{f}	1.36 ± 0.14^{e}	0.40 ± 0.03^{f}
ANOVA	df			P-value			
FS	2	<0.001	<0.001	<0.001	<0.001	0.009	<0.001
VC	1	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
FS×VC	2	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Second season							
Tap water	Without-VC	64.4 ± 3.6^{f}	54.6 ± 2.3^{f}	14.0 ± 1.20^{a}	2.54 ± 0.13^{a}	2.43 ± 0.6^{a}	0.71 ± 0.05^{a}
Tap water	With-VC	77.5 ± 4.2^{e}	68.7 ± 3.6^{e}	8.54 ± 0.68^{b}	1.75 ± 0.10^{b}	2.00 ± 0.15^{b}	0.53 ± 0.06^{b}
Lipoic acid	Without-VC	78.5 ± 4.8^{d}	71.4 ± 4.8^{d}	7.92 ± 0.65^{c}	1.40 ± 0.08^{c}	1.79 ± 0.16^{c}	0.51 ± 0.03^{c}
Lipoic acid	With-VC	80.7 ± 4.4^{b}	76.3 ± 3.9^{b}	7.07 ± 0.75^{f}	1.05 ± 0.04^{e}	1.49 ± 0.13^{e}	0.39 ± 0.02^{f}
Si	Without-VC	79.5 ± 4.9^{c}	74.1 ± 4.1^{c}	7.29 ± 0.78^{d}	1.25 ± 0.07^{d}	1.64 ± 0.17^{d}	0.48 ± 0.02^{d}
Si	With-VC	82.6 ± 4.6^{a}	79.0 ± 4.6^{a}	6.76 ± 0.66^{e}	0.93 ± 0.03^{f}	1.33 ± 0.12^{f}	0.45 ± 0.03^{e}
ANOVA	df			P-value			
FS	2	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
VC	1	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
FS×VC	2	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Nutrient content and K/Na ratio

Individually applied vermicompost-tea, lipoic acid or Si significantly increased N, P, Ca, K, and K/Na ratio while significantly reducing Na content. Integrative treatments involving vermicompost-tea and/or lipoic acid or Si further enhanced N, P, Ca, K, and K^+/Na^+ ratio, and decreased Na compared to untreated control across both seasons (Table 4). The combined application of Si and vermicompost-tea emerged as the most effective treatment, elevating N by 33.7% and 33.1%, P by 70.2% and 73.6%, K by 40.0% and 39.4%, Ca by 89.4% and 90.4%, K/Na by 78.2% and 84.2%, and decreasing Na by 21.8% and 23.5% during the two seasons compared to untreated control and surpassing other integrative treatments. Likewise, the co-application of lipoic acid and vermicompost-tea enriched N by 32.0% and 31.5%, P by 59.5% and 60.5%, K by 33.5% and 33.1%, Ca by 80.7% and 80.9%, and K/Na by 65.2% and 70% while decreased Na by 18.8% and 20.8% during two seasons respectively compared untreated control.

Table 4. Impact of vermicompost-tea application and/or exogenously sprayed lipoic acid or Si on the nutrient content of salt-stressed maize plants across 2021 and 2022 growing seasons. The data presented as mean ± standard error (SE). Same letters within each column for each season reveal nonsignificant variations according to LSD test (P ≤ 0.05).

Foliar spray (FS)	Vermicompost (VC)	N	P	K	Ca	Na	K^+/Na^+ ratio
		%	%	%	%	%	
First season							
Tap water	Without-VC	1.81 ± 0.04^{e}	0.37 ± 0.02^{e}	1.55 ± 0.06^{e}	1.14 ± 0.09^{f}	2.24 ± 0.14^{a}	0.69 ± 0.04^{f}
Tap water	With-VC	2.21 ± 0.06^{d}	0.50 ± 0.07^{d}	1.78 ± 0.05^{d}	1.35 ± 0.11^{e}	1.91 ± 0.12^{b}	0.93 ± 0.07^{e}
Lipoic acid	Without-VC	2.30 ± 0.09^{c}	0.54 ± 0.04^{c}	1.90 ± 0.07^{c}	1.70 ± 0.14^{d}	1.88 ± 0.14^{c}	1.01 ± 0.06^{d}
Lipoic acid	With-VC	2.39 ± 0.08^{ab}	0.59 ± 0.06^{b}	2.07 ± 0.06^{b}	2.05 ± 0.08^{b}	1.82 ± 0.13^{e}	1.14 ± 0.08^{b}
Si	Without-VC	2.36 ± 0.08^{b}	0.57 ± 0.08^{b}	2.03 ± 0.09^{c}	1.97 ± 0.13^{c}	1.86 ± 0.11^{d}	1.09 ± 0.09^{c}
Si	With-VC	2.42 ± 0.06^{a}	0.63 ± 0.05^{a}	2.17 ± 0.08^{a}	2.16 ± 0.12^{a}	1.75 ± 0.11^{f}	1.23 ± 0.09^{a}
ANOVA	Df			P-value			
FS	2	<0.001	0.002	<0.001	<0.001	<0.001	<0.001
VC	1	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
FS×VC	2	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Second season							
Tap water	Without-VC	1.84 ± 0.05^{e}	0.38 ± 0.03^{e}	1.57 ± 0.08^{f}	1.15 ± 0.07^{f}	2.21 ± 0.16^{a}	0.70 ± 0.06^{f}
Tap water	With-VC	2.23 ± 0.06^{d}	0.51 ± 0.04^{d}	1.79 ± 0.09^{e}	1.37 ± 0.08^{e}	1.88 ± 0.12^{b}	0.95 ± 0.08^{e}
Lipoic acid	Without-VC	2.32 ± 0.09^{c}	0.56 ± 0.02^{c}	1.92 ± 0.10^{d}	1.72 ± 0.06^{d}	1.85 ± 0.15^{c}	1.03 ± 0.09^{d}
Lipoic acid	With-VC	2.42 ± 0.09^{ab}	0.61 ± 0.04^{b}	2.09 ± 0.16^{b}	2.08 ± 0.12^{b}	1.75 ± 0.15^{d}	1.19 ± 0.09^{b}
Si	Without-VC	2.38 ± 0.08^{bc}	0.60 ± 0.05^{b}	2.05 ± 0.12^{c}	1.99 ± 0.05^{c}	1.83 ± 0.16^{c}	1.12 ± 0.06^{c}
Si	With-VC	2.45 ± 0.07^{a}	0.66 ± 0.05^{a}	2.19 ± 0.13^{a}	2.19 ± 0.11^{a}	1.69 ± 0.13^{d}	1.29 ± 0.06^{a}
ANOVA	df			P-value			
FS	2	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
VC	1	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
FS×VC	2	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Leaf osmo-protector and antioxidant contents

The co-application of vermicompost-tea and/or lipoic acid or Si significantly increased total soluble sugars, free proline, ascorbate, glutathione, and α-tocopherol contents in maize plants under salt stress (Table 5). The combined application of Si showed the most notable increase in total soluble sugars by 64.5% and 62.9%, proline by 60.4% and 59.6%, ascorbate by 42.9% and 45.9%, glutathione by 84.3% and 82.6%, and α-tocopherol by 73.1% and 70.9% during the two seasons, respectively. Furthermore, the co-application of vermicompost-tea and lipoic acid increased the studied osmo-protectors and antioxidants, demonstrating a considerable response in maize leaves under salt stress. It fortified total soluble sugars by 62.9% and 61.8%, proline by 56.6% and 55.5%, ascorbate by 38.0% and 37.7%, glutathione by 77.1% and 75.5% and α-tocopherol by 68.9% and 68.4% during two seasons respectively compared to untreated control.

All assessed treatments of vermicompost-tea and/or Si or lipoic acid significantly impacted catalase, superoxide dismutase, ascorbate peroxidase, peroxidase, and glutathione reductase in maize leaves levels compared with the untreated control under salt stress (Table 6). The combined application of Si and vermicompost-tea displayed the most substantial increase in catalase by 17.6% and 18.2%, peroxidase by 102.0% and 100.0%, ascorbate peroxidase by 16.1% and 15.8%, superoxide dismutase by 116.0% and 115.0%, and glutathione reductase by 50.1% and 50.0% compared untreated control during the two seasons, respectively. This co-application surpassed other integrative treatments in both seasons. Similarly, the combined application of lipoic acid and vermicompost-tea stimulated eased catalase by 17.7% and 14.5%, peroxidase by 94.0% and 93.9%, ascorbate peroxidase by 14.8% and 14.6%, superoxide dismutase by 110.0% and 109.0%, and glutathione reductase by 47.5% and 47.4% during two seasons respectively compared to untreated control.

Table 5. Impact of vermicompost-tea application and/or exogenously sprayed lipoic acid or Si on leaf osmo-protector and antioxidant contents of salt-stressed maize plants across 2021 and 2022 growing seasons. The data presented as mean ± standard error (SE). Same letters within each column for each season reveal nonsignificant variations according to LSD test (P ≤ 0.05).

Foliar spray (FS)	Vermicompost (VC)	Proline	Total soluble sugars	α-Tocopherol	Ascorbate	Glutathione
		μg g^{-1}	mg g^{-1}	μmol g^{-1}	μmol g^{-1}	μmol g^{-1}
First season						
Tap water	Without-VC	23.5 ± 1.6^{f}	17.6 ± 1.2^{e}	1.90 ± 0.15^{e}	1.21 ± 0.11^{f}	0.96 ± 0.07^{e}
Tap water	With-VC	32.5 ± 1.8^{e}	24.5 ± 1.3^{d}	2.98 ± 0.21^{d}	1.46 ± 0.12^{e}	1.45 ± 0.12^{d}
Lipoic acid	Without-VC	34.2 ± 2.3^{d}	26.6 ± 1.5^{c}	3.10 ± 0.32^{c}	1.50 ± 0.16^{d}	1.60 ± 0.16^{c}
Lipoic acid	With-VC	36.8 ± 2.6^{b}	28.5 ± 1.8^{a}	3.21 ± 0.32^{b}	1.67 ± 0.15^{b}	1.70 ± 0.12^{b}
Si	Without-VC	35.9 ± 1.5^{c}	27.6 ± 1.6^{b}	3.19 ± 0.31^{b}	1.59 ± 0.13^{c}	1.67 ± 0.15^{b}
Si	With-VC	37.7 ± 2.7^{a}	28.8 ± 1.7^{a}	3.29 ± 0.36^{a}	1.73 ± 0.13^{a}	1.77 ± 0.17^{a}
ANOVA	df			P-value		
FS	2	<0.001	<0.001	<0.001	<0.001	<0.001
VC	1	<0.001	<0.001	<0.001	<0.001	<0.001
FS×VC	2	<0.001	<0.001	<0.001	<0.001	<0.001
Second season						
Tap water	Without-VC	23.8 ± 1.3^{f}	17.8 ± 1.2^{e}	1.93 ± 0.16^{d}	1.22 ± 0.14^{f}	0.98 ± 0.05^{e}
Tap water	With-VC	32.7 ± 2.2^{e}	24.8 ± 1.3^{d}	2.87 ± 0.19^{c}	1.48 ± 0.16^{e}	1.47 ± 0.09^{d}
Lipoic acid	Without-VC	34.5 ± 1.3^{d}	26.8 ± 1.7^{c}	3.00 ± 0.21^{abc}	1.53 ± 0.19^{d}	1.61 ± 0.13^{c}
Lipoic acid	With-VC	37.0 ± 2.6^{b}	28.8 ± 1.9^{a}	3.25 ± 0.28^{a}	1.68 ± 0.13^{b}	1.72 ± 0.15^{b}
Si	Without-VC	36.2 ± 1.5^{c}	27.8 ± 1.8^{b}	3.22 ± 0.25^{ab}	1.65 ± 0.16^{c}	1.69 ± 0.11^{b}
Si	With-VC	38.0 ± 2.7^{a}	29.0 ± 1.8^{a}	3.30 ± 0.26^{a}	1.78 ± 0.14^{a}	1.79 ± 0.14^{a}
ANOVA	df			P-value		
FS	2	<0.001	<0.001	<0.001	0.002	<0.001
VC	1	<0.001	<0.001	<0.001	<0.001	<0.001
FS×VC	2	<0.001	<0.001	<0.001	<0.001	<0.001

Table 6. Impact of vermicompost-tea application and/or exogenously sprayed lipoic acid or Si on leaf antioxidant enzyme activities of salt-stressed maize plants across 2021 and 2022 growing seasons. The data presented as mean ± standard error (SE). Same letters within each column for each season reveal nonsignificant variations according to LSD test (P ≤ 0.05).

Foliar spray (FS)	Vermicompost (VC)	Catalase	Peroxidase	Ascorbate peroxidase	Superoxide dismutase	Glutathione reductase
		A_{564} min^{-1} g^{-1} protein				
First season						
Tap water	Without-VC	60.6 ± 3.6^{e}	0.84 ± 0.07^{f}	56.9 ± 2.5^{e}	3.34 ± 0.25^{f}	34.1 ± 2.6^{f}
Tap water	With-VC	64.3 ± 3.8^{d}	1.16 ± 0.11^{e}	60.9 ± 3.1^{d}	4.74 ± 0.26^{e}	45.2 ± 3.6^{e}
Lipoic acid	Without-VC	67.9 ± 4.1^{c}	1.33 ± 0.11^{d}	62.8 ± 3.2^{c}	6.32 ± 0.65^{d}	47.5 ± 3.8^{d}
Lipoic acid	With-VC	69.5 ± 4.6^{b}	1.63 ± 0.16^{b}	65.3 ± 3.6^{b}	7.03 ± 0.55^{b}	50.3 ± 3.9^{b}
Si	Without-VC	69.4 ± 3.9^{b}	1.56 ± 0.16^{c}	63.0 ± 3.8^{c}	6.76 ± 0.65^{c}	48.4 ± 3.7^{c}
Si	With-VC	71.3 ± 4.5^{a}	1.70 ± 0.15^{a}	66.1 ± 3.9^{a}	7.22 ± 0.45^{a}	51.2 ± 3.6^{a}
ANOVA	df			P value		
FS	2	<0.001	<0.001	<0. 001	<0.001	<0.001
VC	1	<0.001	<0.001	<0.001	<0.001	<0.001
FS×VC	2	<0.001	<0.001	<0.001	<0.001	<0.001
Second season						
Tap water	Without-VC	60.8 ± 4.2^{e}	0.87 ± 0.06^{e}	57.3 ± 3.1^{d}	3.37 ± 0.15^{e}	34.3 ± 2.5^{f}
Tap water	With-VC	63.7 ± 4.3^{d}	1.20 ± 0.12^{d}	61.3 ± 4.2^{c}	4.77 ± 0.25^{d}	45.6 ± 3.7^{e}
Lipoic acid	Without-VC	68.4 ± 4.6^{c}	1.37 ± 0.14^{c}	63.0 ± 4.5^{b}	6.40 ± 0.54^{c}	47.7 ± 3.5^{d}
Lipoic acid	With-VC	70.1 ± 4.9^{b}	1.66 ± 0.13^{ab}	66.1 ± 4.3^{a}	7.11 ± 0.81^{b}	50.6 ± 4.2^{b}
Si	Without-VC	69.8 ± 4.8^{b}	1.58 ± 0.13^{b}	63.3 ± 3.9^{b}	6.84 ± 0.33^{b}	48.8 ± 3.6^{c}
Si	With-VC	71.9 ± 4.8^{a}	1.74 ± 0.14^{a}	66.4 ± 4.9^{a}	7.26 ± 0.63^{a}	51.5 ± 4.1^{a}
ANOVA	df			P value		
FS	2	<0.001	<0.001	<0.001	<0.001	<0.001
VC	1	<0.001	<0.001	<0.001	<0.001	<0.001
FS×VC	2	<0.001	<0.001	<0.001	<0.001	<0.001

Agronomic traits

Compared to untreated maize plants exposed to salt stress, all applied treatments of vermicompost-tea and/or lipoic acid or Si significantly enhanced plant height, number of rows per ear, number of grains per row, grain yield, biological yield, and 1000-grain weight across both 2021 and 2022 seasons (Table 7). The greatest improvements were observed with the integrative application of vermicompost-tea and Si or lipoic acid. The co-application of Si and vermicompost-tea was the most effective, followed by lipoic acid and vermicompost-tea under salinity conditions compared to untreated control. The co-application of lipoic acid and vermicompost-tea ameliorated plant height by 23.5% and 26.7%, number of grains per row by 44.1% and 42.4%, number of rows per ear by 31.8% and 21.3%, grain yield by 45.3% and 45.4%, biological yield by 42.8% and 42.7%, and 1000-grain weight by 30.3% and 21.5% across both seasons of 2021 and 2022 respectively compared to untreated control under salinity stress.

Association among assessed treatments and studied parameters under salinity stress

Exploring the relationship between the studied treatments and observed parameters is a crucial aspect that can provide beneficial insights. The relationship among the studied biochemical components, antioxidant defenses, growth, and yield of maize plants was examined using the heatmap. This analysis grouped the treatments into distinct clusters (Figure 1). The co-application of Si and vermicompost-tea, followed by lipoic acid and vermicompost-tea, possessed the most favorable performance across the majority of evaluated traits. Conversely, the untreated control exhibited unfavorable performance.

Table 7. Impact of vermicompost-tea application and/or exogenously sprayed lipoic acid or Si on agronomic traits of salt-stressed maize plants across 2021 and 2022 growing seasons. The data presented as mean ± standard error (SE). Same letters within each column for each season reveal nonsignificant variations according to LSD test ($P \leq 0.05$).

Foliar spray (FS)	Vermicompost (VC)	Plant height	Number of grains per row	1000 grain weight	Grain yield	Biological yield
		cm		g	kg ha^{-1}	kg ha^{-1}
			First season			
Tap water	Without-VC	171.2 ± 7.3^{f}	22.3 ± 1.3^{f}	171.8 ± 6.3^{f}	4000 ± 9.3^{f}	9180 ± 13^{e}
Tap water	With-VC	186.9 ± 8.2^{e}	26.4 ± 1.6^{e}	202.6 ± 6.9^{e}	4903 ± 9.8^{e}	11903 ± 16^{d}
Lipoic acid	Without-VC	202.5 ± 9.8^{d}	29.1 ± 2.7^{d}	208.8 ± 8.3^{d}	5075 ± 8.6^{d}	12437 ± 19^{c}
Lipoic acid	With-VC	211.4 ± 7.6^{b}	32.1 ± 2.6^{b}	223.8 ± 8.9^{b}	5811 ± 12^{b}	13110 ± 18^{b}
Si	Without-VC	201.4 ± 9.3^{c}	30.8 ± 2.2^{c}	216.2 ± 8.6^{c}	5491 ± 9.4^{c}	13001 ± 16^{b}
Si	With-VC	217.1 ± 9.5^{a}	35.5 ± 2.4^{a}	230.3 ± 8.5^{a}	6004 ± 10^{a}	13855 ± 15^{a}
ANOVA	df			P-value		
FS	2	<0.001	<0.001	<0.001	<0.001	<0.001
VC	1	<0.001	<0.001	<0.001	<0.001	<0.001
FS×VC	2	<0.001	<0.001	<0.001	<0.001	<0.001
			Second season			
Tap water	Without-VC	170.6 ± 8.4^{f}	23.7 ± 1.7^{e}	193.2 ± 9.3^{f}	4042 ± 11^{d}	9205 ± 16^{e}
Tap water	With-VC	190.7 ± 8.5^{e}	28.4 ± 1.4^{d}	213.7 ± 9.6^{e}	4970 ± 12^{c}	11926 ± 13^{d}
Lipoic acid	Without-VC	196.2 ± 9.2^{d}	30.7 ± 2.8^{c}	219.4 ± 8.7^{d}	5142 ± 16^{c}	12483 ± 20^{c}
Lipoic acid	With-VC	216.2 ± 8.9^{b}	33.6 ± 2.3^{b}	234.8 ± 9.2^{b}	5878 ± 15^{a}	13143 ± 21^{b}
Si	Without-VC	205.2 ± 7.3^{c}	32.4 ± 2.5^{b}	228.3 ± 8.9^{c}	5557 ± 14^{b}	13025 ± 19^{b}
Si	With-VC	220.6 ± 8.8^{a}	37.4 ± 2.7^{a}	240.8 ± 9.3^{a}	6071 ± 13^{a}	13878 ± 23^{a}
ANOVA	df			P-value		
FS	2	<0.001	0.003	<0.001	<0.001	<0.001
VC	1	<0.001	<0.001	<0.001	<0.001	<0.001
FS×VC	2	<0.001	<0.001	<0.001	<0.001	<0.001

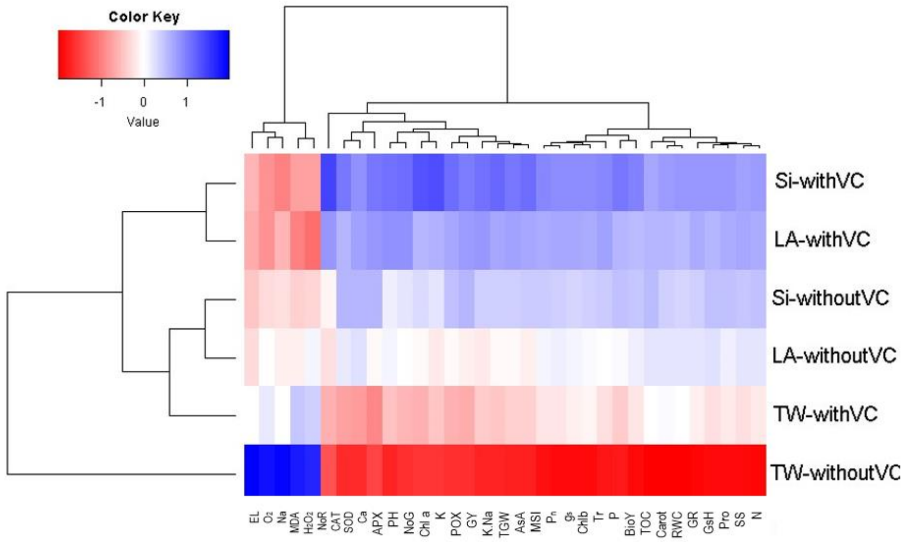

Figure 1. Heatmap for the evaluated physiological, biochemical, and agronomic traits of salt-stressed maize plants treated with vermicompost-tea and/or exogenously sprayed Si or lipoic acid. LA: Lipoic acid; VC: vermicompost; TW: tap water; EL: electrolyte leakage; O_2: superoxide radical; MDA: malondialdehyde; H_2O_2: hydrogen peroxide; CAT: catalase; SOD: superoxide dismutase; APX: ascorbate peroxidase; PH: plant height; NoG: number of grains per row; Chl a: chlorophyll *a*; POX: peroxidase; GY: grain yield; TGW: 1000 grain weight; AsA: ascorbate; MSI: membrane stability index; P_n: net photosynthetic rate; g_S: stomatal conductance; Chlb: chlorophyll *b*; Tr: transpiration rate; BioY: biological yield; TOC: α-tocopherol; Carot: carotenoids; RWC: relative water content; GR: glutathione reductase; GSH: glutathione; Pro: proline; SS: total soluble sugars.

DISCUSSION

Maize is highly sensitive to salt stress, posing a threat to its production, especially with predicted increases in salinity due to climate change. Soil salinity diminishes water availability to plant roots, affecting metabolic processes, meristematic activity, and cell elongation. This leads to decreased photosynthesis and increased respiration rates due to the osmotic stress induced by salt, resulting in nutritional imbalance, toxicity, and oxidative stress. Furthermore, salinity stress induces the generation of reactive oxygen species (ROS) in plants, resulting in oxidative damage to cellular components such as chlorophyll, DNA, membranes, and proteins. These negative impacts lead to inhibiting growth and productivity in plants grown under saline conditions. Accordingly, enhancing the salt tolerance of maize plants is critical to mitigating these adverse effects to sustain maize production and ensure global food security. The present work aimed to explore the impact of exogenously applied lipoic acid or Si, alone or in integration with soil application of vermicompost-tea on physiological parameters, antioxidant defense systems, biochemical components, growth, and yield of maize plants exposed to salt stress. The results exhibited that the application of vermicompost-tea, Si, or lipoic acid significantly enhanced physiological attributes, antioxidant enzyme activities, and agronomic traits in salt-stressed maize plants compared with the untreated control.

The application of soil-based vermicompost-tea significantly enhanced all studied physiological parameters compared to untreated control under salinity conditions. The remarkable effects of vermicompost can be attributed to its rich microbial activity, fostering the production of growth regulators such as gibberellins, auxins, and cytokinins from bacteria, fungi, actinomycetes, yeasts, and algae. Furthermore, vermicompost contains humic substances that enhance the availability of critical nutrients such as N, P, K, Mg, Fe, and Zn, facilitating the synthesis of tryptophan, a precursor to auxins crucial for rooting and growth regulators. This directly and indirectly leads to increased chlorophyll synthesis and consequent enhancement in chlorophyll levels. In this context, previous reports indicate an increase in total chlorophylls due to the application of vermicompost under stress conditions (Aslam et al., 2023). Additionally, Tammam et al. (2023) elucidated that vermicompost regulates protein expression at the translational level, potentially safeguarding photosynthetic components and mitigating the detrimental effects induced by salt stress.

The combined application of soil-based vermicompost with exogenously sprayed Si or lipoic acid with compost-tea exhibited the most favorable outcomes among the treatments. Applied Si significantly enhanced photosynthetic pigments, photosynthetic efficiency, water relation, nutrient content, accumulation of osmoprotectants, and antioxidant enzymes. It played a crucial role in bolstering the stability of cell membranes in plants facing water deficit stress, safeguarding these membranes from structural and functional degradation. This function was vital in maintaining membrane integrity and preserving the functions of water deficit-stressed plants, ultimately contributing to improved plant growth and production by mitigating salinity stress. Likewise, previous reports highlighted the positive impact of Si on physiological functions and growth parameters under stressful conditions (Zargar et al., 2019). In this respect, Bhardwaj et al. (2023) elucidated that Si influence on the growth of stressed plants is multifaceted, such as improving cell wall metabolism, increasing tissue extensibility, adjusting cell wall metabolism, increases leaf rigidity by altering its texture, and maintaining nutrient balance thereby enhancing tissue extensibility and improving cell physiological and biochemical processes. Furthermore, Arif et al. (2021) disclosed that Si enhances plant tolerance to stressors by improving photosynthesis rates related to leaf ultrastructure and chlorophyll content, boosting water uptake and transport under stress, improving the uptake of essential elements and the activity of essential enzymes. Besides, Misra et al. (2023) manifested that Si promotes leaf blade erectness, preserving elevated water use efficiency and leaf water potential and facilitating better light penetration for improved photosynthesis under stressed conditions.

Stressed maize plants treated with lipoic acid showed significant improvement in chlorophyll content, photosynthetic process in maize plants in the antioxidant defense system, and reductions in MDA and electrolyte leakage contents, hence enhancing growth and production maize plants subjected to salinity stress. The foliar application of lipoic acid mitigated salt-induced MDA and electrolyte leakage accumulation while improved the membrane stability index and relative water content. In this context, Gorcek and Erdal (2015) demonstrated that its foliar application significantly enhanced plant growth and productivity under salinity conditions by modifying ion homeostasis and bolstering the antioxidant defense system, thereby reducing ROS ies levels. Gorcek and Erdal (2015) also highlighted the role of lipoic acid in reducing salt-induced MDA and electrolyte leakage accumulation, participating in several enzyme complexes, serving as a redox couple and aiding in electron transfer processes under saline conditions. Those mentioned above practical enhancements of combined soil-based vermicompost-tea with exogenously sprayed Si or lipoic acid on photosynthetic pigments, photosynthetic efficiency, water relation, nutrient content, accumulation of osmoprotectants, and enzymatic and non-enzymatic antioxidant activities exhibited the most favorable outcomes against salinity stress.

CONCLUSIONS

Vermicompost-tea, Si, and lipoic acid applications appeared as potent natural biostimulants to enhance salt tolerance mechanisms in maize plants subjected to saline conditions compared to untreated plants under salinity stress. The combined soil-based vermicompost-tea with exogenously sprayed Si or lipoic acid exhibited effectively enhanced photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, and carotenoids), photosynthetic efficiency (net photosynthetic rate, transpiration rate, and stomatal conductance), antioxidant mechanisms; both enzymatic (catalase, peroxidase, ascorbate peroxidase, superoxide dismutase, and glutathione reductase) and non-enzymatic (soluble sugars, free proline, ascorbate, glutathione, and α-tocopherol), water relation (relative water content and membrane stability index), nutrient content (N, P, Ca and K), accumulation of osmoprotectants and antioxidant enzymes. Moreover, these combined applications considerably reduced the presence of harmful reactive oxygen species such as H_2O_2 and $O_2^{\bullet-}$ radicals. Hence these applications fortified plant growth and yield traits enabling better salt tolerance under salt-affected soil. Accordingly, this study highlighted the potential of these applications to attenuate the adverse effects of soil salinity on maize production under arid environments. Consequently, employing soil-based vermicompost-tea with exogenously applied Si or lipoic acid is an efficient strategy for enhancing salt tolerance in arid environments.

Author contributions

Conceptualization: K.A., E.M.D., M.M.R., E.S. Methodology: K.A., E.M.D., M.M.R., E.S. Software: K.A., E.M.D., M.M.R., E.S. Validation: K.A., E.M.D., M.M.R., E.S. Formal analysis: K.A., E.M.D., M.M.R., E.S. Investigation: K.A., E.M.D., M.M.R., E.S. Resources: K.A., E.M.D., M.M.R., E.S. Data curation: K.A., E.M.D., M.M.R., E.S. Writing-original draft preparation: K.A., E.M.D., M.M.R., E.S. Writing-review and editing: E.M.D., M.M.R., E.S. Visualization: K.A., E.M.D., M.M.R., E.S. Supervision: E.M.D., M.M.R., E.S. Funding acquisition: K.A., E.M.D. All authors have read and agreed to the published version of the manuscript.

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