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EXOGENOUS APPLICATION OF COMPOST EXTRACT AND VERMIWASH ENHANCES GROWTH, NUTRIENT UPTAKE AND ANTIOXIDANT ACTIVITY IN SPRING MAIZE UNDER HEAT STRESS

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Abstract

Heat stress is a threat to maize growth, development, and productivity. The application of compost extract and vermiwash has been proven to be an efficient approach to combat the heat-induced growth and developmental limitation of maize crop and to promote sustainable crop production. Maize is a significant cereal crop and its yield is significantly reduced due to heat stress. Thermotolerance in the maize crop can be improved by the application of exogenously applied compost extract and vermiwash. With the objective to cope with heat stress, a wire-house experiment was performed where two maize hybrids, viz., YH-5507 and DK-6724 (heat-tolerant and -sensitive hybrids, respectively) were grown in pots under heat stress and control conditions. Five exogenously applied organic amendments levels C0 (control, no amendment), CE1 (8% compost extract), CE2 (10% compost extract), VW1 (6% vermiwash) and VW2 (8% vermiwash), were used in soil-filled pots under heat stress and control conditions. The data depicted that compared with control condition, heat stress recorded a significant reduction in morphological, physiological, biochemical and nutritional attributes. Nonetheless, it was observed that exogenously applied compost extract and vermiwash, particularly VW1 and CE2 followed by VW2 ameliorated the negative effects of heat stress. Under heat stress condition, a significant and positive influence on all attributes was recorded for VW1 treatment, which increased nitrate by 22.54%, phosphate by 32.60%, sulphate by 81.45%, SOD by 35.41%, POD by 49.41% and CAT by 36.19%, on average of two maize hybrids, compared to control condition. Among hybrids, YH-5507 showed comparatively more resistance against heat stress. The findings of this work revealed that heat stress drastically decreased the growth and productivity of maize; however, exogenously applied compost extract and vermiwash positively influenced the performance of maize hybrids.

Key words: Antioxidant activity; Compost extract; Heat stress; Maize; Nutrient uptake; Sustainable crop production; Vermiwash

Introduction

Heat stress is a serious threat to food security and maize productivity (Tiwari & Yadav, 2019). However, maize hybrids response varies for morphological and physiological attributes during heat stress (Yousaf *et al.*, 2022). Under heat stress, plants show different responses such as orientation of leaf, closing of stomata, membrane lipid composition, early maturation, accumulation of osmo-protectants, antioxidants defense and protein synthesis against the impacts of heat stress (Singh *et al.*, 2015; Hassan *et al.*, 2021; Mustafa *et al.*, 2021). These responses are imperative indicator for selecting heat tolerant maize hybrids (Deva *et al.*, 2020). So, abiotic factors impacts the maize productivity (Wamatsembe *et al.*, 2017; Sabagh *et al.*, 2021). Among abiotic stresses, heat stress reduces growth and yield of maize crop (Chavez-Arias *et al.*, 2021; Krasilnikov *et al.*, 2022). Maize is a highly heat sensitive crop which causes major losses in crop productivity (Niu *et al.*, 2021). So, heat stress effects change with plant life cycle stages and reduces maize growth and productivity due to pollen abortion, silk drying, and limiting physiological processes (Lizaso *et al.*, 2018; Ullah *et al.*, 2022; Wang *et al.*, 2024). Furthermore, heat stress enhances electrolyte leakage and reactive oxygen species production and causing the oxidative stress, cell membrane damages and lipid peroxidation (Mustafa *et al.*, 2021; Han *et al.*, 2022).

Maize (*Zea mays* L.) belongs to Poaceae family; is a third most important cereal crop and has a premier nutritive value, and ranks as the third most important cereal crop in Pakistan. Moreover, it plays a substantial role in food demand and value addition to the economy (Ali *et al.*, 2021). It is widely cultivated in autumn and spring seasons for animal feed and used as a raw material for industrial purposes. Maize production during the spring season is severely constrained by heat stress, particularly at reproductive stages such as silking, anthesis, and grain development, leading to reduced photosynthetic efficiency, impaired crop growth, poor seed quality, and significant yield losses (Bassu *et al*., 2014; Ostmeyer *et al*., 2020; Ravikiran *et al*., 2020; Noor *et al*., 2019; Ahsan *et al*., 2020; Ullah *et al*., 2018). In Pakistan, heat stress is a major limitation for spring-sown maize, where an increase of 2°C in global temperature may reduce maize yield by 8–14% (Lana *et al*., 2017; Yousaf *et al*., 2021). Previous studies have consistently reported the adverse effects of heat stress on maize growth, productivity, and crop health (Yousaf *et al*., 2017; Yousaf *et al*., 2020; Riaz *et al*., 2021). Therefore, it is necessary to reduce heat stress impacts in maize plants by using some strategies, including use of organic amendments and heat tolerant varieties. In this context,

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exogenously applied compost extract and vermiwash were applied for alleviating adverse impacts of high temperature in maize. Vermiwash improves abiotic stress tolerance by enhancing fresh and dry weight, relative water content, shoot and root length, antioxidant activities, protein content, carotenoid content and chlorophyll content (Riya *et al.*, 2024; Oyege *et al.*, 2025). It reduces H_2O_2, MDA, superoxide anion, ion leakage and proline levels while, enhanced antioxidant and photosynthetic efficiency, ion homeostasis and water relations of plants. Riya *et al*., (2024) reported that it also reduced superoxide accumulation, lipid peroxidation, cell death and cells protection to mitigate oxidative stress during abiotic stress in plants. In another study, it was also demonstrated that vermiwash minimized electrolyte leakage and oxidative stress, while improved antioxidant enzyme activities to alleviate abiotic stress (Ruiz-Lau *et al.*, 2020).

Compost extract is a liquid organic fertilizer which increases nutrient contents in soil to enhance plant nutrient uptake, growth and yield (Welke, 2005). It reduces oxidative stress, promoted plant growth and development and enhanced agronomic attributes i.e root development under abiotic stress condition (Lerma-Moliz *et al.*, 2025). It has remarkable biofertilizing and antioxidant properties which enhance growth attributes, osmolyte accumulation while decreased MDA and its effects depends upon crop sensitivity (Samet *et al.*, 2018). Therefore, compost extract increases microbial diversity in soil leading to increased availability of nutrients for plants (Lerma-Moliz *et al.*, 2024). Compost extract is an eco-friendly fertilizing strategy which increases nitrogen content, vegetative growth of plants, antioxidant capacity, quality of the crop and soil fertility (Mohd Din *et al.*, 2017). Compost extract also enhanced photosynthetic rate, stomatal conductance, chlorophyll content, soluble sugar content, SOD, CAT, POD, APX, while reducing singlet oxygen and hydrogen peroxide in seedling roots which maintained integrity of the mitochondria (Talei, 2018; Li *et al.*, 2024).

Materials and Methods

Source of maize seeds: Seeds of two maize hybrids were used i.e., YH-5507 were obtained from Maize and Millet Research Institute, Yusafwala, Sahiwal, Pakistan and DK-6724 from Monsanto Pakistan Private Limited, Lahore.

Application of vermiwash and compost extract: Vermiwash was prepared by extraction of vermicompost by dissolving 500g of solid vermicompost in 2 L of distilled water and kept for two days. Later on, vermiwash was extracted by using sieve cloth and was applied to plants by foliar application (Aslam & Ahmad, 2020; Aslam *et al*., 2020). For preparation of compost extract, 500 g solid compost was dissolved in 2.0 L of distilled water and kept for two days. Then, compost extract was extracted by using sieve cloth and compost extract was foliar sprayed (Aslam *et al*., 2022). Plants were foliar sprayed with vermiwash and compost extract (Table 1).

Glass canopy experiment: Pot experiment was conducted in the wirehouse at Student Research Farm, Department of Agronomy, University of Agriculture, Faisalabad. Each plastic pot size was i.e., 31 cm depth with 24 cm diameter and containing 8 kg loamy soil. Then normal irrigated water was given to plants and after the soil moisture contents were at their field capacity, sowing was done. In each pot, ten seeds of maize hybrids were sown with a depth of 1-1.5 inches. After germination, six plants were maintained after thining in each pot. Each treatment had three replicates and CRD was applied. The plants were allowed to grow for 20 days after germination. After 20 days of germination of plants were divided into two sets. One set was kept in wire house, while other set was shifted into open door plexiglass fitted canopies for 20 days. In wire house and plexiglass fitted canopy, inside and outside temperature was recorded on the daily basis (Table 2). The canopy temperature was 9-11ºC higher than ambient temperature. Plants were foliar sprayed with vermiwash and compost extract after one week of stress imposition (Table 1). Plants were harvested after 15 days of treatments and, data was recorded for morpho-physio-biochemical attributes.

Table 1. Concentrations of vermiwash and compost extract used in the research.

Organic amendments	Concentration
Vermiwash (VW1)	6%
Vermiwash (VW2)	8%
Compost extract (CE1)	8%
Compost extract (CE2)	10%

Table 2. Daily mean temperature of inside and outside canopy during heat stress condition.

Date	Outside Canopy	Inside Canopy
Feb. 27	19.50	28.50
Feb. 28	20.50	30.00
March 1	20.50	31.00
March 2	22.00	30.00
March 3	22.00	29.50
March 4	21.00	29.00
March 5	20.50	27.00
March 6	20.00	28.50
March 7	20.50	29.00
March 8	23.50	31.00
March 9	20.50	27.50
March 10	23.50	32.00
March 11	23.00	31.00
March 12	23.00	32.50
March 13	25.50	34.00
March 14	25.00	33.00
March 15	24.50	33.00
March 16	24.50	33.50
March 17	24.50	34.50
March 18	24.00	35.00

Data recording: The data recording for morphological, biochemical, physiological and nutritional is given below:

Morphological attributes: The plant was uprooted carefully for root length, washed with tap water, root and shoot length was measured. Root and shoot fresh weights were recorded (immediately after uprooting) on analytical balance immediately after uprooting. Plants were packed into separate envelop papers for determination of root and shoot dry weight and oven dried for one week at 70ºC and recorded on analytical balance.

Biochemical and physiological attributes

(i): Photosynthetic pigments contents: Chlorophyll (*a* and *b*) was estimated using Arnon (1949) method and following Davis (1946) carotenoids were calculated. 0.1 g of fresh leaves were grinded in 80% acetone, then filtered with filter paper and volume up to 5 mL was made by adding 80% acetone and the samples were kept at 4°C for overnight. Centrifuged the samples for 5 minutes at 10000 rpm and recorded absorbance at 663, 645 and 480 nm on spectrophotometer (Hitachi-U-2900). Chlorophyll *a*, chlorophyll *b* and carotenoids were calculated by following formulas:

$$\text{Chl. } a \text{ (mg/g f.wt.)} = [12.7 \text{ (OD 663)} - 2.69 \text{ (OD 645)}] \times V/1000 \times W$$

$$\text{Chl. } b \text{ (mg/g f.wt.)} = [22.9 \text{ (OD 645)} - 4.68 \text{ (OD 663)}] \times V/1000 \times W$$

$$\text{Carotenoids (mg/g f.wt.)} = [\text{OD 480} + 0.114 \text{ (OD 6630} - 0.638 \text{ (OD 645)}/ 2500] \times 1000$$

(ii) Relative water content (RWC): According to Barrs and Weatherley (1962), the fresh weight of flag leaves was recorded on an electronic scale. Leave samples were soaked in distilled water for 24-h, and the weight of leaves was recorded. Then the leaves were oven dried at 70°C, then again the dry weight was taken. RWC was determined utilizing the below equation using the data of turgid weight (TW), fresh weight (FW), and dried weight (DW).

$$\text{RWC (\%)} = [(FW - DW)/ (TW - DW)]*100$$

Oxidative stress and antioxidants

(iii) Hydrogen peroxide (H_2O_2): According to Velikova *et al*., (2000) protocol, fresh leaf sample (0.5 g) in prechilled pestle and mortar was homogenized after adding 0.1% TCA (0.5 mL), then allowed to centrifuge the samples for 15 min at 12,000 rpm. An amount of supernatant (500 µL), 1000 µL of 1 molar KI and potassium phosphate buffer (500 µL) were mixed and absorbance was noted at 390 nm.

(iv): Malondialdehyde (MDA): Following Heath and Packer (1968) procedure, leaf sample (0.5 g) was homogenized in trichloroacetic acid (3 mL) and centrifuged for 15 min at 11,500 rpm. Supernatant (2 mL) was mixed with thiobarbutaric acid (4mL) and allowed to heat for 30 min at 95ºC. Again, samples were centrifuged for 10 min at 11500rpm and absorbance was noted at 532 and 600nm. Computed the data values by using following formula where ε is the extinction coefficient.

$$\text{MDA} = (A532\text{-}A600)/\varepsilon$$

(v): Superoxide dismutase (SOD): According to Ries and Giannopolities (1977) protocol, 50 µL enzyme extract, 50 µL NBT, 100 µL Triton X, 100 µL methionine, 400 µL distilled water, 50 µL riboflavin and 250 µL phosphate buffer with pH 7.6. The final volume was made upto 3 mL and exposed to fluorescent light at room temperature for 15 min and absorbance was measured at 560 nm on spectrophotometer.

(vi): Peroxidase (POD): The supernatant was extracted by adding phosphate buffer having pH 7.8 (Chance & Maehly, 1955). In a cuvette, about 100µL of supernatant, 1mL of buffer, 100µL of guaicol, and 100µL of hydrogen peroxide were added. The absorbance was determined at 470nm after 0, 20, 40, 60 and 80 seconds.

(vii) Catalase (CAT): According to Chance & Maehly (1955) protocol, mixed enzyme extract (100 µL) with phosphate buffer (1.9 mL) and 5.9 mM H_2O_2 (1 mL) in a cuvette and absorbance was determined at 240 nm on spectrophotometer.

Elemental analysis: Dried 0.5 gram plant sample was taken in digestion flasks with concentrated nitric acid and digested at 100-150ºC. The total solution volume up to 50 mL was made by adding distilled water. Solution was filtered and elements were determined.

(i): Sulfate-S determination: According to Tendon (1993) protocol, extract (10 mL) was mixed with 6 N HCl (1 mL), 0.5% gum arabica solution in conical flask and twirled for few seconds. Then 0.5 g barium chloride crystals was added and swirled until all crystals dissolved and solution became clear. From 100mg/L potassium sulfate stock solution, to make sulfate-S standard curve 4-20 mg /L graded series were prepared. A buffer solution prepared by dissolving 28 mL ethyl alcohol, 50 g $MgCl_2.6H_2O$ and 4.1 g KNO_3 in distilled water, while the remaining protocol was same. The absorbance was measured at 340 nm.

(ii): Estimation of phosphate-P: The dried plant material (0.5 g) was heated in distilled water (5 mL) for 1 hour, and volume was made up to 50 mL. 1 mL extract was taken from 50 mL, and mixed with molybdate-vanadate reagent (1 mL) and 2 N HNO_3 (2 mL), then the volume was made up to 10 mL and vortexed for few seconds. The mixture was kept for 20 minutes and observed the absorbance at 420 nm (Yoshida *et al*., 1976). Distilled water was used as blank.

(iii): Determination of nitrate-N: Following Kowalenko & Lowe (1973) method, 0.5 g oven dried plant sample was boiled with 5 mL distilled water for one hour and, then distill water was added to make total volume up to 50 mL. From the extract 3mL was taken in test tube, mixed with 7 mL chromotropic acid and vortexed for few seconds. The color intensity was checked at A_{430}.

Chemicals: All chemicals used in this study were purchased from Merck, Fluka and Sigma and Aldrich companies, and were of either ACS or AR of high purity grades as per requirement of the performed procedures.

Statistical Analysis

All the recorded data was analyzed statistically by using ANOVA techniques and Statistix 8.1 software. LSD test was used to compare treatment means at 5% significant level and graphical presentation of data was made on MS Excel (Steel *et al.*, 1997).

Results

Morphological attributes: The change in the morphological attributes were observed. Results showed that heat stress, maize hybrids, compost extract (CE) and vermiwash (VW) application significantly influenced the morphological attributes of maize hybrids. The weights of both fresh and dried of both hybrids ominously declined under heat stress. Nevertheless, the use of vermiwash and compost extract amplified these characteristics in both heat stress and control conditions, with the VW1 level exhibiting the most significant enhancement. Among all exogenously applied vermiwash (VW) and compost extract (CE) doses, VW1 increased physiological attributes in both maize hybrids, followed by CE2, VW2, CE1 and C0 under control and heat stress conditions. Under heat stress, VW1 plant height was increased by 57.56 and 49.31%, shoot length by 75.09 and 85.94%, root length by 53.03 and 56.73%, shoot fresh weight by 54.74 and 75.08%, root fresh weight by 94.77 and 89.19%, shoot dry weight by 77.58 and 59.04%, root dry weight by 55.98 and 50.35%, and number of roots by 67.19 and 47.78% in DK-6724 and YH-5507, respectively. Under heat stress conditions, YH-5507 depicted more biomass than DK-6724 (Tables 3 and 4).

Physiological attributes: The change in the physiological attributes were observed. The physiological characteristics were profoundly impacted by heat stress. To compare cultivars, the DK-6724 decrease was more prominent than the YH-5507. Physiological attributes in both hybrids were greatly enhanced by exogenously applied vermiwash and compost extract under control and heat stress conditions. Among all exogenously applied vermiwash (VW) and compost extract (CE) doses, VW1 increased physiological attributes in both maize hybrids, followed by CE2, VW2, CE1 and C0 under control and heat stress conditions. Under heat stress, VW1 increased chlorophyll *a* by 69.48 and 62.20%, chlorophyll *b* by 74.62 and 64.13%, carotenoids by 80.35 and 37.82% and, relative water content 46.28 and 43.01% in DK-6724 and YH-5507, respectively. Among the maize hybrids in terms of these characteristics, YH-5507 outperformed than DK-6724 under control and heat stress conditions (Fig. 1).

Oxidative stress and antioxidants: The change in the oxidative stress and antioxidants were observed. The leaf H_2O_2, MDA, SOD, POD and CAT contents of both hybrids were strongly impacted by heat stress. Heat stress pretentious a substantially reduction in the above-mentioned attributes with the highest reduction under heat stress. However, vermiwash and compost extract significantly increased these attributes under control and heat stress conditions. Among all exogenously applied vermiwash (VW) and compost extract (CE) doses, VW1 decreased oxidative stress while increased antioxidants activity in both maize hybrids, followed by CE2, VW2, CE1 and C0 under control and heat stress conditions. Under heat stress, Leaf H_2O_2 content was decreased by 30.05 and 29.42% and MDA by 30.42 and 44.60% in the DK-6724 and YH-5507 hybrids respectively, while leaf SOD content enhanced by 40.14 and 30.68%, POD by 68.71 and 30.11%, and CAT by 39.71 and 32.66% under heat stress at VW1 (6%). As expected, under control and heat stress conditions, YH-5507 had more values for these qualities than DK-6724 (Fig. 2).

Table 3. Effect of morphological attributes in two maize (*Zea mays* L.) hybrids treated with different compost extract and vermiwash under heat stress.

Heat levels	Hybrids	OA	Plant height (cm)	No. of roots per plant	Shoot length (cm)	Root length (cm)
H0	DK-6724	C0	80.17 ± 3.15 l	29.67 ± 1.20 k	41.73 ± 1.41 k	24.07 ± 1.53 k
		CE1	83.57 ± 2.34 k	32.33 ± 1.20 j	45.97 ± 1.27 h	27.43 ± 1.33 ij
		CE2	95.83 ± 2.45 f	38.00 ± 1.73 g	50.08 ± 1.39 d	31.47 ± 1.33 fg
		VW1	101.03 ± 3.32 d	43.33 ± 1.45 e	53.17 ± 1.16 b	33.43 ± 1.13 de
		VW2	91.17 ± 3.09 h	34.33 ± 1.45 i	48.65 ± 1.50 e	29.87 ± 1.27 gh
	YH-5507	C0	89.57 ± 2.97 i	38.00 ± 1.15 g	43.77 ± 1.16 j	26.47 ± 1.39 j
		CE1	96.07 ± 3.32 f	44.67 ± 0.88 d	47.23 ± 1.39 g	30.27 ± 1.10 g
		CE2	108.53 ± 2.48 b	55.33 ± 0.88 b	53.17 ± 1.18 b	37.80 ± 1.00 b
		VW1	113.13 ± 2.54 a	58.00 ± 1.15 a	57.30 ± 1.21 a	40.00 ± 1.20 a
		VW2	99.23 ± 2.68 e	48.67 ± 1.45 c	50.77 ± 1.39 c	34.67 ± 1.18 cd
H1	DK-6724	C0	56.47 ± 2.68 r	21.33 ± 1.45 n	25.30 ± 1.21 r	19.80 ± 0.98 l
		CE1	66.83 ± 2.45 q	24.33 ± 1.45 m	29.10 ± 1.39 p	22.40 ± 1.35 k
		CE2	74.47 ± 2.97 n	32.33 ± 1.20 j	35.19 ± 1.10 n	28.57 ± 0.77 hi
		VW1	88.97 ± 2.80 i	35.67 ± 0.88 h	44.30 ± 1.27 i	30.30 ± 0.46 g
		VW2	68.67 ± 3.18 p	28.33 ± 1.45 l	32.78 ± 1.44 o	25.93 ± 1.21 j
	YH-5507	C0	70.30 ± 2.86 o	30.00 ± 1.15 k	27.03 ± 1.39 q	22.80 ± 0.98 k
		CE1	77.67 ± 3.03 m	33.67 ± 1.45 i	36.30 ± 1.07 m	26.90 ± 0.96 ij
		CE2	93.93 ± 2.40 g	41.00 ± 1.15 f	41.33 ± 1.39 k	32.17 ± 1.21 ef
		VW1	104.97 ± 3.03 c	44.33 ± 1.45 d	47.77 ± 1.67 f	35.73 ± 1.17 c
		VW2	84.83 ± 3.15 j	35.67 ± 0.88 h	37.72 ± 1.50 l	30.13 ± 0.87 gh

Table 4. Effect of morphological attributes in two maize (*Zea mays* L.) hybrids treated with different compost extract and vermiwash under heat stress.

Heat levels	Hybrids	OA	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)
H0	DK-6724	C0	14.13 ± 0.90 f	5.11 ± 0.21 m	3.38 ± 0.16 h	1.95 ± 0.11 i
		CE1	15.40 ± 0.75 e	5.44 ± 0.24 kl	3.78 ± 0.27 f	2.12 ± 0.14 h
		CE2	17.23 ± 0.66 b	6.62 ± 0.16 g	4.49 ± 0.21 d	2.65 ± 0.06 d
		VW1	18.27 ± 0.84 a	7.52 ± 0.27 e	4.87 ± 0.12 b	2.82 ± 0.14 c
		VW2	16.17 ± 0.95 d	6.25 ± 0.17 i	4.21 ± 0.24 e	2.38 ± 0.08 f
	YH-5507	C0	14.07 ± 0.66 f	5.36 ± 0.21 l	3.89 ± 0.25 f	2.28 ± 0.10 fg
		CE1	16.67 ± 0.49 c	6.62 ± 0.23 g	4.45 ± 0.21 d	2.58 ± 0.10 de
		CE2	18.17 ± 0.72 a	9.24 ± 0.32 b	4.90 ± 0.26 b	2.97 ± 0.14 ab
		VW1	18.57 ± 0.66 a	9.79 ± 0.28 a	5.37 ± 0.17 a	3.04 ± 0.10 a
		VW2	18.13 ± 1.13 a	8.16 ± 0.23 c	4.67 ± 0.25 c	2.90 ± 0.12 bc
H1	DK-6724	C0	8.23 ± 0.84 l	2.87 ± 0.16 r	1.90 ± 0.27 l	1.23 ± 0.12 m
		CE1	10.67 ± 0.66 j	3.50 ± 0.18 q	2.57 ± 0.20 k	1.46 ± 0.12 l
		CE2	12.07 ± 0.84 h	4.83 ± 0.24 n	3.08 ± 0.23 i	1.81 ± 0.24 j
		VW1	14.13 ± 0.72 f	5.58 ± 0.24 j	3.38 ± 0.18 h	1.91 ± 0.13 ij
		VW2	10.17 ± 0.84 k	3.91 ± 0.31 p	2.76 ± 0.18 j	1.58 ± 0.10 kl
	YH-5507	C0	8.53 ± 0.95 l	4.16 ± 0.18 o	2.70 ± 0.21 j	1.69 ± 0.16 k
		CE1	11.43 ± 1.13 i	5.52 ± 0.19 jk	3.28 ± 0.20 h	1.82 ± 0.10 j
		CE2	14.33 ± 0.84 f	6.94 ± 0.26 f	3.81 ± 0.21 f	2.18 ± 0.18 gh
		VW1	15.87 ± 0.90 d	7.88 ± 0.21 d	4.29 ± 0.18 e	2.53 ± 0.11 e
		VW2	13.13 ± 0.72 g	6.46 ± 0.18 h	3.54 ± 0.16 g	2.11 ± 0.11 h

Table 5. Effect of nutritional attributes in two maize (*Zea mays* L.) hybrids treated with different compost extract and vermiwash under heat stress.

Heat levels	Hybrids	OA	Nitrate-N (mg/g d.w.)	Phosphate-P (mg/g d.w.)	Sulphate-S (mg/g d.w.)
H0	DK-6724	C0	11.30 ± 0.36 gh	7.91 ± 0.39 i	1.43 ± 0.09 g
		CE1	11.57 ± 0.41 f	8.15 ± 0.38 h	1.56 ± 0.08 f
		CE2	11.85 ± 0.33 cd	9.62 ± 0.36 cd	1.74 ± 0.09 c
		VW1	11.97 ± 0.44 c	9.88 ± 0.39 b	1.88 ± 0.10 ab
		VW2	11.70 ± 0.45 ef	8.84 ± 0.31 f	1.68 ± 0.06 d
	YH-5507	C0	11.40 ± 0.51 g	8.22 ± 0.32 h	1.52 ± 0.10 f
		CE1	11.77 ± 0.34 de	8.99 ± 0.38 e	1.72 ± 0.10 cd
		CE2	12.18 ± 0.41 b	9.72 ± 0.39 c	1.83 ± 0.10 b
		VW1	12.34 ± 0.47 a	10.08 ± 0.42 a	1.91 ± 0.08 a
		VW2	12.12 ± 0.37 b	9.58 ± 0.43 d	1.84 ± 0.10 b
H1	DK-6724	C0	9.16 ± 0.39 o	6.24 ± 0.28 m	0.82 ± 0.10 l
		CE1	9.85 ± 0.42 m	6.90 ± 0.31 l	0.92 ± 0.12 k
		CE2	10.25 ± 0.40 k	7.85 ± 0.27i	1.12 ± 0.10 i
		VW1	11.17 ± 0.42 h	8.51 ± 0.36 g	1.52 ± 0.07 f
		VW2	10.09 ± 0.45 l	7.39 ± 0.32 k	1.04 ± 0.11 j
	YH-5507	C0	9.46 ± 0.35 n	6.92 ± 0.40 l	0.91 ± 0.11 k
		CE1	10.25 ± 0.37 k	7.5 ± 0.36 k	1.16 ± 0.07 i
		CE2	10.97 ± 0.45 i	7.97 ± 0.39 i	1.42 ± 0.12 g
		VW1	11.65 ± 0.40 ef	8.92 ± 0.38 ef	1.62 ± 0.09 e
		VW2	10.65 ± 0.40 j	7.67 ± 0.40 j	1.26 ± 0.12 h

Nutritional attributes: The change in the nutritional attributes were observed. Results revealed that the leaf phosphate, sulphate and nitrate contents of both hybrids were significantly decreased due to exposure of heat stress. However, under both control and heat stress conditions, soil amendments of compost and vermicompost doses substantially increased the nutritional attributes of both maize hybrids, with larger values observed for VW1. Among all exogenously applied vermiwash (VW) and compost extract (CE) doses, VW1 recorded highest increase in nutritional attributes in both maize hybrids, followed by CE2, VW2, CE1 and C0 under control and heat stress conditions. Under heat stress, VW1 increased level of nitrate by 21.92 and 23.17%, phosphate by 36.41 and 28.78%, sulphate by 91.65 and 98.85%, compared to controls where no organic amendment was used. When comparing hybrids, YH-5507 outperformed DK-6724 (Table 5).

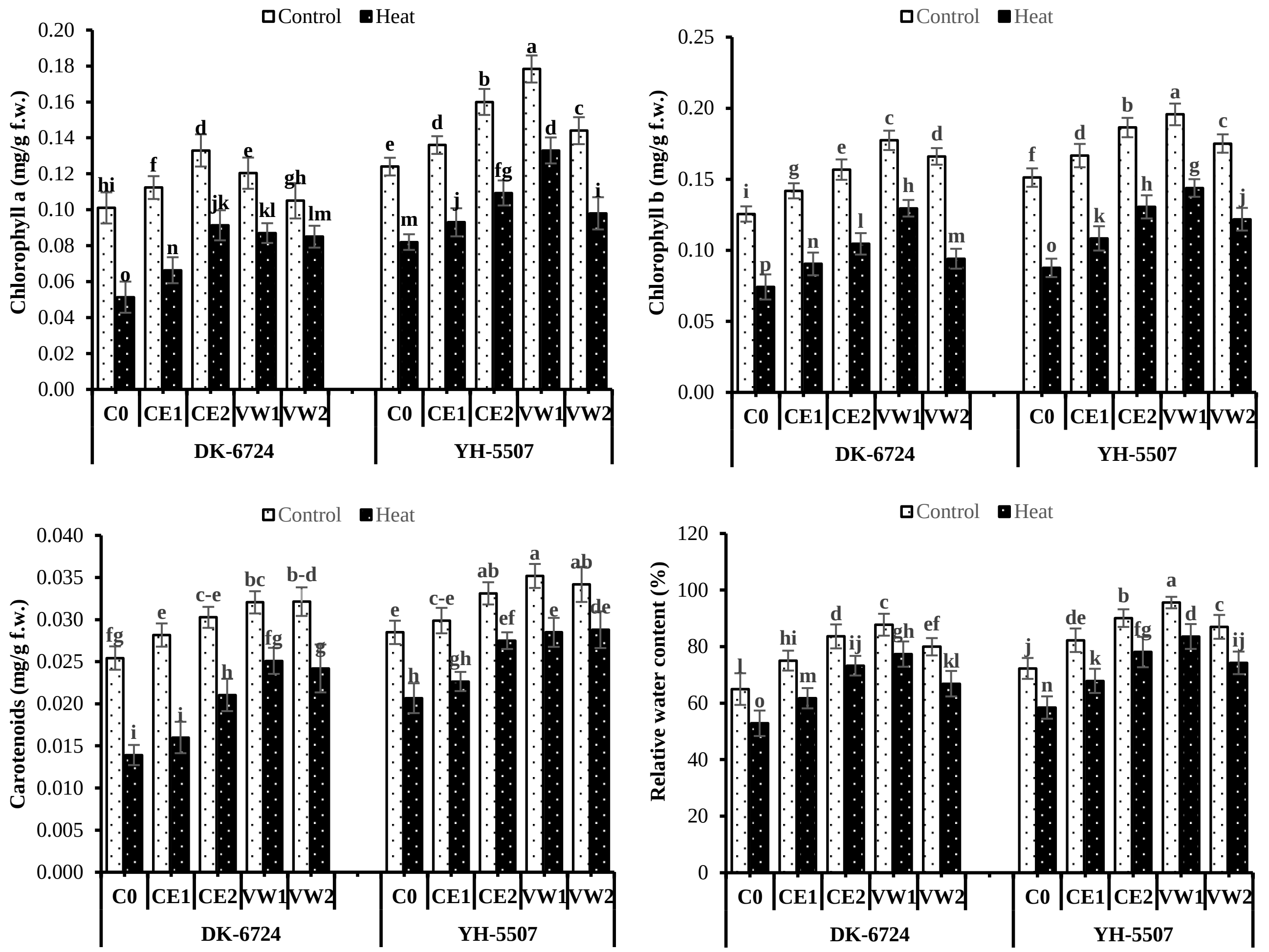

Fig. 1. Chlorophyll *a*, chlorophyll *b*, carotenoids and relative water content in two maize (*Zea mays* L.) hybrids treated with compost extract and vermiwash under heat stress. C0 (control), CE1 (compost extract 8%), CE2 (compost extract 10%), VW1 (vermiwash 6%), VW2 (vermiwash 8%).

Chord diagram: The chord diagram illustrates the interactive relationships between maize growth, physiological, biochemical and nutrient uptake attributes under different treatments of compost extract (CE) and vermiwash (VW) in both hybrids (YH-5507 and DK-6724). A strong positive association was observed between antioxidant enzymes (SOD, POD and CAT) and organic amendment treatments, particularly VW1 and CE2, under heat stress conditions. These treatments also showed pronounced connections with nutrient uptake variables, including nitrate (NO_3^-), phosphate (PO_4^{3-}), and sulphate (SO_4^{2-}), signifying their synergistic role in enhancing metabolic activity under stress. Furthermore, chlorophyll content (Chl a, Chl b and total chlorophyll), relative water content (RWC) and photosynthetic pigments displayed strong correlations with VW1 and CE2, indicating improved photosynthetic efficiency and water balance. In contrast, control treatments (C0) exhibited weak linkages with most physiological and biochemical parameters, confirming the adverse effects of heat stress without organic amendments. Comparatively, the heat-tolerant hybrid YH-5507 formed denser interactions with beneficial traits and treatments than the sensitive DK-6724, demonstrating its superior adaptive potential. Overall, the chord diagram highlighted that exogenous application of compost extract and vermiwash, particularly VW1 and CE2, substantially enhanced inter-parameter coordination, contributing to improved stress tolerance and maize performance (Fig. 3.).

Heatmap analysis: The heatmap illustrates the hierarchical clustering of morphological, physiological and biochemical traits of two maize hybrids (YH-5507 and DK-6724) subjected to control and heat stress conditions with exogenous application of compost extract (CE) and vermiwash (VW). The color gradient from blue to red indicates the magnitude of trait response, where blue shades represent lower values and red shades represent higher values. Treatments with CE and VW markedly improved performance under heat stress, as indicated by elevated intensity in growth (PH, RL, RWC, RWF), physiological (Chl.a, Chl.b, NAR, SL) and biochemical attributes (N, P, S, SDW, RDW, Chl.b, Carotenoids). Under stress, higher antioxidant enzyme activities (SOD, CAT, POD) and reduced oxidative markers (MDA, H_2O_2) were observed in CE2 and VW1 treatments, particularly in the heat-tolerant hybrid (YH-5507). Clustering patterns clearly differentiated the tolerant (V_1) and sensitive (V_2) hybrids and showed the positive impact of optimized CE and VW doses on mitigating heat-induced oxidative stress while enhancing growth and nutrient acquisition efficiency (Fig. 4).

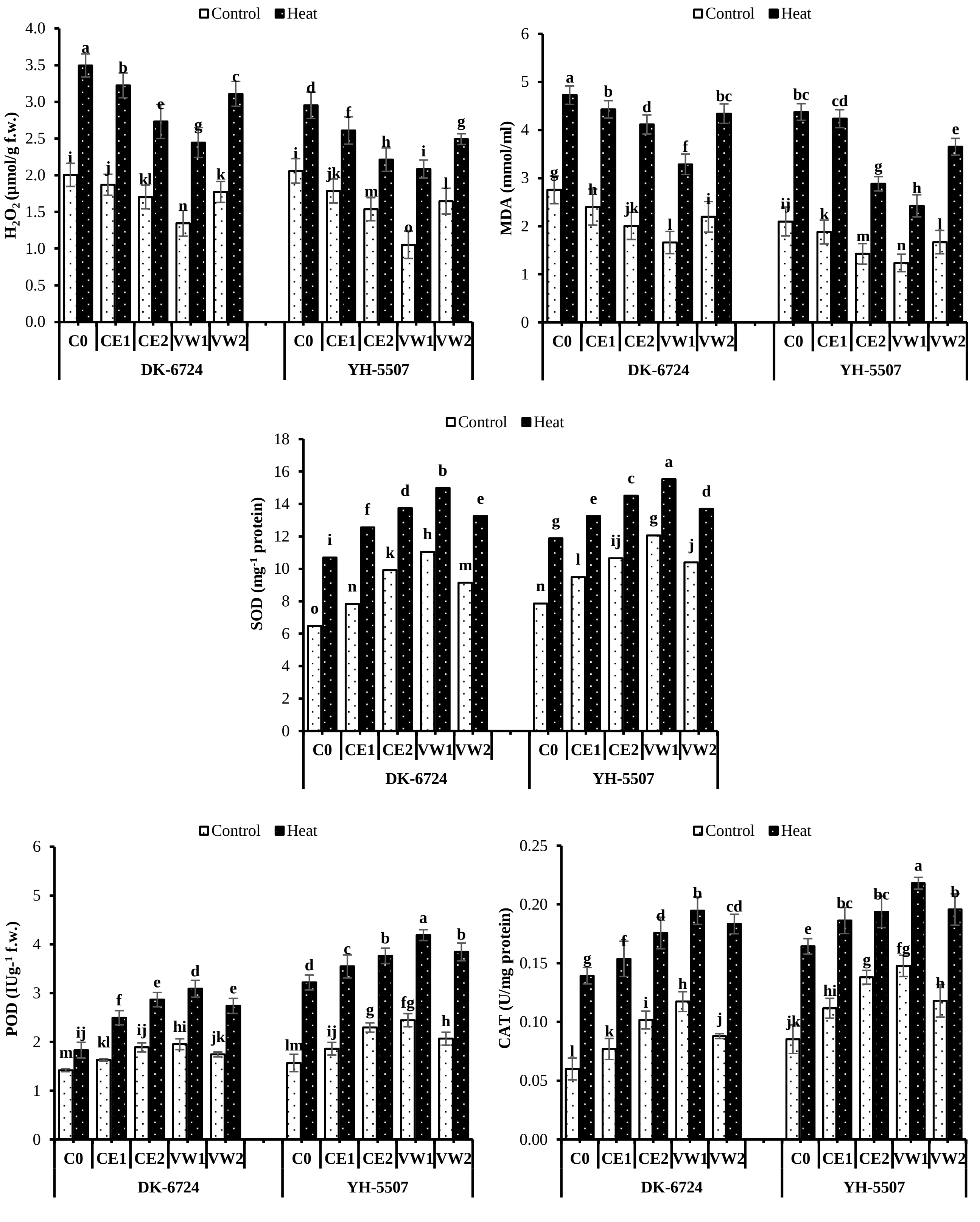

Fig. 2. H_2O_2, MDA, SOD, POD and CAT content in two maize (*Zea mays* L.) hybrids treated with compost extract and vermiwash under heat stress. C0 (control), CE1 (compost extract 8%), CE2 (compost extract 10%), VW1 (vermiwash 6%), VW2 (vermiwash 8%).

Discussion

Global warming is a serious problem, with significant negative impacts on agricultural productivity (Taratima *et al.,* 2022). When temperature rises beyond an optimum level, it leads to heat stress in maize crop by disturbing the physiological and biochemical processes (Naveed *et al.,* 2014; Djalovi *et al.*, 2024). Heat stress is one of the most detrimental abiotic stresses, causing significant reduction in plant growth and yield in tropical and sub-tropical regions (Hussain *et al.*, 2025). Maize is very sensitive to heat stress at reproductive stage and extreme temperature decreases pollen germination ability, and thus, decreases grain yield (Azizi & Rahimi-Moghaddam, 2020).

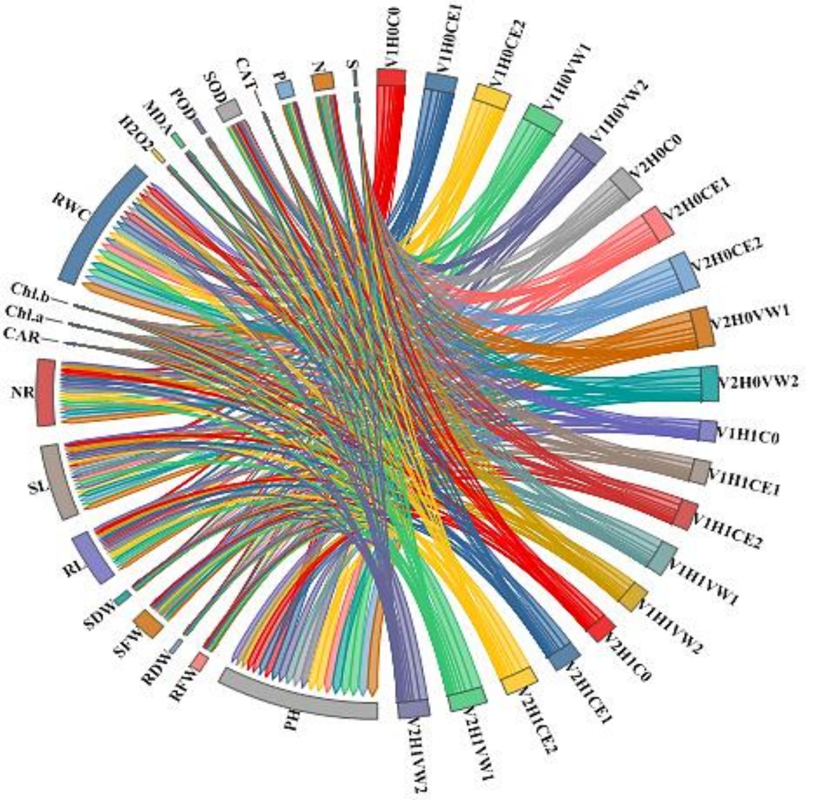

Fig. 3. Chord diagram of morpho-physio-biochemical attributes under exogenously applied compost extract and vermiwash on two maize hybrids during control and heat stress conditions. PH (plant height), RWC (relative water content), RFW (root fresh weight), RL (root length), NR (number of roots per plant), Chl.a (chlorophyll a), RDW (root dry weight), SDW (shoot dry weight), Chl.b (chlorophyll b), P (phosphate contents), SFW (shoot fresh weight), SL (shoot length), S (sulphate contents), N (nitrate contents), CAR (carotenoids contents), H_2O_2 (hydrogen peroxide), MDA (malondialdehyde), POD (peroxidase), SOD (superoxide dismutase), CAT (catalase).

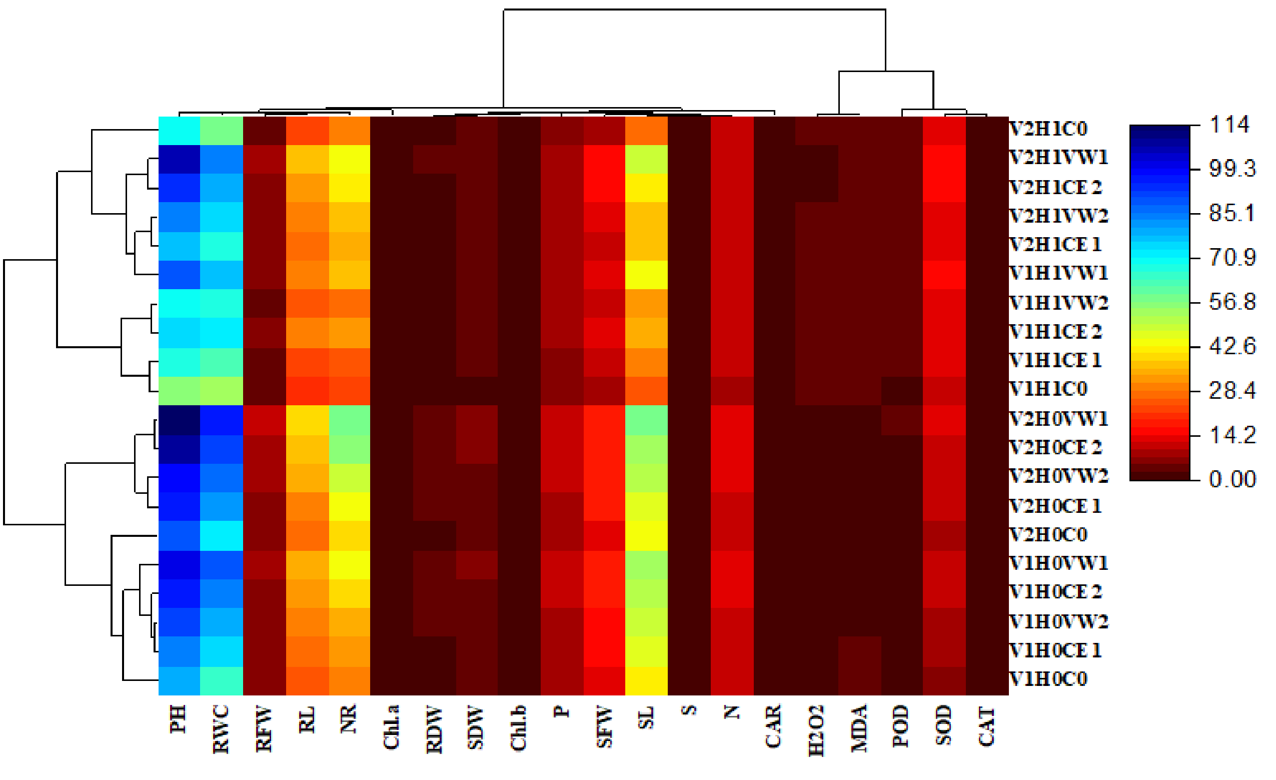

Fig. 4. Clustered heatmap of morpho-physio-biochemical attributes under exogenously applied compost extract and vermiwash on two maize hybrids during control and heat stress conditions. PH (plant height), RWC (relative water content), RFW (root fresh weight), RL (root length), NR (number of roots per plant), Chl.a (chlorophyll a), RDW (root dry weight), SDW (shoot dry weight), Chl.b (chlorophyll b), P (phosphate contents), SFW (shoot fresh weight), SL (shoot length), S (sulphate contents), N (nitrate contents), CAR (carotenoids contents), H_2O_2 (hydrogen peroxide), MDA (malondialdehyde), POD (peroxidase), SOD (superoxide dismutase), CAT (catalase).

Heat stress changes the thylakoid membrane structure and inhibit photosynthesis leads to chlorophyll contents degradation which affect Calvin cycle Rubisco carboxylation activity to fix CO_2 (Marutani *et al.,* 2014; Zou *et al.,* 2017; Hussain *et al.,* 2019; Lal *et al.,* 2022). Heat stress induced ROS accumulation, causes oxidative stress leading to lipid peroxidation which leads to membrane leakage and ultimately loss of membrane integrity (Taratima *et al.,* 2022; Sharma *et al.,* 2012; Mishra *et al.,* 2011). Heat stress also reduces stomatal conductance and transpiration rate under high temperature which limits CO_2 uptake and decreases relative water content leading to dehydration (Yuan *et al.,* 2017; Habibi *et al.,* 2024). Heat stress decreases root growth, nutrient uptake and photosynthetic efficiency which affects nutrient concentration in plants (Mishra *et al.,* 2023). As reported in previous studies that heat stress retarded plant growth and biomass under stress causing a significant reduction in the size, weight and quality of maize grains. (Marias *et al.,* 2017; Ullah *et al.,* 2018; Hussain *et al.,* 2025).

Mitigating the adverse effects is crucial for sustainable crop production and global food security (Hussain *et al.,* 2025). Recorded data showed that heat stress effected all these characters in shoot by declining the contents of these ions. Thus, the application of foliar spray of CE1, CE2, VW1 and VW2 on maize plants showed improvement in these nutrient contents under both glass canopy and normal conditions. Likewise, the exogenous application of organic amendments proved beneficial under heat stress and enhanced the level of minerals. Results were obtained by foliar spray of CE1, CE2, VW1 and VW2 under heat stress and control conditions. The result foliar spray was better with VW1 followed by CE2, VW2 and CE1 than control condition.

Vermiwash provides nutrients, hormones and enzymes to promote chlorophyll stability and photosynthetic activity (Hudda *et al.,* 2020; Jaysawal *et al.,* 2020; Riya *et al.,* 2024). It enhances stomatal conductance to improve CO_2 assimilation and transpiration rate. It activates antioxidant enzymes (SOD, POD and CAT) to scavenge ROS and prevents membrane injury (Riya *et al.,* 2024; Oyege *et al.,* 2025). It induces osmolytes accumulation to ensures osmotic adjustment and RWC maintenance which strengthens root growth and nutrient absorption to support water balance under stress condition (Elumalai *et al.,* 2013; Benazzouk *et al.,* 2018; Oyege *et al.,* 2025). It enhances photosynthesis and growth which results in better physiological performance, biomass and enhances yield (Jaysawal *et al.,* 2020; Nahar *et al.,* 2020; Oyege *et al.,* 2025).

The compost extract has high content of humic acids, beneficial microorganism and nutrient contents such as nitrogen and potassium to improve soil quality which stimulates chlorophyll synthesis (Morales-Corts *et al.,* 2018; Gonzalez-Hernandez *et al.,* 2022; Riya *et al.,* 2024). It improves stress tolerance by enhancing antioxidants defense to minimize ROS accumulation and oxidative damage (Lerma-Moliz *et al.,* 2024). It promotes osmolyte contents to maintain osmotic potential (Li *et al.,* 2024). It enhances root development to enhance nutrient and water uptake (Pant *et al.,* 2012; Lerma-Moliz *et al.,* 2025). Increase in antioxidants reduces malondialdehyde (MDA) levels to minimize electrolyte leakage (Ruiz-Lau *et al.,* 2020; Osman *et al.,* 2022**;** Lerma-Moliz *et al.,* 2024), supporting photosynthetic rate, RWC and gas exchange traits to ensures metabolic balance under stress which improves growth, yield and overall stress tolerance (Pant *et al.,* 2012; Osman *et al.,* 2022; Pacheco Govea *et al.,* 2025). It is important to mention that there is some interaction to modulate the plant growth. However foliar spray of organic amendments such as vermiwash (VW) and compost extract (CE) reduced these effects.

Conclusion

The present study demonstrated that exogenous application of compost extract and vermiwash effectively mitigated the detrimental effects of heat stress in maize. Heat stress significantly impaired morphological, physiological, biochemical and nutritional traits; however, treatments, particularly 6% vermiwash (VW1) and 10% compost extract (CE2), markedly enhanced growth performance, chlorophyll content, relative water content and nutrient uptake while stimulating antioxidant defense mechanisms (SOD, POD, CAT). Vermiwash improved osmotic balance, photosynthetic efficiency and stress resilience by enhancing enzymatic and metabolic activities, whereas compost extract enriched nutrient availability and promoted root development, leading to improved nutrient acquisition under heat stress. Among the two maize hybrids, YH-5507 exhibited superior thermotolerance compared to DK-6724. Overall, the findings emphasize that foliar application of compost extract and vermiwash offers an eco-friendly, sustainable strategy to alleviate heat-induced oxidative damage, enhance physiological efficiency and maintain maize productivity under high-temperature stress conditions, ensuring resilient and sustainable crop production.

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