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Research Article

Hermetic effect of *Moringa oleifera* leaf extract mitigates salinity stress in maize by modulating photosynthetic efficiency, and antioxidant activities

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Abstract

Salinity poses a significant constraint to cereal productivity particularly in arid and semiarid regions. The application of allelochemical has shown promising results in mitigating the intensity of abiotic stresses. A pot experiment was conducted to assess the efficacy of different concentrations of aqueous allelopathic extract derived from moringa leaves in mitigating the adverse impacts of salinity on the germination and growth of maize cultivars via seed priming. The study involved three variables: two cultivars of maize, 'Pioneer 30Y87' (salt tolerant) and 'Pioneer 30T60' (salt sensitive) e seed priming with moringa leaf extract (MLE) at varying concentrations of 0.5%, 1.0%, 1.5%, 2.0%, 2.5%, 3.0%, and hydro-priming as control; and different salinity levels of 0, 6, and 12 dS m^{-1}. Salinity had a negative impact on the germination process, leading to delayed and suboptimal growth of seedlings. Additionally, salinity reduced the synthesis of photosynthetic pigments (20-50%), photosynthesis, transpiration, internal carbon, and stomatal conductance. Further, MLE also improved the antioxidant activities (catalase: CAT and peroxidase: POD) by 22-56% which reduced the hydrogen peroxide production. Moreover, 'P-30Y87' exhibited favorable performance in terms of better germination, growth, photosynthesis and antioxidant activities. The application of moringa leaf extract (3%) resulted in a more notable hermetic effect in elevating salinity stress thereby enhancing germination, growth, photosynthesis and antioxidant activities. In the conclusion, application of MLE (3%) is a promising approach to mitigate the adverse impacts of salinity by improving germination, growth, photosynthesis and antioxidant activities.

Keywords: abiotic stress; allelopathic hormesis; *Moringa oleifera*; priming; salinity

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Introduction

The phenomenon of soil salinization exerts a significant influence on more than 800 million hectares of arable land across the globe (Anil Kumar *et al*., 2022). The extent of salinity stress is significantly increasing around the globe due to rapid climate change which is adversely affecting crop productivity. Soil salinity induces ionic disequilibrium and osmotic perturbation, leading to a concomitant manifestation of physiological aridity and nutrient paucity in plants (Anil Kumar *et al*., 2022). In addition, the plant experiences an elevation in reactive oxygen species (ROS) levels, leading to nucleic acid damage, protein oxidation, and lipid peroxidation. The cascade of processes within the plant system culminates in the suppression of plant growth and subsequent reduction in crop productivity. The annual reduction in crop productivity due to salinity-induced stress amounts to approximately 10%, resulting in a staggering economic loss of USD 27.3 billion (Bandehagh and Taylor, 2020).

Zea mays L. is a member of the Poaceae family and is a prominent cereal crop in Pakistan owing to its exceptional nutritional properties. Additionally, it exerts a significant influence on the country's dietary patterns and economic worth amplification. The crop *Zea mays* exhibit susceptibility to soil salinity. The adverse effects of salinity on plant growth and establishment are attributed to physiological, biochemical, and morphological mechanisms, which culminate in stunted growth and suboptimal stand establishment (Garcia *et al*., 2021). The growth of plants is impeded in saline environments because of imbalances in plant hormones and nutrients, toxicity induced by ions, osmotic stress, electrolyte leakage, disturbance of cellular functions, and membrane damage. Significant endeavors are underway to mitigate the deleterious effects of high salinity levels on crops in agriculture (Etesami and Noori, 2019). Salinity stress also causes ionic imbalance and increases the production of ROS that cause oxidation of chlorophyll, DNA, lipids and proteins resulting in significant growth losses (Radi, 2018; Seleiman *et al*., 2020). Plants possess an excellent antioxidant defense system to scavenge the ROS and ensures plant survival under saline conditions (Balasubramaniam *et al*., 2023). The accumulation of different osmolyte including proline, sugars, and glycine betain is also an important response used by plants to mitigate the adverse impacts of salinity. The accumulation of these solutes maintains the osmotic adjustment and detoxify the ROS which protects the cellular metabolism and membrane structure (Balasubramaniam *et al*., 2023).

Several agronomic methodologies and selective breeding initiatives have been directed towards conferring halophytic resilience in agricultural produce (Suprasanna, 2020). The utilization of allelopathic water extracts is becoming increasingly favored within the field of agronomy due to its accessibility, eco-friendliness, and sustainability. Moreover, these have been employed to augment the yield of diverse plant species. The allelopathic properties of Sorghum make it a promising crop for the extraction of allelochemicals. However, it is comprised of phenolic compounds including p-hydroxybenzoic acid, ferulic acid, and p-cumeric acid which, at minimal concentrations, exhibit growth-promoting effects on other plant species (Cheema *et al*., 2013). The exogenous administration of natural phytohormones, such as aqueous extracts of sorghum and moringa, stimulates plant growth and development, augments photosynthesis, and fortifies the plant's defense mechanism, thereby mitigating diverse abiotic stresses (Pal *et al*., 2023).

Moringa oleifera is an edible plant, and it possesses a huge potential to mitigate the adverse impacts of abiotic stresses. For instance, the application of moringa leaf extract reduces the uptake of toxic ions (sodium: Na and chloride: Cl), malondialdehyde, electrolyte leakage and hydrogen peroxide (H_2O_2) production and increases the antioxidant activities which in turn reduce the toxic effects of salinity (Azeem *et al*., 2023). Other authors also found a significant increase in antioxidant activities, nutrient uptake, reduction in oxidative stress markers and improvement in subsequent plant growth and development (Ahmed *et al*., 2021; Al-Taisan *et al*., 2022). In another study, Al-Taisan *et al*. (2022) found that MLE mitigate the adversities of salinity on *Mentha* species by increasing photosynthetic efficiency, proline synthesis, soluble sugars and ascobate peroxide (APX), catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPX) activities. The need to

address the harmful impact of salinity on maize due to escalating environmental degradation has led to the exploration of sustainable and eco-friendly methods. The situation implies a significant opportunity for the utilization of allelopathic aqueous extracts to augment agricultural productivity in soils susceptible to salinity. The literature lacks sufficient reports on the impact of the exogenous application of MLE on the growth and biomass production of maize cultivated in saline soils. Thus, we hypothesized that MLE can mitigate the adverse impacts of salinity on maize in dose dependent manner. Therefore, this study aimed to determine the efficacy of different concentrations of MLE on germination, growth, photosynthesis and antioxidant activities in maize cultivars.

Materials and Methods

Experiment site

The study aimed to assess the efficacy of *Moringa oleifera leaf* extract (MLE) as a natural crop growth enhancer in the presence of salt stress. Pot experiments were conducted at the Green House located within the Faculty of Agriculture at the University of Agriculture, Faisalabad. The experiments were carried out during the autumn season of 2018, at a geographical location with a latitude of 31°-44' N, longitude of 73°-06' E, and an altitude of 184.4 m. The meteorological data (including average temperature, relative humidity, and rainfall) was obtained from the Agricultural Meteorology Cell at the Department of Agronomy, University of Agriculture, Faisalabad as shown in Figure 1. The soil used for this study was collected with soil auger and it had a sandy loam texture with pH 7.83, organic carbon 2.54 g kg^{-1}, EC 2.09 dSm^{-1}, SAR 12.02, and TSS 45.05 mM $L^{-1.}$

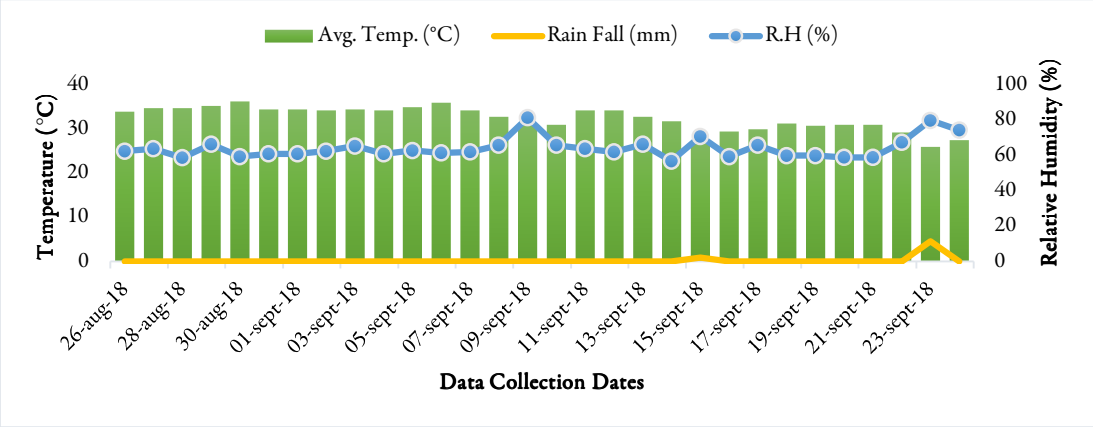


Figure 1. Metrological data presentation during study

Experimental treatments and preparation of moringa leaf extract

Seeds of two maize cultivars, 'Pioneer-30Y87' and 'Pioneer-30T60', were obtained from the maize and Millets Research Institute, Sahiwal. The seeds underwent surface sterilization using a solution of sodium hypochlorite (2.63%) for a duration of 30 minutes, followed by triple rinsing with distilled water. The seeds were primed with 0, 0.5, 1, 1.5, 2, 2.5 and 3% moringa leaf extract and were hydro-primed. The study involved conducting experiments in pots by maintaining salinity at 0, 6 and 12 dSm^{-1}, which were arranged in a completely randomized design (CRD) with a factorial arrangement and 3 replications. The moringa leaves were taken, dried and ground to make the powder. The powdered leaves were dissolved in water for 24 hours, thereafter, the material was filtered and moringa leaf extract was obtained. The moringa leaf extract was prepared according to treatments and seeds were primed with prepared extract.

Data collection

Determination of germination related traits

The data regarding the emergence of seeds was recorded on a daily basis. No. of emerged seeds were counted on a daily basis to calculate the start time of emergence, Handbook of Association of Official Seed Analysts (Baalbaki *et al*., 2009). The time taken to 50% emergence (E_{50}) was calculated by a formula modified by Farooq *et al*. (2005)

$$T_{50} = t_i + \frac{\left(\frac{N}{2} - n_i\right)(t_j - t_i)}{n_j - n_i} \text{----------(i)}$$

Where, N = final number of emerged seeds, and n_i and n_j = cumulative number of seeds emerged by adjacent counts at times t_i and t_j where $n_i < N/2 < n_j$.

Mean emergence time (MET) was calculated according to the equation of Ellis et al. (1981).

$$MET = \frac{\sum DN}{\sum n} \text{----------(ii)}$$

Where, n = number of seeds germinated on days D (Days from the beginning of germination)

Final emergence percentage (FEP) will be taken at the end of experiment.

$$FEP = \frac{\text{No.of seeds germinated}}{\text{No.of seeds sown}} \times 100 \text{----------(iii)}$$

Determination of leaf gas exchange characteristics and photosynthetic pigments

Physiological parameters such as net photosynthetic rate, transpiration rate, carbon flux and stomatal conductance were recorded one day before harvesting by using a photosynthetic system (Lci 4225).

Chlorophyll contents were measured by following the procedure of Arnon (1949). For chlorophyll pigments, leaf samples (0.5 g) from each treatment were homogenized with 8 mL of 80% acetone (v/v). The homogenate was filtered through filter paper. The absorbance of the filtrate was read by spectrophotometer (UV-4000, ORI, Germany) at 663, 645 and 480 nm for chlorophyll a, b and carotenoids, respectively. Chlorophyll a, b and carotenoids were calculated with the following formula:

$$\text{Chl a contents} = \frac{(0.0127 \times A663 - 0.00269 \times A645) \times 100}{0.5} \text{----------(iv)}$$

$$\text{Chl b contents} = \frac{(0.0229 \times A645 - 0.00468 \times A663) \times 100}{0.5} \text{----------(v)}$$

$$\text{Total chlorophyll} = \text{chlorophyll a} + \text{chlorophyll b} \text{----------(vi)}$$

$$\text{Total Carotenoids} = \frac{A^{car}}{Em} \times 100 \text{----------(vii)}$$

Where, Em = 2500 and A^{car} = O.D 480 + 0.114 (O.D 663) – 0.638 (O.D 645)

Determination of oxidative stress markers and antioxidant activities

The fresh leaf samples (0.5 g) were taken and ground in 5 ml of TCA (5%) solution and then it was centrifuged and the extract was collected. Thereafter, 1 ml extract, 100 ml of phosphate buffer and 1 ml of potassium iodide was added and absorbance was noted at 600 nm (Velikova *et al*., 2000). To determine CAT activity fresh leaves (1 g) were taken and ground in 10 ml of potassium phosphate buffer (PPB: 50 mM) and centrifuged for 30 minutes and supernatant was collected. Then 100 µl of enzyme extract was treated with 100 µl H_2O_2 and absorbance was taken at 240 nm (Chance and Maehly, 1955). To determine POD activity, 0.5 g fresh leaves were ground in 5 ml PPB (50 mM) and then centrifuged (12000 rpm) for 10 minutes at 5 °C. Then 100 µl of enzyme extract was treated with 100 µl of each guaiacol and H_2O_2 and absorbance was recorded at 470 nm (Guan *et al*., 2009). Lastly, to determine SOD activity enzyme extract was obtained and a reaction mixture contained H_2O_2 (400 µl), triton (100 µl), NBT (50 µl), enzyme extract (50 µl) and riboflavin (50 µl) ad absorbance was noted at 560 nm (Zhang, 1992).

Statistical analysis

All the collected information on the emergence index, plant development, physiological, and biochemical factors were analyzed by three-way analysis of variance with Statistix 8.1. Sigma plot 10.0 was used for the graphical presentation, and Microsoft Excel 2016 was used for the calculations. The data were examined using the analysis of variance technique, and Tukey's honestly significant difference test (as a post-hoc test) was used to differentiate the mean values at the 5% confidence level (Steel *et al.,* 1997).

Results

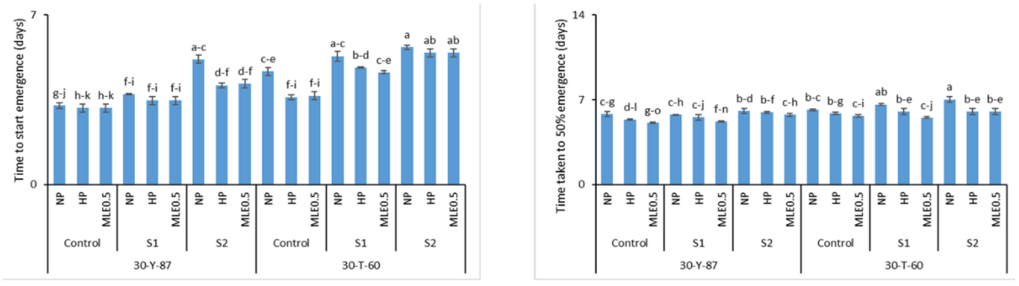
Germination traits

All significant interactions were observed (Table 1). The findings indicate that subjecting unprimed seeds of 'P-30Y87' cultivars to salinity levels of 6 dS m^{-1} and 12 dS m^{-1} resulted in a 13% and 56% increase in emergence time, respectively, compared to the control group with no salinity (Figure 2 A). The application of hydro-priming and priming using varying concentrations of MLE resulted in a notable reduction in the emergence time of maize cultivar ('P-30Y87'). This reduction ranged from 4-32% in the absence of salinity. However, under salinity levels of 6 dS m^{-1} and 12 dS m^{-1}, the reduction in emergence time was more pronounced, ranging from 6-33% and 21-41%, respectively. Similar observations were made in the case of the maize variety 'P-30T60', wherein the duration of emergence was reduced by 23-48% under control conditions, 8-33% under 6 dS m^{-1} salinity stress, and 5-30% under 12 dS m^{-1} salinity stress. The maize cultivar 'P-30Y87' exhibited the highest reduction in emergence time when its seeds were primed with a concentration of 2.5% MLE (Figure 2A).

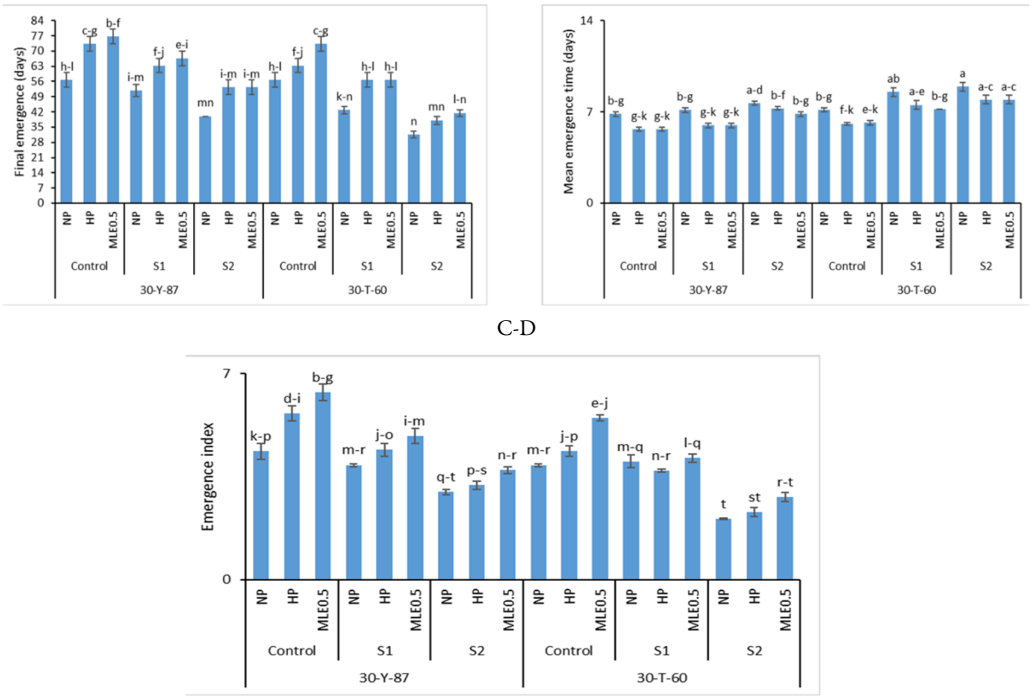
Table 1. Summary of analysis of variances regarding the effect of seed priming with moringa leaf extract on emergence traits of maize cultivars under different salinity levels

Source of Variation	Df	TSE	TFE	MET	FEP	EI
Cultivars	1	7.06**	7.06**	30.34**	2817.8**	22.01*
Salinity levels	2	5.12**	5.12**	25.55**	10081.8**	85.50**
Seed priming	7	9.90**	9.90**	10.73**	2166.5**	14.32**
Cultivars × Salinity levels	2	0.540**	0.540**	4.425**	84.5*	0.634**
Cultivars × Seed priming	7	0.207**	0.207**	0.190^{ns}	39.0^{ns}	0.183^{ns}
Salinity levels × Seed priming	14	0.123*	0.123**	0.195^{ns}	45.1*	0.553**
Cultivars × Salinity levels × Seed priming	14	0.097**	0.097*	0.126^{ns}	23.5^{ns}	0.152^{ns}

TSE: time to start emergence, TFE: time to 50% emergence, MET: mean emergence time, FEP: final emergence percentage, EI: emergence index. * and ** indicates the significant at *P≤0.05* and *P≤0.01* respectively.



A-B



C-D

E

Figure 2. Effect of moringa leaf extract seed priming levels time to start emergence (days: A), time to 50% Emergence (days: B), final emergence percentage (C), mean emergence time (days: D), and emergence index (days: E) of maize cultivars under salinity stress

The data is mean of three replication and different letters indicating the significance (HSD) at 5% confidence level.

The germination process of 'P-30Y87' and 'P-30T60' non-primed seeds was delayed by 2-5% and 6-13% at salinity levels of 6 and 12 dS m^{-1}, respectively, in comparison to their control counterparts that were grown under non-stressful conditions. The implementation of hydropriming and seed priming techniques using MLE resulted in a 50% reduction in the emergence time for both cultivars. The highest decrease in the duration of 50% emergence was noted in 'P-30Y87' when subjected to 2.5% MLE and in 'P-30T60' when exposed to 3% MLE.

The MET of both cultivars was significantly increased upon exposure to salt stress in non-primed seeds, as depicted in Figure 2. The highest rise in MET was noted at a salinity level of 12 dS m^{-1}, with 6 dS m^{-1} following closely behind. In non-primed seeds, the salinity levels of 6 dS m^{-1} and 12 dS m^{-1} resulted in a 2% and 10% increase, respectively, in the MET of P-30Y87 when compared to the control. An analogous rise in MET was noted in 'P-30T60' at salinity levels of 6-12 dS m^{-1}, with an increase of 22-28%. The application of MLE for seed priming resulted in a significant decrease in the mean emergence time (MET) of both cultivars under salinity stress and the effect was more pronounced with higher levels, as shown in Figure 4. The dilutions of 2.5% and 3% MLE exhibited the highest efficacy in reducing the MET of 'P-30Y87' and 'P-30T60', respectively (Figure 2D).

A decline in the ultimate emergence rate was noted for unprimed seeds of 'P-30Y87' (9-30%) and 'P-30T60' (25-45%) at salinity levels of 6-12 dS m^{-1}, respectively. The highest enhancement in the ultimate emergence ratio was detected in 'P-30Y87' and 'P-30T60' strains at 2.5% and 3% MLE concentrations, respectively. (Figure 2D).

A reduction of 11-32% ('P-30Y87') and 3-47% ('P-30T60') under salinity conditions of 6 dS m^{-1} and 12 dS m^{-1}, respectively, in comparison to the control group. The application of hydropriming and seed priming techniques, utilizing varying concentrations of moringa leaf extract, resulted in a significant increase in the

emergence index of 'P-30Y87' and 'P-30T60' cultivars. The observed increase ranged from 28-71% and 12-84% respectively, under controlled conditions. 'P-30Y87' exhibited a significant increase in emergence index of 7-82%, 13-64%, and 28-72% under control, 6 dS m^{-1}, and 12 dS m^{-1} conditions, respectively, in comparison to their corresponding controls. An increase in the emergence index was noted in 'P-30T60' when compared to their respective controls. The increase ranged from 12-84% in the control group, 7-36% in the 6 dSm^{-1} group, and 9-91% in the 12 dSm^{-1} group ((Figure 2E).

Leaf gas exchange characteristics

The imposition of salinity stress (6 dS m^{-1} and 12 dS m^{-1}) on non-primed seeds resulted in a significant (Tables 2 and 3) decline in the photosynthetic rate of 'P-30Y87' (28% and 56%) and 'P-30T60' (45% and 75%) when compared to non-primed seeds grown under normal conditions (no stress). It was observed that all the seed priming techniques exhibited efficacy in mitigating the salt-induced decline in the photosynthetic rate. 'P-30Y87' exhibited an increase of 9.66%, 2.88% and 13.119% in response to control, 6 dS m^{-1}, and 12 dS m^{-1} salinity levels. Similarly, 'P-30T60' demonstrated an improvement of 1.50%, 4.11% and 27.18% under the same salinity conditions. The 'P-30Y87' strain exhibited the highest rate of photosynthesis at 2.5% MLE (Table 4).

Table 2. Summary of analysis of variances regarding the effect of seed priming with moringa leaf extract on biochemical parameters of maize cultivars under different salinity levels

Source of variation	Df	Chl. a	Chl. b	Total chl.	Car.	Cat.	H_2O_2	SOD	POD
Cultivars	1	0.206**	0.206**	0.879**	0.011**	3.76**	6.75**	1589.8**	24.40**
Salinity levels	2	2.96**	2.96**	5.50**	0.232**	10.01**	24.80**	12045.4**	49.23**
Seed priming	7	2.72**	2.72**	4.82**	0.325**	7.41**	18.10**	7424.7**	66.48**
Cultivars × Salinity levels	2	0.023^{ns}	0.023**	0.049**	0.004^{ns}	1.136**	0.446**	510.4**	15.50**
Cultivars × Seed priming	7	0.009^{ns}	0.009^{ns}	0.003^{ns}	0.002^{ns}	0.024^{ns}	0.183^{ns}	64.3^{ns}	0.520*
Salinity levels × Seed priming	14	0.01476*	0.01476*	0.02517^{ns}	0.00698**	0.015^{ns}	0.224**	213.4**	0.855*
Cultivars × Salinity levels × Seed priming	14	0.00112^{ns}	0.00112^{ns}	0.01200^{ns}	0.00119^{ns}	0.042^{ns}	0.274**	31.4^{ns}	0.742*

Chl. a: chlorophyll a, Chl. b: chlorophyll b, Total Chl: total chlorophyll, Car: carotenoids, Cat: catalase content, H_2O_2: hydrogen peroxide, SOD: superoxide dismutase activity, POD: peroxidase activity. * and ** indicates the significant at $P \leq 0.05$ and $P \leq 0.01$ respectively.

Table 3. Summary of analysis of variances regarding the effect of seed priming with moringa leaf extract on physiological parameters of maize cultivars under different salinity levels

Source of variation	Df	PR	TR	Int. CO_2	SC
Cultivars	1	3.73**	2.66**	17187.2**	0.036**
Salinity levels	2	96.54**	67.73**	86746.8**	0.202**
Seed priming	7	12.70**	9.41**	11204.0**	0.046**
Cultivars × Salinity levels	2	2.15**	0.743**	2408.9**	0.001*
Cultivars × Seed priming	7	0.55**	0.366**	1100.5**	0.002**
Salinity levels × Seed priming	14	0.092^{ns}	0.107**	33.9^{ns}	0.0001**
Cultivars × Salinity levels × Seed priming	14	0.160**	0.054*	34.4^{ns}	0.00036*

PA: rate of photosynthesis, TR: rate of transpiration, internal CO_2: internal carbon, SC: stomatal conductance. * and ** indicates the significant at $P \leq 0.05$ and $P \leq 0.01$ respectively.

The application of salinity levels ranging from 6-12 dS m^{-1} resulted in a reduction of transpiration rate by 27% and 55% in non-primed seeds of the cultivar 'P-30Y87' and 47% and 74% of the cultivar 'P-30T60' in comparison to the control group that was not subjected to salinity. The application of hydro priming and priming with varying concentrations of moringa leaf extract resulted in improvements ranged from 9-67% in the absence of salinity, while under salinity levels of 6-12 dS m^{-1}, the improvements ranged from 2-88% and 9-119%, respectively (Table 4).

A reduction of internal carbon concentration up to 63% was observed in non-primed seeds of 'P-30T60', followed by 'P-30Y87' with a reduction of 39%, when exposed to a salinity level of 12 dS m^{-1}. 'P-30T60' and 'P-30Y87' experienced a reduction in their internal carbon concentration of 41% and 21%, respectively, when subjected to a salinity level of 6 dS m^{-1}, as shown in Table 1. All priming treatments exhibited a significant impact on enhancing the internal carbon concentration of both cultivars. The maximum increase was observed at a seed priming level of 2.5% MLE in 'P-30Y87' and 3% MLE in 'P-30T60' across all salinity levels (Table 4).

Table 4. Effect of seed priming levels on physiological traits of maize cultivars under different salinity stress

Variety	Treatment	Priming	Photosynthesis (μ mol CO^2 m^2 S^{-1})	Transpiration (μ mol CO^2 m^2 S^{-1})	Internal CO_2 (µmol per mol)	Stomatal conductance (μ mol m^2 S^{-1})
Pioneer-30Y87	Control	NP	4.95±0.16gh	2.47±0.04no	248.1±6.2lmn	0.25±0.004n
		HP	5.38±0.06f	2.69±0.1oklm	265.4±2.4jkl	0.27±0.010lm
		MLE0.5	5.47±0.12f	2.82±0.02jk	287.9±13.6i	0.29±0.006jkl
		MLE1.0	6.30±0.00d	3.00±0.09hi	313.8±14.3h	0.32±0.009ghi
		MLE1.5	7.13±0.16b	3.52±0.14de	332.7±18.4efg	0.39±0.014cd
		MLE2.0	8.15±0.24a	3.73±0.11bc	348.4±9.6cde	0.41±0.011bc
		MLE2.5	8.25±0.06a	4.13±0.14a	380.3±2.4a	0.45±0.014a
		MLE3.0	8.15±0.03a	3.78±0.12b	366.8±12.5abc	0.42±0.012b
	Salt-EC6	NP	3.58±0.07lm	1.79±0.07st	195.4±2.9vwx	0.18±0.007rst
		HP	3.66±0.02klm	1.83±0.06q-t	209.9±9.2s-v	0.18±0.006stu
		MLE0.5	4.23±0.14i	2.11±0.06p	226.1±3.4p-s	0.21±0.006pq
		MLE1.0	4.84±0.10h	2.42±0.1o	245.7±3.9mno	0.24±0.011no
		MLE1.5	5.30±0.17f	2.65±0.04lm	264.1±6.5j-m	0.25±0.004mn
		MLE2.0	5.87±0.06e	2.94±0.06hij	287.2±2.4i	0.30±0.006ij
		MLE2.5	6.73±0.14c	3.37±0.03ef	321.6±5.6fgh	0.35±0.003fg
		MLE3.0	6.36±0.16d	3.18±0.08g	306.8±6.4h	0.33±0.008gh
	Salt-EC12	NP	2.13±0.05d	1.11±0.04wx	149.6±4.7a	0.11±0.004xy
		HP	2.42±0.04rs	1.21±0.03w	158.0±2.1a	0.12±0.006xy
		MLE0.5	2.58±0.06qr	1.73±0.02t	179.1±8.5xyz	0.18±0.009stu
		MLE1.0	3.43±0.06mn	1.72±0.04t	198.4±2.9uvw	0.19±0.007rst
		MLE1.5	3.84±0.17jkl	1.92±0.08qrs	214.7±7.1r-u	0.20±0.008pqr
		MLE2.0	4.12±0.07ij	2.09±0.08p	228.5±6.2o-r	0.22±0.008op
		MLE2.5	4.73±0.15h	2.44±0.09o	272.2±6.8ij	0.25±0.009mn
		MLE3.0	4.68±0.23h	2.34±0.10o	244.2±12.5nop	0.24±0.010no
Pioneer-30T60	Control	NP	5.44±0.09f	2.82±0.01jkl	255.8±3.5j-n	0.29±0.006jkl
		HP	5.49±0.06f	2.85±0.08ijk	265.9±3.1jkl	0.28±0.008kl
		MLE0.5	6.23±0.14d	3.22±0.04fg	287.5±5.4i	0.32±0.004hi
		MLE1.0	6.29±0.16d	3.25±0.15fg	308.1±5.5h	0.34±0.011fg
		MLE1.5	6.92±0.14bc	3.56±0.05d	314.9±5.3gh	0.36±0.005ef
		MLE2.0	7.18±0.01b	3.59±0.07cd	335.4±4.4def	0.38±0.007de
		MLE2.5	8.12±0.21a	4.12±0.01a	353.1±8.4bcd	0.39±0.009cd
		MLE3.0	8.18±0.04a	4.20±0.09a	369.9±6.6ab	0.42±0.009b
	Salt-EC6	NP	2.97±0.06op	1.49±0.04v	150.1±2.6a	0.15±0.006vw
		HP	3.10±0.10op	1.53±0.02uv	166.6±4.2xz	0.16±0.009tuw
		MLE0.5	3.96±0.06ijk	1.98±0.04pq	185.0±4.6wxy	0.20±0.004qrs
		MLE1.0	4.18±0.11i	2.13±0.09p	198.6±4.5t-w	0.22±0.009op
		MLE1.5	4.64±0.10h	2.32±0.02o	217.0±3.9rst	0.24±0.002no

		MLE2.0	5.22±0.06fg	2.61±0.08mn	240.1±2.2n-q	0.27±0.008lm
		MLE2.5	5.28±0.02f	2.64±0.09m	251.6±8.0k-n	0.29±0.009jk
		MLE3.0	6.20±0.12d	3.11±0.11gh	269.4±4.8ijk	0.32±0.011hi
	Salt-EC12	NP	1.38±0.04u	0.73±0.03z	92.4±0.9c	0.07±0.003z
		HP	1.76±0.03t	0.89±0.03yz	105.3±1.1c	0.10±0.006y
		MLE0.5	2.50±0.06r	0.96±0.02xy	130.2±4.8b	0.12±0.006xy
		MLE1.0	2.85±0.14pq	1.51±0.04v	149.0±6.1a	0.13±0.005wx
		MLE1.5	3.23±0.12no	1.69±0.04tu	164.5±5.3a	0.16±0.006uv
		MLE2.0	3.60±0.11lm	1.81±0.04rst	179.1±4.7xyz	0.17±0.008tu
		MLE2.5	3.61±0.11lm	1.81±0.02q-t	191.2±3.8wx	0.20±0.008pqr
		MLE3.0	3.83±0.09jkl	1.97±0.03pqr	223.8±9.3qrs	0.22±0.006op

The data is mean of three replication and different letters indicating the significance (HSD) at 5% confidence level

Salinity caused significant reduction in stomatal conductance of non-primed seeds of 'P-30Y87' (28% at 6 dS m^{-1}, 55% at 12 dS m^{-1}) and 'P-30T60' (48% at 6 dS m^{-1} ,75% at 12 dS m^{-1}). Under unstressed condition, seed priming with distilled water + MLE enhanced the stomatal conductance of 'P-30Y87' and 'P-30T60' up to 81% and 44% respectively. At 6 dS m^{-1} MLE maximized the stomatal conductance of 'P-30Y87' and 'P-30T60' up to 92% and 45% as compared with non-primed seeds. At 12 dS m^{-1} seed priming with MLE enhanced the stomatal conductance of 'P-30Y87' and 'P-30T60' up to 131% and 213% as compared with non-primed seeds (Table 4).

Photosynthetic pigments

Unprimed seeds of cultivars 'P-30Y87' to salinity concentrations of 6 dS m^{-1} and 12 dS m^{-1} resulted in a reduction of 27% and 58%, respectively, in the chl a content when compared to the control group that was not exposed to salinity. The 'P-30T60' exhibited a reduction of 41 and 68% in its chl a concentration (Figure 3A). The application of hydro priming and priming using 2.5% ('P-30Y87') and 3% ('P-30T60') moringa leaf extract resulted in a notable increase in the chl a content of maize cultivars. Similar observations were made for the maize variety 'P-30T60', where the chlorophyll-a concentration showed an increase of 12-68% under control conditions, 7-91% under 6 dS m^{-1} salinity stress, and 24-198% under 12 dS m^{-1} salinity stress. The findings indicate that the application of moringa leaf extracts had a hermetic effect on the chl a content in the maize cultivar ('P-30T60'), particularly in the presence of salinity stress.

The effect of salinity on non-primed and primed seeds of both maize cultivars. Salinity levels 6 dS m^{-1} and 12 d Sm^{-1} restricted the chl b content of non-primed seeds of 'P-30Y87' by 20-49% and 'P-30T60' by 42-69% as compared with chl b content of non-primed seeds grown under control conditions. Hydro priming and seed priming with 2.5% MLE in 'P-30Y87' improved the Chl b content by 16-60% (in control), 14-50% (at 6 dS m^{-1}) and 22-91% (at 12 dS m^{-1}). Similarly, maximum improvement in chl b content of 'P-30T60' was recorded with 3% MLE under control, 6 dS m^{-1} and 12 dS m^{-1} by 17-55%, 16-74%, 60-164% respectively (Figure 3B).

The total chlorophyll content of both cultivars ('P-30Y87', 'P-30T60') was significantly suppressed by different levels of salinity. At 6 dS m^{-1}, the suppression was 24% and 36% for 'P-30Y87' and 'P-30T60', respectively, compared to their respective control. Under a salinity level of 12 dS m^{-1}, both cultivars ('P-30Y87', 'P-30T60') exhibited comparable behavior. The salinity level caused a reduction in the total chlorophyll content of both cultivars, with a decrease of 45% and 55% observed in 'P-30Y87' and 'P-30T60', respectively, as compared to the control. The analysis indicated that the overall chlorophyll levels in 'P-30T60' were 17% lower than those in 'P-30Y87' (Figure 3C).

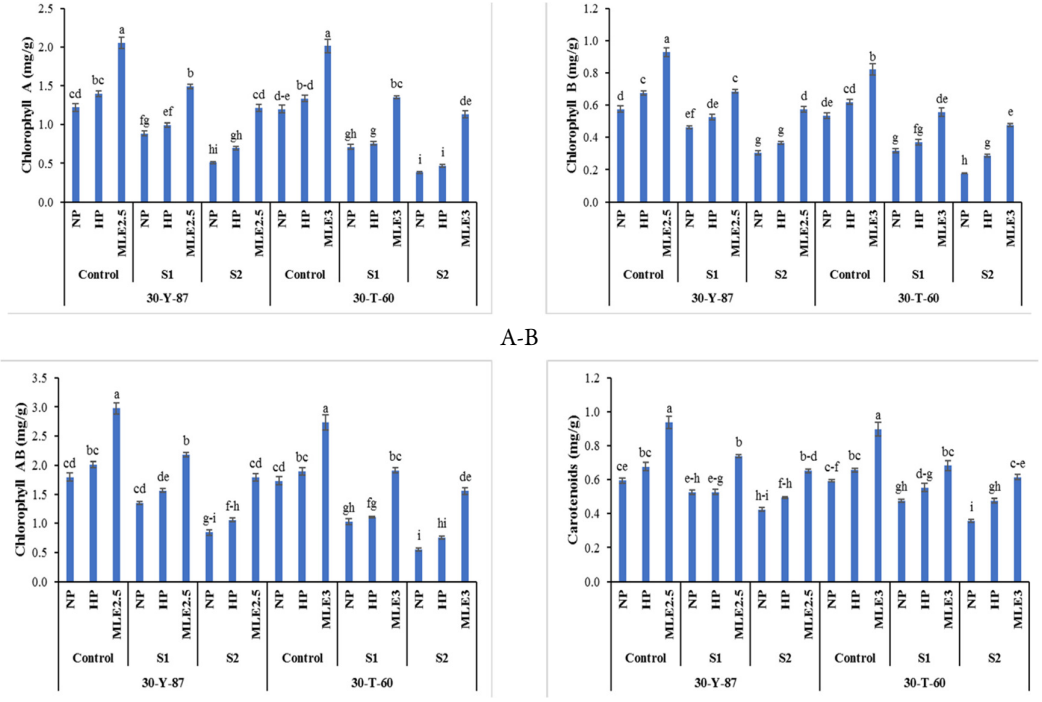


Figure 3. Effect of moringa leaf extract seed priming levels on chlorophyll a (A), chlorophyll b (B), total chlorophyll (C), and carotenoids (D) concentration of maize cultivars under salinity stress
The data is mean of three replication and different letters indicating the significance (HSD) at 5% confidence level.

Compared to the control, it was observed that salinity had a negative impact on the chlorophyll content of non-primed seeds from both cultivars ('P-30Y87' and 'P-30T60'). The reduction in chlorophyll content ranged from 25-53% for 'P-30Y87' and 41-68% for 'P-30T60', with an increase in salinity levels at 6 and 12 dS m^{-1}, respectively. The application of priming treatments, specifically hydro priming with 2.5-3% MLE, resulted in a statistically significant improvement in the overall chlorophyll levels of 'P-30Y87' and 'P-30T60'. The increase in chlorophyll content was observed across varying levels of salinity stress, with 'P-30Y87' showing an increase of 12-66% at control, 16-62% at 6 dS m^{-1}, and 25-111% at 12 dS m^{-1}, while 'P-30T60' showed an increase of 10-58% at control, 8-86% at 6 dS m^{-1}, and 38-183% at 12 dS m^{-1}, as compared to their respective control groups. While 'P-30Y87' exhibited a 17% increase in chlorophyll content, 'P-30T60' (a salt-sensitive cultivar) demonstrated the greatest enhancement in overall chlorophyll content under saline conditions, surpassing that of 'P-30Y87' (a salt-tolerant crop).

At an electrical conductivity level of 6 dS m^{-1}, the carotenoid content of non-primed seeds of 'P-30Y87' and 'P-30T60' was limited by salinity, with reductions of 11% and 20%, respectively. At an electrical conductivity level of 12 dS m^{-1}, the carotenoid content of non-primed seeds of 'P-30Y87' and 'P-30T60' was further restricted by salinity, with reductions of 28% and 40%, respectively. The application of priming treatments, specifically hydro-priming in combination with 2.5% MLE, resulted in a significant increase in carotenoid levels in 'P-30Y87' under control conditions (15-59%), as well as under salt stress conditions of 6 dS m^{-1} (1-42%) and 12 dS m^{-1} (15-52%). Similarly, in 'P-30T60', hydro-priming and seed priming with 3% MLE were found to be the most effective treatments for enhancing carotenoid levels under control conditions (11-52%), as well as under salt stress conditions of 6 dS m^{-1} (15-42%) and 12 dS m^{-1} (33-71%) (Figure 3D).

Antioxidant activities

The application of salinity levels at 6 dS m^{-1} on non-primed seeds of cultivars 'P-30Y87' and 'P-30T60' resulted in a 44% and 11% increase in catalase content, respectively, compared to the control group with no salinity (Table 3). A reduction in catalase levels was observed in 'P-30Y87' and 'P-30T60' at a salinity level of 12 dS m^{-1}, with decreases of 12% and 35% respectively compared to the control (Figure 4A). The application of hydro priming and priming with 2.5% of moringa leaf extract resulted in a significant increase in catalase content in the maize cultivar ('P-30Y87'). The observed improvements ranged from 14-46% in the absence of salinity stress. Under salinity stress levels of 6 dS m^{-1} and 12 dS m^{-1}, the improvements in catalase content were observed to be 8-34% and 6-36%, respectively. Similar observations were made in the case of the maize variety 'P-30T60', wherein the catalase levels were found to have increased by 13-47% under control conditions, 9-38% under 6 dS m^{-1} conditions, and 21-77% under 12 dS m^{-1} conditions.

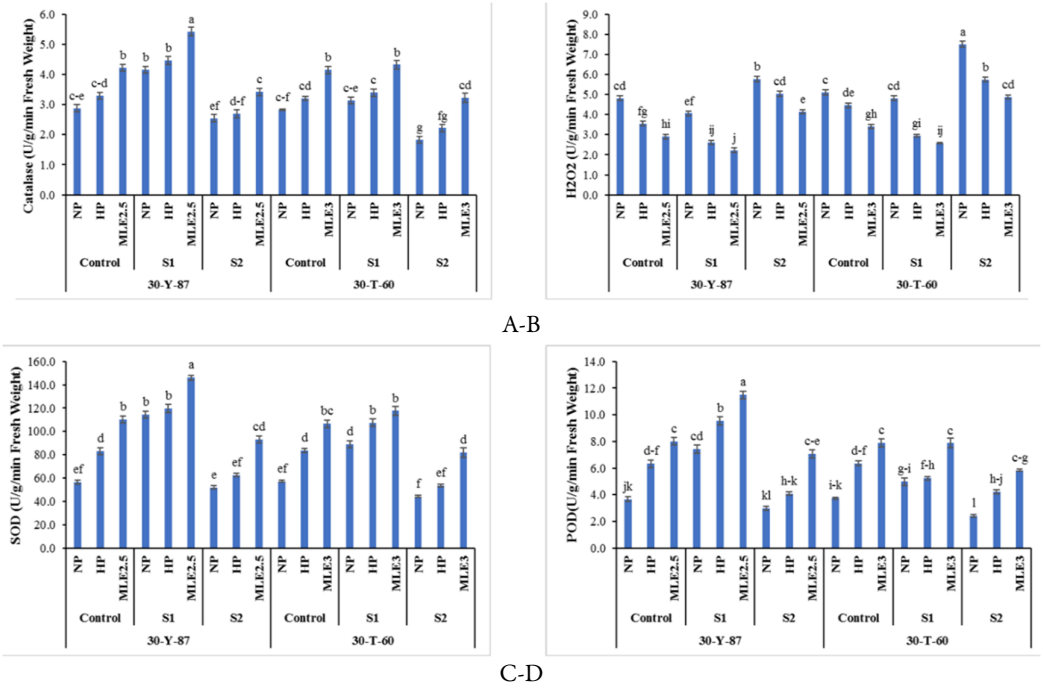


Figure 4. Effect of moringa leaf extract seed priming levels on CAT A), H_2O_2 (B), SOD (C), and POD (D) concentration of maize cultivars under salinity stress

CAT: catalase, H_2O_2: hydrogen peroxide, SOD: superoxide dismutase, POD: peroxidase. The data is mean of three replication and different letters indicating the significance (HSD) at 5% confidence level.

Primed and non-primed seeds of both cultivars showed different behavior under different salinity levels. Non-primed seeds under no stress showed more growth as compared with non-primed seeds under salt stress. Salinity increased the superoxide dismutase activity of non-primed seeds of 'P-30Y87' and 'P-30T60' at Ec level of 6 dSm^{-1} (101%, 56%) and decreased the activity at 12 dS m^{-1} (9%, 23%) respectively. However, priming treatments (Hydro-priming +2.5% MLE) significantly enhanced the superoxide dismutase content of 'P-30Y87' under control (46-93%), 6 dS m^{-1} (5-28%) and 12 dS m^{-1} (20-79%) whereas in 'P-30T60', hydro-priming and seed priming with 3% MLE maximized the superoxide dismutase under control (45-86%), 6 dSm^{-1} (20-32%) and 12 dS m^{-1} (22-86%) (Figure 4C).

The salinity levels at 6 dS m^{-1} on non-primed seeds of 'P-30Y87' and 'P-30T60' cultivars resulted in a 103% and 33% increase in peroxidase contents, respectively, compared to the control group with no salinity. A reduction in peroxidase levels of 'P-30Y87' and 'P-30T60' was noted at a salinity level of 12 dS m^{-1}, with decreases of 19% and 35%, respectively. The application of hydro priming and priming with 2.5% of moringa

leaf extract resulted in a significant increase in peroxidase contents of maize cultivar ('P-30Y87'). The observed improvements were 72-118% under normal conditions, while under salinity levels of 6 dS m^{-1} and 12 dS m^{-1}, the improvements ranged from 28-55% and 38-138%, respectively. Similar observations were made for the maize variety 'P-30T60' when subjected to 3% MLE (Figure 4D).

Oxidative stress markers

The non-primed seeds of cultivars 'P-30Y87' and 'P-30T60' that were subjected to salt levels of 6 dS m^{-1} experienced a reduction in hydrogen peroxide content of 16% and 6%, respectively, when compared with the control group, which did not experience any salinity ((Figure 4B). At a salinity level of 12 dS m^{-1}, an increase in hydrogen peroxide content of 19% and 47%, respectively, was detected when compared to the control. This was the case for both 'P-30Y87' and 'P-30T60'. These decreases ranged from 36-45% and 13-28% under salinity levels of 6 dS m^{-1} and 12 dS m^{-1} respectively. Hydro priming and priming 2.5% of moringa leaf extract decreased the hydrogen peroxide concentration of maize cultivar ('P-30Y87') by 26-40% in control (no salinity) whereas such decrease ranged from 26-40% to 36-45% under salinity levels of no salinity. The same pattern of behaviour was observed for the maize cultivar 'P-30T60', in which there was a reduction in the amount of hydrogen peroxide present by 13-33% (control), 39-46% (6 dS m^{-1}), and 23-35% (12 dS m^{-1}).

Discussion

The utilization of inorganic growth enhancers such as chemical and nutrient agents, as well as organic bio-stimulants, are considered feasible methods to mitigate crop yield reduction. Bio-stimulants are organic compounds that facilitate the growth of crops by enhancing nutrient absorption and utilization, promoting rhizosphere activity, and increasing resistance to both biotic and abiotic stressors. The utilization of bio-stimulants to enhance plant growth and yield has garnered significant interest among experts in plant physiology. The utilization of moringa leaf extract as a seed priming agent has been documented to augment the seedling vigor of various crops under ideal circumstances. However, the impact of stressful conditions has not been previously taken into account. Furthermore, the biochemical and physiological mechanisms underlying stress tolerance in response to allelopathic hormesis remain unclear from a biological perspective. Consequently, the present investigation was designed to examine the physiological and biochemical underpinnings of the enhancements in stress tolerance observed maize cultivars subjected to stress conditions.

Reduced germination rates and suboptimal stand establishment are significant factors that impede achieving maximum yield. The application of various priming agents, such as moringa leaf extract, has been found to enhance germination and promote stand establishment in numerous crops, as reported by Farooq *et al.* 2006; Farooq *et al.* (2008) and Wajid *et al.* (2018). Seed priming has been observed to enhance seedling vigor, improve dormancy breakdown and increase productivity, as reported by Muhammad Farooq *et al.* (2015) and Hussein *et al.* (2007). The results of the experiment indicate that seed priming with varying concentrations of MLE had a positive impact on the final emergence percentage and emergence index. Additionally, it was observed that the time required for emergence was significantly reduced, including the time taken to reach 50% emergence and the mean emergence time, under salinity stress. The observed phenomenon could potentially be associated with enhanced uptake of water, rupture of the seed coat, and hastened biochemical reactions due to seed priming, as reported by Anjum *et al.* (2011). This investigation aligns with numerous prior studies (Zheng *et al*., 2016 and Farooq *et al*., 2008) that have demonstrated the capacity of seed priming to enhance germination and seedling vigor. According to Wajid *et al.* (2018), MLE possesses compounds that promote plant growth and enhance the emergence index of crops. MLE, or Maternal Leachate Extract, is a source of essential vitamins and nutrients that are transferred to the developing embryo. This transfer leads to the activation of metabolic repair during the priming lag phase, as reported by Farooq *et al.*

(2010).As a result, seed germination is improved and subsequent growth of seedlings growing under salinity is enhanced. The alterations in salinity levels lead to a decline in the biochemical and physiological processes, ultimately resulting in a reduction of plant biomass and the final crop yield, as reported by Naz *et al.* (2015).

According to Kolbert *et al.* (2012), plants tend to allocate a significant portion of their resources towards enhancing their defense mechanisms rather than focusing on growth and development when subjected to stressful conditions. The growth and physiological processes of various plant organs were impacted by changes in salinity levels. According to Shoresh *et al*. (2011) plant's photosynthetic capacity was significantly impaired by salinity, primarily because of stomatal closure and reduced leaf expansion. The observed decrease in leaf expansion in the current investigation under high salinity conditions could be attributed to osmotic stress and nutritional imbalance. This may result in nutrient deficiencies and ion toxicities, ultimately leading to leaf damage. The reduction in chlorophyll content due to salt stress leads to a decrease in photosynthesis rate. Nevertheless, the application of calcium effectively alleviates the detrimental impacts of salt stress. The leaves of Moringa plant exhibit a higher concentration of calcium, approximately four times greater than that of milk. Additionally, the leaves contain three times more potassium than bananas and seven times more ASA than oranges. The findings of the current investigation indicate that maize exhibited comparable responses to priming interventions. An augmentation in the pace of photosynthesis, carbon flux, transpiration rate, and stomatal conductance was noted as the levels of moringa leaf extract were elevated. According to Abdalla *et al.* (2015) findings, the utilization of MLE resulted in enhancements in the stomatal conductance and photosynthesis rate of *Erusa vesicaria*, as well as the plant's total chlorophyll contents, ascorbic acid, and total proteins. Additionally, the application of MLE led to an increase in the levels of plant growth hormones such as auxins, cytokinin, and gibberellins.

Insufficient potassium availability in saline environments results in a reduction of photosynthetic pigments and an elevation of oxidative damage, ultimately constraining crop development (Gong *et al*., 2011). Higher seedling vigor is strongly correlated with chlorophyll contents. The process of priming seeds can result in consistent and rapid germination, leading to the development of robust seedlings with elevated levels of chlorophyll in their leaves (Ghassemi-Golezani *et al.* 2008). El-Baky *et al.* (2008) observed a rise in the chlorophyll levels of leaves in response to salinity when bioregulators were administered. The application of moringa leaf extract on foliage has been observed to impede the premature senescence of leaves, thereby promoting the development of a greater leaf surface area that contains a higher concentration of photosynthetic pigments.

The presence of salinity induces an excessive generation of reactive oxygen species (ROS) that ultimately triggers oxidative harm to the cellular structure of plants, as per the findings of Yu *et al.* (2015). Plants activate their antioxidant defense mechanism, leading to an increase in SOD activity. The antioxidants produced can effectively mitigate oxidative stress by efficiently eliminating ROS. In the case of malfunctioning scavenging system against reactive oxygen species (ROS), the plant metabolism may undergo alterations, leading to structural modifications of proteins that increase their susceptibility to proteolytic degradation. Numerous antioxidants or molecules that scavenge water and lipids are responsible for the detoxification of ROS, as stated by Foyer *et al.* (1994). Superoxide dismutase, peroxidase, and catalase enzymes are crucial antioxidants. Superoxide dismutase (SOD) is accountable for the process of dismutation of O^{2-} into H_2O_2 and O_2. On the other hand, catalase (CAT) and peroxidase (POD) are involved in the suppression of H_2O_2, as stated by Farooq *et al*. (2008). Superoxide dismutase (SOD) exhibits the capability to mitigate oxidative stress-induced harm, as reported by Sun and Tao (2011). According to the report of Wang *et al.* (2009) the cultivars of lucerne that are tolerant to salt exhibited elevated levels of SOD activity. Inter-cultivar variability was observed in the biochemical response, as per the findings of this investigation. The salinity level of 6 dS m^{-1} was found to increase the SOD activity in both salt tolerant and salt sensitive cultivars of maize ('P-30Y87', 'P-30T60'). However, cultivars did not show an increase in SOD contents at 12 dS m^{-1}.

The study demonstrated that the enzymatic antioxidants in maize cultivars were enhanced when subjected to salt stress in salt-tolerant cultivars. However, the antioxidant activities were reduced in non-primed seeds exposed to 12 dS m^{-1} as compared to the control and 6 dS m^{-1}. The salt tolerant cultivars exhibited a higher rate of increase in antioxidant activities in comparison to the salt sensitive cultivars. The findings suggest that the maize cultivar 'P-30Y87' exhibits a greater capacity to stimulate the antioxidant system in response to salinity. These findings are consistent with the research conducted by Neto *et al.* (2006). The utilization of MLE as a seed priming agent resulted in favorable synergistic effects in biochemical parameters. The application of MLE to the seeds resulted in a significant enhancement of SOD, CAT, and APX activities across all growth conditions. Elevated catalase (CAT) levels observed in plants subjected to salinity stress suggest a heightened ability to scavenge reactive oxygen species, thereby conferring greater protection against oxidative stress induced by salinity. Enhanced antioxidant activities result in a decrease in reactive oxygen species (ROS)-induced damages, as demonstrated by the reduction in H_2O_2 concentration within the plant system. The presence of diverse secondary metabolites and allelochemicals, such as ascorbate, phenols, and zeatin, may be associated with enhanced efficiency of biochemical parameters (Khan *et al*., 2019).

Conclusions

The presence of high salinity levels had a significant negative impact on the germination and growth of maize. The application of hydro-priming and seed-priming techniques using varying concentrations of moringa has been observed to effectively alleviate the detrimental impact of salinity. The germination rate under salinity was enhanced by all the seed priming treatments. The observed hermetic response of MLE was particularly notable at concentrations of 2.5% ('P-30Y87') and 3% MLE ('P-30T60'). The observed enhancement in growth parameters can be attributed to augmented antioxidant activities, photosynthetic pigments, and leaf gas exchange characteristics. However, more in-depth studies are molecular studies are needed to explore the mechanism of how MLE can mitigate the adverse impacts of salinity. Besides this field studies are also direly needed to optimize the concentration of MLE while considering the soil and climate conditions.

Authors' Contributions

Conceptualization: M and AK. Writing-original draft: M and AK. Writing-reviewing and editing: FM, HS, SAA, SA, MA, MA and ZB.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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