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# Impact of foliarly applied morigna leaf extract for the mitigation of salt stress in *Chenopodium quinoa* genotypes: growth, physiological and ionic modifications

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**Contribution**

**Naz**, **T.** & **M. M. Iqbal** has designed, conceived the research idea supervised the present research and written the initial main body of manuscript, **U. Ali** & **M. Shafeeq** has conducted and carried out the present research study, **S. Nawaz & M. Nawaz** has helped in research experimentation by providing the availability of seeds, fertilizers and inputs, **Noor ul Ain & F. Amin** helped in data tabulation and statistically analysed the obtained data, **A. Nadeem & M. Kanwal** helped in determination of different chemical parameters in laboratory, **Z. Iqbal & R. Nawaz** considerably helped in crop protection measures and agronomic production systems as well as provided funds, **M. B. Khan & M. I. Hussain** critically reviewed the manuscript and facilitated in introduction, methodology and updated literature citation and references, **M. Imran & M. A. Ghafoor** significantly contributed in improving results and discussion with mechanistic and logical reasoning, **A. Afzal** & **K. Shahzad** critically checked the plagiarism via Turnitin of the manuscript, removed any repetition, edited and improved the manuscript in the light of reviewers comments.

## ABSTRACT

Salinity poses a significant threat to crop production and food security, particularly in regions like Pakistan where salt-tolerant crop options are limited. While some salt-tolerant forage species exist, the need for salt-tolerant cereal crops is crucial. Quinoa, a pseudo-cereal renowned for its high nutritional value and tolerance to abiotic stresses including salinity, presents a promising solution. Under saline conditions, quinoa's protein content can increase, but overall productivity is often reduced. This limitation can potentially be addressed by applying moringa leaf extract (MLE), a natural biostimulant rich in micronutrients, antioxidants, and growth promoters. To investigate this, a greenhouse experiment was conducted at two salinity levels (ECe = 10 and 20 dS $m^{-1}$) to assess the effects of foliar MLE application on quinoa grain production and nutritional attributes. The experiment utilized a completely randomized design with three replicates. Post-harvest analysis revealed that foliar MLE application positively influenced quinoa growth, significantly enhancing its salt tolerance. This improvement is attributed to MLE's chemical composition, which facilitated reduced sodium ($Na^+$) accumulation and increased potassium ($K^+$) and calcium ($Ca^{2+}$) concentrations in both leaves and seeds, ultimately improving plant growth and yield. Notably, among the genotypes tested, UAF-Q7 exhibited greater inherent salt tolerance than UAF-Q9, with MLE application further augmenting this resilience. This study highlights the potential of foliar MLE application as a viable strategy to mitigate salinity stress in quinoa, especially in salt-affected agricultural regions of Pakistan, contributing to enhanced food security.

**Keywords**: Moringa leaf extract, quinoa, salt tolerance, growth, physiology.

**INTRODUCTION:** Salinity has been noted as one of the utmost destructive agricultural problems among all the abiotic stresses, around the globe. Salinity reduce the plant physiological characters (Zadeh and Naeini, 2007), lowers productivity, shortens canopy and minimizes crop producing. Salinity negatively affects the germination of seed, limits the growth of plant and deteriorates plant health and physiology that ultimately lessens overall yield (Parida *et al.*, 2005). Intensive agriculture though increases our production but highly affects fertility of the lands and stimulates soil degradation. Another effect of this system is reduction of agro-biodiversity. One main reason is that we are highly dependent upon our few crop species one of the example is that our 75% of demand is fulfilled by just 12 species and maize, wheat and rice contributes almost 50% to our world diet. Food security globally depends upon consideration on different options but these are limited due to our dependence on few species and more terrible situation occurs due to biotic stresses and more insect pest attacks on these conventional crops (Ghous *et al.*, 2022). Now, it is a need of the hour to introduce the new alternate crops because these are getting attraction due to its numerous benefits that includes tolerance to stress conditions, nutritional profile and better profits properties (Akram *et al.*, 2021). Sustainability can be achieved by the use of these crops that can acclimatize in varying environment (Ravi *et al.*, 2010). Apart from these crops which can also be grown on the marginal lands where the main crops are being failed due to certain reasons. Therefore, introduction of new salt-resistant genotypes and halophytes species is needed to counter this problem (Mazhar *et al.*, 2023). In future, the introduction of new plants species/varieties which are resistant to salinity and give reasonable yield which are to be compared to other species/varieties in the same condition is needed to ensure food safety for increasing population. Among newly introduced non-conventional crops, *Chenopodium quinoa* is a lesser-known halophyte; from Chenopodiaceous family and common halophyte genera. Quinoa is a dicot halophyte plant with broad leaves ((Jacobsen *et al.*, 2005). It can tolerate drought, soil salinity, biotic stress and it have a high nutritious value which attracts the world to fulfill the future food security depends on its genotypes (Ruiz *et al.*, 2014). Quinoa is better than rice, wheat and maize in having protein content of 12.5 to 16.7% and fats also that ranges from 5.5 to 8.5% (Vega-Gálvez *et al.*, 2010). Quinoa is recognized for its gluten-free property that enables that food suitable for celiac disease patient (Valcárcel-Yamani and Lannes, 2012). Information about it morphological and phenological features can be helpful for the breeders to develop such genotypes that have desired characters. This is the reason that in a specific country we need more genotypes. Recently, some studies show that quinoa performs well in the stress conditions and also show its mechanism to tolerate these stress conditions (Bazile *et al.*, 2015). The *C. quinoa* plants which grow under high salt ($Na^+$) stress, increase its protein contents but their yield affected (Wu *et al.*, 2016). High sodium levels can have a significant impact on yield, seed hardness, 1000-grain weight, plant growth, but its total protein contents might increase significantly. On the other hand, significant reductions in yield and carbohydrate contents have been observed (Koyro and Eisa, 2008).

At high salt stress levels, quinoa growth can be corrected by using certain plant growth regulators (PGRs) which improved the salt tolerance capability of the plants as well as PGRs might control stress-related plant biochemical and internal physiological changes (Noor *et al.*, 2016). A variety of PGRs is available but most of them are synthetic, expensive and unsafe for the environment. Among the various plant sources that can be used to treat stress-related problems in plants, the juice extracted from *Moringa oleifera* leaves might have excellent properties (Bakhtavar *et al.*, 2015). The MLE attained from fresh moringa leaves can contain small amounts of

auxiliary metabolites, antioxidants and osmo-protective agents (Rady *et al.*, 2013). In MLE, cytokianin derivative "zeatin", and minerals are present. Due to its composition and role in plant growth MLE has been declared as potential bio-stimulant (Rehman *et al.*, 2015). The MLE can be used as a foliar spray having the positive effect on plant physiology and can increase the stress tolerance in quinoa plants (Abdel Latef *et al.*, 2017). In addition, research work linked to the tolerance of quinoa to salt stress has been reported. However, studies must also be carried out to assess salinity and MLE impact on quinoa growth.

**OBJECTIVES:** The objective of the current study was to explore the consequence of salinity on growth, yield and seed quality of available quinoa genotypes and *in vitro* evaluation of foliar MLE for mitigation of salt stress**.**

**MATERIALS AND METHODS:** A pot trial was conducted in the control condition at Institute of Soil and Environmental Sciences (ISES) at University of Agriculture Faisalabad (UAF), Punjab Pakistan. The soil was collected from the ISES UAF farm, air dried, sieved 2mm. and basic physico-chemical properties was computed (table 1).

| Parameter | Obtained value | Unit |
|---|---|---|
| pHs | 7.68 | - |
| ECe | 2.61 | dS $m^{-1}$ |
| N | 0.09 | % |
| P | 7.1 | ppm |
| K | 100 | ppm |
| Organic matter | 0.91 | % |
| Saturation percentage | 31.5 | % |
| Textural class | Sandy clay loam | - |

Table 1: Physico-chemical properties of the soil used for experiment.

For preparing MLE, healthy leaves were taken and were extracted after overnight freezing. The extract was sieved and distilled water was used to prepare dilution. Two genotypes UAF-Q7 and UAF-Q9 were used. The treatments used were: $T_1$ = Control, $T_2$ = EC10 dS $m^{-1}$, $T_3$ = EC20 dS $m^{-1}$, $T_4$ = Control + Foliarly sprayed MLE at 3%, $T_5$ + EC 10 dS $m^{-1}$ + Foliarly sprayed MLE at 3%, $T_6$ = EC20 dS $m^{-1}$ + Foliarly sprayed MLE MLE at 3%. Soil salinity was developed using NaCl salt thirty days prior to sowing. The quantity of salt was calculated succeeding US Salinity Lab (Naz *et al.* 2023) as:

$$Amount\ of\ NaCl\ (g\ kg-1) = TSS \times Eq.weight\ of\ NaCl \times Saturation\ percentage\ /1000$$

The MLE was foliarly sprayed at 4 leaf stage, 20 days after 1st spray and at flowering. The treatments having three replications each were arranged in Completely Randomized Design (CRD). The recommended rates of NPK fertilizer were applied at 60:40:30 Kg $ha^{-1}$ using fertilizers such as Urea, DAP and SOP respectively.

**Physiological properties:** The second leaf from apex was restrained at 3 dissimilar spots for total chlorophyll contents (TCC) using Chlorophyll Meter. The photosynthetic and transpiration rates as well as stomatal conductance were determined (from 10 a.m. to 1 p.m.) on flag leaves of 3 randomly selected quinoa plants from each treated pot using a portable Infrared Gas Analyzer. At reaping, plant height was recorded, samples were incubated at 65 ± 0.5°C and dry weight of shoots and seeds were recorded. After 7 days, seeds were separated from panicles and stored for chemical analysis.

**Chemical analysis:** The seed and shoot (g) were dipped in 15 mL of di-acid ($HNO_3$ and $HClO_4$) mixture (3:1) and incubated overnight at room temperature. The mixture was incubated at 150° C for 20 min. and lastly at 250° C for 30 min. until nitric acid fumes were changed to transparent. After cooling and filtering, the volume was made 50 ml. The digested samples were used to determine $Na^+$ and $K^+$ in the shoot and $Ca^{+2}$ in the seed with the help of. flame photometer and atomic absorption spectrophotometer (Mazhar *et al.*, 2023), respectively. Flour of quinoa seed samples was made using grinder machine. Nitrogen in flour samples was estimated by Kjeldahl's method (Sáez-Plaza *et al.*, 2013). Seed protein percentage was calculated by multiplying % N with 6.25.

**Statistical analysis:** The study data was subject to statistical review in the CRD-factorial system. An Analysis of Variance (ANOVA) technique assessed the effect of salt, variety and MLE on quinoa. The LSD test was pragmatic to isolate significance among different treatment means (Steel *et al.,* 1997) via Statistics 8.1.

**RESULTS AND DISCUSSION: Physiology of quinoa: Total chlorophyll contents:** Analysis of Variance (ANOVA, table 2) showed substantial consequence of salt stress and the application of MLE on TCC (figure 1a) of quinoa genotypes. The maximum (75 SPAD Value) TCC were recorded in UAF-Q7 in T4 where MLE was applied under non-saline condition. Contrarily, the minimum (65) TCC were recorded in UAF-Q7 in T3 where plants were visible to salt stress (EC 20 dS $m^{-1}$). As compared to control, salt stress reduced TCC by 5.79% at EC 10 dS $m^{-1}$ and 9.7% at EC 20 dS $m^{-1}$ in UAF-Q7 genotype. While in UAF-Q9, TCC were reduced by 4.6% at EC 10 dS $m^{-1}$ and 10% at EC 20 dS $m^{-1}$. The foliarly applied MLE had a progressive impression on TCC. In UAF-Q7, MLE spray increased the TCC by 4% at EC 10 dS $m^{-1}$ and by 3% at EC 20 dS $m^{-1}$ as compared to respective control. Likewise, in UAF-Q9, MLE spray increased TCC by 3.72 % at EC 10 dS $m^{-1}$, 4.5% at EC 20 dS $m^{-1}$. The variety UAF-Q7 showed greater improvement in TCC with applied MLE. Our outcomes are in-line with (Derbali *et al.*, 2023) whom quantified that salinity has prominent impact on chlorophyll contents. The drop in the chlorophyll contents at 300 mM NaCl was elucidated by an increased chlorophyllase enzyme activity and with a restricted nitrogen uptake. Previously, a decline in SPAD-Value and TCC in quinoa under saline conditions was confirmed (Long, 2016). Salinity affects the chlorophyll contents while chlorophyll contents in leaves were enhanced by the application of MLE (Rashid *et al.*, 2018).

**Photosynthetic rate:** The salt stress and application of MLE significantly (table 2) impacted the photosynthetic rate (figure 1b). The maximum (9.4 µmol $CO_2$ $m^{-2}$ $s^{-1}$) photosynthetic rate was recorded in UAF-Q9 in T4 where MLE was applied under non-saline condition. Contrarily, the minimum (1.2 µmol $CO_2$ $m^{-2}$ $s^{-1}$) photosynthetic rate was recorded in UAF-Q7 in T3 where plants were exposed to salt stress (EC 20 dS $m^{-1}$). As compared to control, salt stress reduced photosynthetic rate by 41 % at EC10 and 82 % at EC20 in variety UAF-Q7. While in UAF-Q9 photosynthetic rate was reduced by 30 % at EC 10 dS $m^{-1}$ and 81% at EC 20 dS $m^{-1}$. The foliar application of MLE had a positive impact on photosynthetic rate. In UAF-Q7, MLE spray increased photosynthetic rate by 45% at EC10 and by 100% at EC 20 as compared to respective control. Likewise, in UAF-Q9, MLE spray increased photosynthetic rate by 38% at EC10, 100% at EC20. The variety UAF-Q7 showed greater improvement in photosynthetic rate with the application of MLE and salt stress reduced the photosynthesis significantly. As salt increase in soil it also decreases the available water for up taken by plants. Water is a key component of photosynthetic process, and role uptake of nutrients, activation of enzyme, and stomatal actions. Salt stress also diminishes specific ion toxicity in pant e.g. sodium. Iqbal *et al.* (2017) observed decrease in photosynthesis of quinoa plant under salt stress. The MLE spray improves photosynthesis in quinoa plant due to its chemical composition under stress (Rashid *et al.*, 2018). The MLE has osomo-protectants, anti-oxidants, enzymes and nutrients specifically K which regulates stomata opening and closing as well as responsible for more than 100 enzymes. As MLE applied on crop it improves water uptake and provide nutrients to plant which results in increase in photosynthesis (Abdalla, 2013).

**Transpiration rate:** A significant ($P \leq 0.05$, table 2) effect of salt stress and the foliar application of MLE on transpiration rate (figure 1c) of quinoa genotypes was observed. The maximum (53.8 mmol $H_2O$ $m^{-2}$ $s^{-1}$) transpiration rate was recorded in UAF-Q9 in T1. Contrarily, the minimum (29.8 mmol $H_2O$ $m^{-2}$ $s^{-1}$) transpiration rate was recorded in UAF-Q7 in T6 where plants were exposed to salt stress (EC 20 dS $m^{-1}$) and MLE was applied. As compared to control, salt stress reduced transpiration rate by 21% at EC10 and 33% at EC 20 dS $m^{-1}$ in UAF-Q7. While in UAF-Q9, transpiration rate was reduced by 13% at EC10 and 32% at EC20. In UAF-Q7, MLE spray increased transpiration rate by 8% at EC 10 dS $m^{-1}$ and by 11% at EC 20 dS $m^{-1}$ as compared to respective control. Likewise, in UAF-Q9, MLE spray increased transpiration rate by 20% at EC 10 dS $m^{-1}$, 15% at EC 20 dS $m^{-1}$. The UAF-Q9 showed greater improvement in transpiration rate with the application of MLE. Salt stress reduced the transpiration rate significantly. As salt increase in soil it also decreased the available water for plant uptake. As water level lower down in plant body transpiration rate decreases due to decreased water level in plant body. Transpiration process is a source of nutrients for pant growth. Iqbal *et al.* (2017) also observed decrease in transpiration rate of quinoa plant under salt stress. The MLE spray improves transpiration rate in quinoa plant due to its chemical composition under stress (Rashid *et al.*, 2018). The MLE has osomo-protectants, antioxidants, enzymes and nutrients specifically K which regulates stomata opening and closing. As MLE applied on crop it improves water uptake and provide nutrients to

plant and maintain plant temperature which results in increased transpiration (Abdalla, 2013).

**Stomatal conductance:** The application of MLE significantly ($p \leq 0.05$, table 2) reduced the negative effect of salt stress on stomatal conductance (figure 1d) of quinoa genotypes. The maximum (0.61 mol $m^{-2}$ $s^{-1}$) stomatal conductance was recorded in UAF-Q9 in T4 where MLE was applied under non-saline condition. Contrarily, the minimum (0.03 mol $m^{-2}$ $s^{-1}$) stomatal conductance was recorded in UAF-Q7 in T3 where plants were exposed to salt stress (EC 20 dS $m^{-1}$). As compared to control, salt stress reduced stomatal conductance by 44% at EC 10 dS $m^{-1}$ and 91% at EC 20 dS $m^{-1}$ in UAF-Q7. While in UAF-Q9 stomatal conductance was reduced by 37% at EC 10 dS $m^{-1}$ and 87% at EC 20 dS $m^{-1}$. The foliar application of MLE had a positive impact on stomatal conductance. In UAF-Q7, MLE spray increased stomatal conductance by 85% at EC 10 dS $m^{-1}$ and by 88% at EC 20 dS $m^{-1}$ as compared to respective control. Likewise, in UAF-Q9, MLE spray increased stomatal conductance by 61% at EC 10 dS $m^{-1}$, 100% at EC 20 dS $m^{-1}$. The UAF-Q9 showed greater improvement in stomatal conductance with the application of MLE.

Salt stress reduced the stomatal conductance significantly. As salt increase in soil it decreased the available water for plant uptake. The decreased water contents in plant body lead to reduced stomatal conductance as a result of stomata closure. Stomata are responsible for gaseous exchange in plant body. Iqbal *et al.* (2017) also reported decrease in stomatal conductance under salt stress. The MLE spray improves stomata conductance in quinoa plant due to its chemical composition under salt stress (Rashid *et al.*, 2018). As MLE applied on crop it improves water uptake and provide nutrients to plant and improves water uptake which results in increased stomata conductance (Abdalla, 2013). Structural changes are produced by abiotic stresses in photosynthic machinery leading to reduced photosynthesis in plants (Iqbal *et al.*, 2017). The reduction in chlorophyll contents due to salinity may be caused as a result of degradation and loss in the integrity of membranes. The enzymes are destroyed and thylakoid membranes may be disrobed in response to salt stress. The chlorophyll contents enhanced in response to the exogenous application of MLE as the moringa leaves contain high amount of chlorophyll and carotenoids which have high antioxidant activity. The synthesis of cytokinins is accelerated by the application of MLE which prevents the leaf senescence leading to higher leaf area with more chlorophyll contents (Yasmeen *et al.*, 2013)

**Growth and yield of quinoa: Plant height:** Salt stress negatively impacted the plant growth while a significant ($p \leq 0.05$, table 2) positive effect of MLE on plant growth was observed. The maximum (24 cm) plant height (figure 2a) was recorded in UAF-Q9 in T4 where MLE was applied under non-saline condition. Contrarily, the minimum (37.7 cm) plant height was recorded in UAF-Q7 in T3 where plants were exposed to stress (EC 20 dS $m^{-1}$). As compared to control, salt stress reduced plant height by 5.79% at EC 10 dS $m^{-1}$ and 18% at EC 20 dS $m^{-1}$ in UAF-Q7. While in UAF-Q9 plant height was reduced by 7% at EC10 dS $m^{-1}$ and 14% at EC 20 dS $m^{-1}$. The foliar application of MLE had a positive impact on plant height. In UAF-Q7, MLE foliar spray increased plant height by 5.7% at EC10 and by 4% at EC20 as compared to respective control. Likewise, in UAF-Q9, MLE spray increased plant height by 3.72 % at EC 10, 4.5 % at EC20. The UAF-Q7 showed greater improvement in plant height with the application of MLE. Our results are in line with Iqbal *et al.* (2017) whom observed a significant decline in plant height of quinoa. In another study, a significant decline in plant height in quinoa was observed under saline conditions (Long, 2016). The quinoa cultivars exhibited more reduction in plant height at higher salinity levels as compared to lower salt concentration. Our results are in accordance with Rashid *et al.* (2018) whom reported that MLE has significant impact on quinoa. In conformity, MLE improved the plant height and productivity of wheat in salt affected soil by improving senescence and source linked relationships.

**Spike length:** The maximum (24 cm) spike length (figure 2b) was recorded in UAF-Q9 in T4 where MLE was applied under non-saline condition. Contrarily (table 2), the minimum (17 cm) spike length was recorded in UAF-Q7 in T3 where plants were exposed to salt stress (EC-20). As compared to control, salt stress reduced spike length by 13% at EC 10 dS $m^{-1}$ and 21% at EC 20 dS $m^{-1}$ in UAF-Q7. While in UAF-Q9 spike length was reduced by 7% at EC 10 dS $m^{-1}$ and 10% at EC 20 dS $m^{-1}$. The foliar application of MLE had a positive impact on spike length. In UAF-Q7, MLE foliar spray increased spike length by 4% at EC 10 dS $m^{-1}$ and by 7% at EC 20 dS $m^{-1}$ as compared to respective control. Likewise, in UAF-Q9, MLE spray increased spike length by 8% at EC10, 18 % at EC20. The UAF-Q9 showed greater improvement in spike length with the application of MLE. As salinity increase the spike length decreases. The results are also in accordance with Kaya *et al.* (2007) reported a major decline in spike length of wheat plants as the salinity increased. Rashid *et al.* (2021) also observed that MLE improved quinoa plants due to its chemical composition. Rehman *et al.* (2017) also described that MLE improved the spike length and productivity of wheat under salt affected soils by improving senescence and source linked relationships. The MLE contains high amount of antioxidants, minerals cytokinins and secondary metabolites. Therefore, its exogenous application is helpful in protecting plants from damage caused by environmental stresses. It improves the morphological attributes of plants not only in normal conditions but also in stress environment (Yasmeen *et al.*, 2012).

**Seed yield:** The seed yield was negatively (table 2) impacted by salt stress while the application of MLE proved effective for enhancing the seed yield (figure 2c) under salt stress conditions. The maximum (24 g $pot^{-1}$) seed yield was recorded in UAF-Q7 in T4 where 3% MLE was applied under control conditions. Contrarily, the minimum (12 g $pot^{-1}$) seed yield was recorded in UAF-Q9 in T3 where no MLE was applied under EC 20 dS $m^{-1}$. As compared to control, salt stress reduced seed yield concentration by 20% at EC 10 dS $m^{-1}$ and 35% at EC 20 dS $m^{-1}$ in UAF-Q7. While in UAF Q9, seed yield concentration was increased by 25% at EC 10 dS $m^{-1}$ and 40% at EC 20 dS $m^{-1}$. The foliar application of MLE had a positive impact on seed yield. In UAF-Q7, MLE spray increase seed yield by 18.7% at EC10 and by 18% at EC20 as compared to respective control. Likewise, in UAF-Q9, MLE spray increased seed yield concentration by 19 % at EC10, 18% at EC 20. The UAF-Q7 showed greater improvement in seed yield with the application of MLE. Salt stress reduces the seed yield of quinoa. Previously, a positive impact of MLE on yield of quinoa was recorded (Rashid *et al.*, 2018) . The use of plant growth promoting substances is associated with the growth enhancing compounds which increase cell division, elongation and resultant enhanced growth of plants. The presence of substances which promote plant growth such as proteins, cytokinins and auxins makes MLE very suitable for enhancing plant growth in salt stress environments as these substances promote the formation of cell protoplasm, enhance cell division and enlargement (Abdalla, 2013). The higher plant growth leads to better yield under normal as well as saline conditions. Our results are also supported by Rashid *et al.* (2018) who reported an enhancement in quinoa seed yield and yield related parameters by foliar application of MLE in two years of field experiments. The foliar application of MLE enhances yield due to the presence of high concentration of secondary metabolites and essential minerals in MLE which help plants bear stress conditions through improved water uptake and source sink activity.

**Ionic composition in quinoa: Leaf Na:** The concentration of sodium (figure 3a) in leaves was significantly (table 2) enhanced in plants grown in the salt-affected soil while the application of MLE proved effective in reducing Na concentration in plants exposed to salt stress**.** Statistically, maximum (5.8%) leaf Na was recorded in UAF-Q9 in T3 where no MLE was applied under 20 dS $m^{-1}$ salt stress. Contrarily, the minimum (1.4%) leaf Na was recorded in UAF-Q7 in T4 where MLE was applied under normal soil conditions. As compared to control, salt stress increase leaf Na concentration by 44% at EC 10 dS $m^{-1}$ and 49% at EC 20 dS $m^{-1}$ in UAF-Q7. While in UAF-Q9 leaf Na concentration was increased by 40% at EC10 and 59% at EC20. The foliar application of MLE had a positive impact on leaf Na concentration. In UAF-Q7, MLE spray reduces leaf Na concentration by 40% at EC10 dS $m^{-1}$ and by 18% at EC 20 dS $m^{-1}$ as compared to respective control. Likewise, in UAF-Q9, MLE spray increased leaf Na concentration by 21% at EC 10 dS $m^{-1}$, 15% at EC 20 dS $m^{-1}$. The UAF-Q7 showed greater improvement in leaf Na concentration with the application of MLE. The concentration of $Na^+$ in shoots is more due to its elevated amount in soil under salinity due to which plant growth is severely affected. Parida and Das, (2005) described that higher concentration of salt in the exterior elucidation reasons the ion imbalance or disturbances in ion homeostasis. Earlier scientific reports have exposed that competition among $Na^+$ and $K^+$ occurred, which resulted in reduced level of internal $K^+$ at high exterior NaCl level (Kaya *et al.*, 2007). The persuaded $K^+$ uptake through NaCl prominent to deficiency of

$K^+$,and it can boost the $Na^+/K^+$ ratio that diminish the growth of plant and sourced ionic toxicity (Kaya *et al.*, 2007). The Na concentration was reduced in quinoa plant tissue through the applied MLE. Shalaby (2024) recorded reduction in

| Parameter | Salinity | Treatments (MLE) | Genotype | Salinity × MLE | Salinity × Genotype | MLE × Genotype | CV value |
|---|---|---|---|---|---|---|---|
| Total chlorophyll contents | 1.08 ns | 0.05 ns | 0.15 ns | 2.72 ns | 0.22 ns | 0.0069 ns | 6.41 |
| Photosynthetic rate | 64.18** | 0.01 ns | 842.61** | 0.100 ns | 0.01 ns | 0.01 ns | 6.53 |
| Transpiration rate | 320.32** | 212.18** | 24.26** | 1.08 ns | 1.96 ns | 0.12 ns | 3.80 |
| Stomatal conductance | 969.52** | 582.25** | 61.34** | 0.12 ns | 1.71 ns | 0.31 ns | 5.92 |
| Plant height | 54.56** | 7.27** | 9.41** | 0.09 ns | 3.82** | 0.7 ns | 3.93 |
| Spike length | 15.43** | 6.74** | 0.38** | 1.15 ns | 1.94 ns | 0.02 ns | 8.37 |
| Seed yield | 337.43** | 157.87** | 39.17** | 4.56** | 0.34 ns | 4.42 ** | 7.85 |
| Leaf Na | 233.26 ** | 31.3 ** | 103.98 ** | 0.28 ns | 19.77 ** | 2.35 ns | 10.95 |
| Leaf K | 299.41** | 331.98** | 5.19** | 1.1 ns | 2.33 ns | 0.01 ns | 4.82 |
| Seed Na | 8807.08** | 477.69** | 3831.67** | 76.22** | 0.02 ns | 0.02 ns | 1.52 |
| Seed K | 956.96** | 282.99** | 498.74** | 3.6** | 11.4** | 13.49** | 0.32 |
| Seed Ca | 771.83** | 40528.1** | 1918.97** | 21.69** | 3.45** | 216.34** | 0.82 |
| Seed protein | 851.01** | 165.9** | 23.6** | 2.13 ns | 0.73 ns | 1.72 ns | 2.91 |

Table 2: F-values of ANOVA and coefficient of variation (CV) values for the Impact of foliar application of MLE on quinoa genotypes under salt stress. NS = Non-significant (P > 0.05); * = Significant (P ≤ 0.05); ** = Highly significant (P ≤ 0.01).

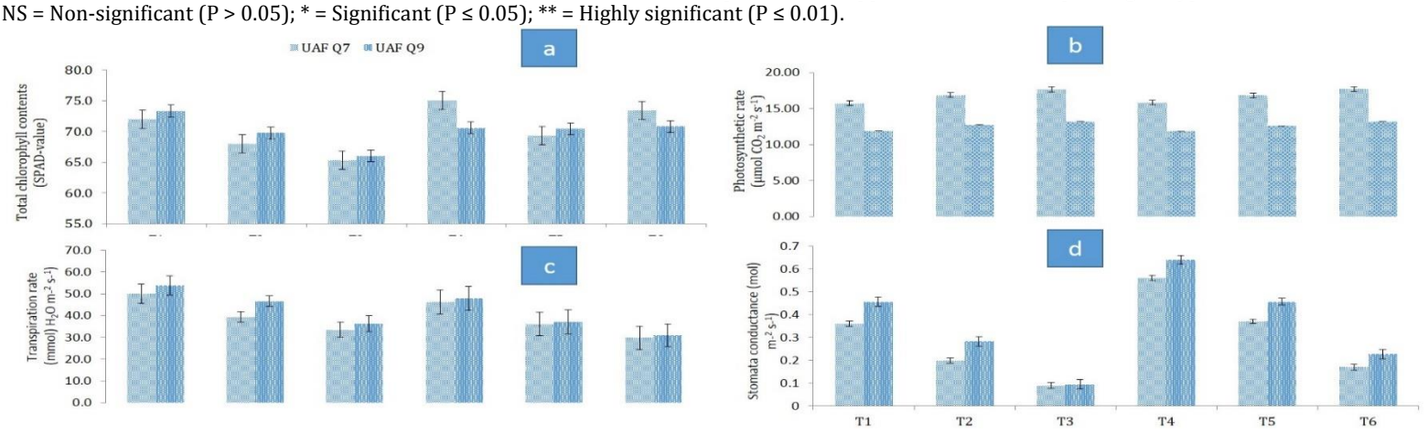


Figure 1: Impact of foliar application of MLE on quinoa genotypes (a = total chlorophyll contents, b = photosynthetic rate, c = transpiration contents, d = stomatal conductance) under salt stress.
(Each value is a mean, n = 3 statistically significant at p ≤ 0.05, T bars represents ± SE of means i.e., SEMs).
T1 = Control, T2 = EC10 dS $m^{-1}$, T3 = EC20 dS $m^{-1}$, T4 = Control + 3% MLE spray, T5 = EC10 dS $m^{-1}$ + 3% MLE spray, T6 = EC20 dS $m^{-1}$ + 3% MLE spray

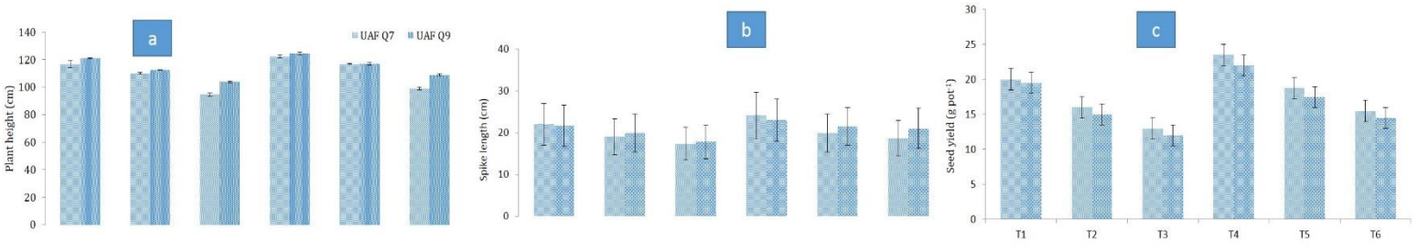


Figure 2: Impact of foliar application of MLE on quinoa genotypes (a = plant height, b = spike length, and c = seed yield) under salt stress.
(Each value is a mean, n = 3 statistically significant at p ≤ 0.05, T bars represents ± SE of means i.e., SEMs).
T1 = Control, T2 = EC10 dS $m^{-1}$, T3 = EC20 dS $m^{-1}$, T4 = Control + 3% MLE spray, T5 = EC10 dS $m^{-1}$ + 3% MLE spray, T6 = EC20 dS $m^{-1}$ + 3% MLE spray

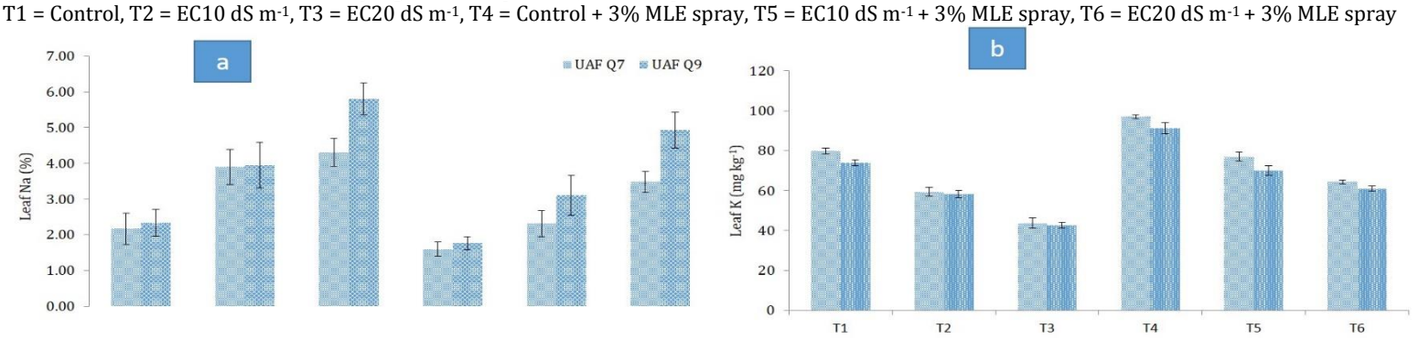


Figure 3: Impact of foliar application of MLE on quinoa genotypes (a = leaf Na, b = leaf K) under salt stress.
(Each value is a mean, n = 3 statistically significant at p ≤ 0.05, T bars represents ± SE of means i.e., SEMs).
T1 = Control, T2 = EC10 dS $m^{-1}$, T3 = EC20 dS $m^{-1}$, T4 = Control + 3% MLE spray, T5 = EC10 dS $m^{-1}$ + 3% MLE spray, T6 = EC20 dS $m^{-1}$ + 3% MLE spray

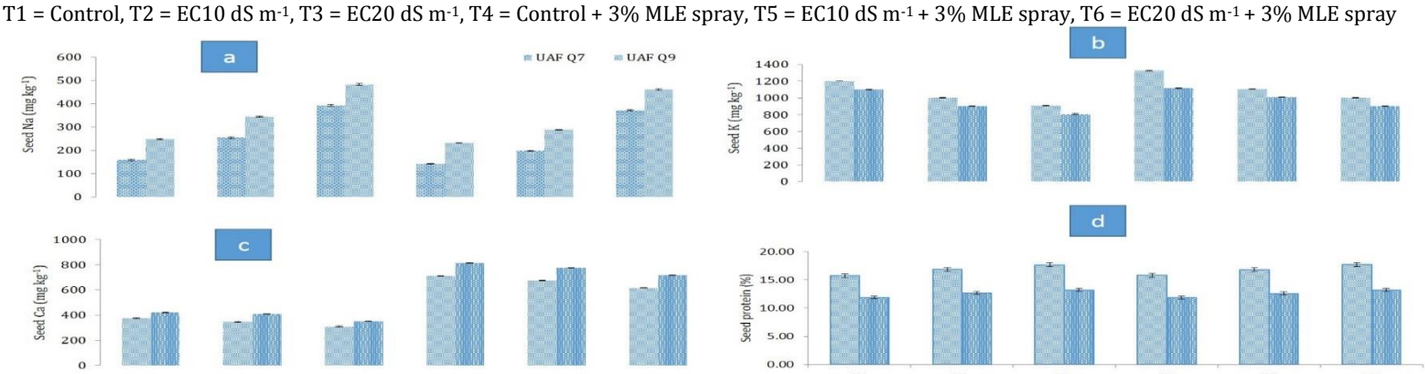


Figure 4: Impact of foliar application of MLE on quinoa genotypes (a = seed Na, b = seed K, c = seed Ca, d = seed protein) under salt stress.
(Each value is a mean, n = 3 statistically significant at p ≤ 0.05, T bars represents ± SE of means i.e., SEMs).
T1 = Control, T2 = EC10 dS $m^{-1}$, T3 = EC20 dS $m^{-1}$, T4 = Control + 3% MLE spray, T5 = EC10 dS $m^{-1}$ + 3% MLE spray, T6 = EC20 dS $m^{-1}$ + 3% MLE spra.

accumulation of Na by MLE application in lettuce plants. Rady *et al*. (2013) established that MLE treated seeds abridged the Na and $Cl^{-1}$ in common bean in salt stress, and lessened the Na in seedlings of wheat exposed to salt stress (Ahmed *et al*. 2021). Merwad (2020) described that foliar spray of moringa declined the Na content in wheat when exposed to salinity. The MLE treatments abridged the accretion of $Na^+$, hence sustaining the integrity of cell membrane and utility in salinity hassle plants, diminishing the leakage of electrolyte, and aggregate the relative water content, thus relieving the salinity and keeping away plants from damage of oxidative stress (Shalaby, 2024).

**Leaf K:** A significant effect (table 2) of salt stress, the applied MLE, quinoa genotypes and their interaction was observed on leaf K (figure 3b) concentration. Statistically, maximum (89 mg $kg^{-1}$) leaf K was recorded in UAF-Q7 in T3 where no MLE was applied under control conditions. Contrarily, the minimum (41 mg $kg^{-1}$) leaf K was recorded in UAF-Q9 in T4 where MLE was applied under normal soil conditions. As compared to control, salt stress reduces leaf K concentration by 26 % at EC10 and 45% at EC20 in UAF-Q7. While in UAF-Q9, leaf K concentration was decreased by 21% at EC10 and 42 % at EC 20 dS $m^{-1}$. The foliar application of MLE had a positive impact on leaf K concentration. In UAF-Q7, MLE spray enhances leaf K concentration by 34 % at EC 10 dS $m^{-1}$ and by 58 % at EC 20 dS $m^{-1}$ as compared to respective control. Likewise, in UAF-Q9, MLE spray increased leaf K concentration by 37% at EC10, 56% at EC20. The UAF-Q7 showed greater improvement in leaf K concentration with the application of MLE. In present study salinity reduces leaf K concentration due to presence of excessive amounts of Na in soil, Na replace K which results less uptake of K in leaves of quinoa. Our outcomes are in accordance with results of Iqbal *et al.* (2017). The K concentration in quinoa tissue was enhanced by foliar spray of MLE. The MLE is an ordinary plant biostimulant having essential minerals and osmoprotectant molecules that preserve osmotic pressure and metabolic processes and safeguard the plants from stress, which lessens the detrimental impacts of abiotic stress, rises nutrient and hinders degradation of chlorophyll of treated plants (Taha, 2016). Thus, MLE rises water and uptake of nutrient by salinity hassle plants owing to the conservation of integrity of cell membrane and nutrient availability, which resulted in improved N, P, K, and levels of chlorophyll (Shalaby, 2024).

**Seed Na:** The application of MLE reduced the seed sodium concentration (figure 4a). Statistically (table 2), maximum (490 mg $kg^{-1}$) seed Na was recorded in UAF-Q9 in T3 where no MLE was applied under 20 dS $m^{-1}$ salt stress. Contrarily, the minimum (144 mg $kg^{-1}$) seed Na was recorded in UAF-Q7 in T4 where MLE was applied under normal soil conditions. As related to control, salt increase seed Na concentration by 37% at EC10 dS $m^{-1}$ and 59% at EC 20 dS $m^{-1}$ in UAF-Q7. While in UAF-Q9, seed Na concentration was increased by 27% at EC 10 dS $m^{-1}$ and 48% at EC 20 dS $m^{-1}$. The foliarly applied MLE had an optimistic impression on seed Na concentration. In UAF-Q7, MLE spray reduces seed Na concentration by 22% at EC10 and by 10% at EC20 as compared to respective control. Likewise, in UAF Q9, MLE spray increased seed Na concentration by 16% at EC10, 4% at EC20. The UAF-Q7 showed greater improvement in seed Na concentration with the application of MLE. (Eisa *et al.*, 2012) pragmatic a significant increase in Na concentration of quinoa leaves and seeds in salt stress. The $Na^+$ in shoots and seeds is more due to its elevated amount in soil under salinity due to which plant growth is severely affected while K replaced the $Na^+$ in soil solution which reduced the concentration of $Na^+$ (Kaya *et al*., 2007).

**Seed K:** The concentration of K in seeds (figure 4b) was significantly (table 2) improved with the application of MLE under salt stress conditions. Statistically, maximum (1326 mg $kg^{-1}$) Seed K was recorded in UAF-Q7 in T4 where MLE was applied under control conditions. Contrarily, the minimum (806 mg $kg^{-1}$) Seed K was recorded in UAF-Q9 in T3 where no MLE was applied and plants were subjected to EC20 dS $m^{-1}$. As compared to control, salt stress decrease seed K concentration by 16% at EC10 and 24% at EC20 in UAF-Q7. While in genotype UAF-Q9 seed K concentration was increased by 18% at EC10 and 26% at EC20. The foliarly applied MLE had a positive impact on seed K concentration. In UAF-Q7, MLE spray enhances seed K concentration by 10% at EC10 and by 11% at EC20 as compared to respective control. Likewise, in UAF-Q9, MLE spray increased seed K concentration by 11.2% at EC10, 11.7% at EC20. The UAF-Q7showed greater improvement in seed K concentration with the applied MLE. In present study the seed K concentration reduced under salt stress conditions due to imbalance in ion concentration due to salinity. Hariadi *et al.* (2011) reported a significant decrease in quinoa seed K concentration under salt stress conditions. Salt stress showed impeded uptake of essential mineral elements in plants. Several studies have witnessed the diminution in seed K in salt stress (Tomar and Agarwal, 2013).

**Seed Ca:** Salt stress negatively impacted the seed Ca (figure 4c) while the seed Ca concentration was improved significantly (table 2) by the application of MLE under salt stress conditions. Statistically, maximum (813 mg $kg^{-1}$) seed Ca was recorded in UAF-Q9 in T4 where 3% MLE was applied under control conditions. Contrarily, the minimum (305 mg $kg^{-1}$) seed Ca was recorded in UAF-Q7 in T3 where no MLE was applied under 20 dS $m^{-1}$ EC. As compared to control, salt stress decrease seed Ca concentration by 7% at EC 10 dS $m^{-1}$ and 17 % at EC 20 dS $m^{-1}$ in genotype UAF-Q7. While in UAF-Q9 seed Ca concentration was reduce by 3 % at EC10 and 16% at EC20. The foliar application of MLE had a positive impression on seed Ca concentration. In UAF-Q7, MLE spray increase seed Ca concentration by 95% at EC 10 dS $m^{-1}$ and by 98% at EC 20 dS $m^{-1}$ as compared to respective control. Likewise, in UAF-Q9, MLE spray increased seed Ca concentration by 90% at EC10, 91% at EC20. The UAF-Q7 showed greater improvement in seed Ca concentration with the application of MLE. Salt stress in plants has shown impeded the absorption of essential mineral elements. Higher $Na^+$ and $Cl^-$ ion concentration are deleterious to planting and cause disturbance in Ca mobility within the plant resulting in lower Ca uptake in seeds.

**Seed protein:** The protein contents (figure 4d) of seeds significantly (table 2) declined under salt stress conditions while the MLE application proved effective in enhancing seed protein. Statistically, maximum (17.6%) seed protein was recorded in UAF-Q7 in T3 where no MLE was applied under 20 dS $m^{-1}$ salt stress conditions. Contrarily, the minimum (11.9 %) seed protein was recorded in UAF-Q9 in T4 where MLE was applied under normal soil conditions. As compared to control, salt stress increase seed protein concentration by 7% at EC10 and 4% at EC 20 dS $m^{-1}$ in UAF-Q7. While in UAF-Q9 seed protein concentration was increased by 9% at EC 10 dS $m^{-1}$ and 6% at EC 20 dS $m^{-1}$. The foliar application of MLE had no impact on seed protein. In UAF-Q7, MLE spray reduced seed protein by 0.3 % at EC 10 dS $m^{-1}$ and increase by 0.1% at EC 20 dS $m^{-1}$ over respective control. Likewise, in UAF-Q9, MLE spray reduces seed protein by 0.5% at EC10, increase by 0.24% at EC20. The UAF-Q7 showed greater improvement in seed protein concentration with the application of MLE. Salt stress has increased seed protein contents. Our outcomes are in consonance with Wu *et al*. (2016), whom conveyed a significant of salt stress on seed protein contents of quinoa. In present study MLE showed impact on seed protein contents. Rashid *et al.* (2018) reported significant influence of foliar applied MLE on protein contents of quinoa.

**CONCLUSION:** In present study, the results indicated that MLE has positive impact on quinoa growth and yield by lowering down Na, increase K and Ca concentration in leaf and seed of quinoa which ultimately improve the plant growth and yield. The foliarly applied MLE considerably increased the plant height, spike length and seed yield as compared to control. IProtein contents of quinoa seeds was affected by stress. The Na concentration in leaf and seeds was considerably increased by salt stress. Whereas, the foliarly applied MLE significantly decreased the Na concentration in leaf and seed, however, the K and Ca concentration in quinoa genotypes were increased.

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