

Morpho-Physiological and Biochemical Responses of *Cymbopogon citratus* and *Asparagus officinalis* L. to Waterlogging and Salinity Stress

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ABSTRACT

Salinity stress is an alarming issue causing a substantial reduction in crop productivity. Waterlogging also limits crop productivity and the extent of both these stresses is increasing due to climate change and global warming. This study investigated the response of *Lemongrass* and *Asparagus* grass under salinity stress and waterlogged conditions. The study was comprised of different treatments: control, salinity stress, waterlogged conditions and salinity stress + waterlogged conditions. The results revealed that salinity + waterlogging pressure negatively affected *cymbopogon citratus* and *Asparagus officinalis*. The physio-morphological, biochemical attributes, enzymatic antioxidants, and nutrient parameters showed a greater reduction under combined salinity and water waterlogged conditions. Waterlogging caused a marked decrease in root growth, leaves production and plant height of both grasses, compared to the control. Salinity stress also resulted in similar morphological modifications, albeit to a lesser extent. Physiological analysis showed a decline in chlorophyll content and RWC, indicating reduced photosynthetic capacity and water uptake efficiency in response to waterlogging and salinity. Electrolyte leakage, increased significantly under waterlogging and salinity stress, suggesting cellular damage and membrane disruption. *C.citratus* exhibited greater resilience to waterlogging and salinity compared to *A. officinalis*. Despite the adverse conditions, *C. citratus* maintained higher chlorophyll content, RWC, and lower electrolyte leakage, indicating better stress tolerance mechanisms. In conclusion, waterlogging and salinity induced significant morpho-physiological modifications in both *C. citratus* and *A. officinalis*. However, *C. citratus* exhibited better tolerance to these stresses, suggesting its potential for cultivation in waterlogged and saline environments.

Keywords: antioxidants, chlorophyll, phenolics, waterlogging, salinity.

INTRODUCTION

Modern agriculture is facing various abiotic stresses including, drought, salinity, heavy metals, and high temperature stress [Jam et al., 2023]. Among these, stress salinity is a serious concern for crop production [Jam et al., 2023]. Globally,

20% of irrigated land is salt-affected and this extent is considered to increase up to 50% by 2050 owing to excessive use of chemicals, unsustainable agricultural practices and climate change [Aksoy et al., 2022]. Salinity stress causes damage to crop production through a substantial increase in the concentration of soluble salts [Seleiman et

al., 2023; Venâncio et al., 2023]. Salinity poses a serious threat to global food security because of its harmful effects on plants [Alkharabsheh et al., 2021; Mukhopadhyay et al., 2021]. Salinity is one of the main abiotic stressors that negatively impact crop output and quality [Zhang et al., 2020]. Salinity toxicity cause osmotic pressure, ionic imbalance, and increases reactive oxygen species [ROS: Kamran et al., 2020]. Waterlogging is defined as the area where free water occupies the soil surface. This is also serious abiotic stress that hampers the gas exchanges between the atmosphere and roots as well as hampers electron transport and the ability of plants to produce ATP [Kaur et al., 2020]. Waterlogging also inhibits the root respiration and enhances the production of toxic compounds that negatively impact plant vegetative and reproductive growth as well as lead to yield losses [Zhou et al., 2020]. Further, waterlogging also induces stomata closing, and reduces chlorophyll synthesis, light harvesting resulting in a reduction in the rate of photosynthesis [Yan et al., 2018]. In addition, waterlogging causes the removal of air from soil pores and reduces oxygen availability which suppresses root activity as well as causes a reduction in nutrient and water uptake [Van Veen et al., 2014].

Asparagus (*Asparagus officinalis* L.) and lemongrass (*Cymbopogon citratus*) are C4 grasses that may thrive under unfavorable environmental conditions. Asparagus grass and lemongrass are the finest remedies for treating waterlogging and salt stress [Striker, 2012]. Lemongrass is cultivated for its essential oil. However, genetic diversity, environment conditions and agronomic practices greatly affect the chemical composition of lemon grass oil [Tzortzakis and Economakis, 2007]. Lemon grass contains an appreciable amount of citral (geranial and neutral isomers) that can be used to prepare beta-carotene, vitamin A, and other compounds. The antimicrobial activity of LGO is used to treat various pathogenic fungi [Zhang et al., 2020]. Asparagus is high in vitamins, steroidal saponins, flavonoids, minerals, and amino acids [Dawid and Hofmann, 2012]. It has long been a popular food source worldwide, making it a plant with high economic value. Asparagus has a high resistance to salt and can thrive in soil that is just mildly alkaline-saline (0.3% or less). Asparagus has beneficial effects in preventing hypertension, heart disease, and some malignancies, because it has a high level of nutrients and antioxidant substances [Doll et al., 2021]. The differentially

expressed genes show the important pathways in the responses of asparagus to salt stress. This plant also possesses excellent antioxidants, regulatory pathways and carbon catabolism activities which play an important role in salt tolerance [Zhang et al., 2020]. Therefore, this study was conducted to ascertain how lemongrass and asparagus grass respond to salinity stress and waterlogged conditions in terms of growth, physiology and morphology.

MATERIALS AND METHODS

Experimental conditions

This study determined the physio-morphological and anatomical responses of lemongrass and asparagus grass subjected to salinity as well as waterlogging stress. The research was performed at the experimental area of Islamia University, Bahawalnagar sub-campus. The plants of lemongrass and asparagus grass were gathered from the Institute of Horticulture Sciences, University of Agriculture, Faisalabad. The study was comprised of different treatments: control, salinity stress, waterlogging, and salinity stress + water logging. The salinity stress was applied by feeding the soil with a 300 mL solution of 0.5 M NaCl, while the waterlogging was developed by saturating the soil with water. The control plants were supplied with deionized. The pots had a capacity of 8 kg soil was filled with soil and five plants of each grass was sown in each pot. The pots were visited regularly and all the management practices were kept constant to obtain a good stand establishment. A completely randomized design was used to perform this study and each treatment had three replicates.

Determination of growth and photosynthetic pigments

The plants were randomly selected to determine the root and shoot length as well as biomass production. For measuring chlorophyll contents, 0.5 g of fresh plant material was prepared in 80% acetone, and concentrations of chl a, chl b, and carotenoids were determined by measuring absorbance at 645, 663, and 480 nm.

Determination of phenolics and osmolytes

First, 0.5 g fresh leaves were taken and ground to obtain the extract by using acetone (80%). Thereafter, samples were centrifuged (12000 rpm) and supernatant was taken. Then, 100 μ L extract was taken and mixed with Folin-'Cio-calteu's phenol reagent (2.5 ml), and volume was increased to 5 ml and absorbance was taken at 750 nm to determine phenolic concentration. For the determination of anthocyanin 0.1 g of fresh leaves of plants was homogenized using 1 ml of methanol and then the mixture was placed in a water bath for 60 minutes at 50°C, afterwards, absorbance was taken at 535 nm.

To examine soluble sugars: fresh leaf samples (0.1 g) were boiled with 5 ml of water filtered and volume was made to 50 ml. Later, 5 ml of anthrone reagent and 1 ml of obtained extract were heated for 20 minutes at 90°C and absorbance was measured at 620 nm. In the case of TSP: 0.5 g fresh samples were taken and ground with phosphate buffer and the obtained extract was added with Bradford reagent and kept under room conditions and absorbance was taken at 595 nm.

Finally, 0.25 g of fresh plant material was extracted in 10 mL of 6% TCA solution to measure ascorbic acid. After that, 4 mL of extract and 2 mL of 2% dinitrophenyl hydrazine were added, along with 1 drop of 10% thiourea solution. After heating the mixture for 20 minutes, allowing it cool, the absorbance was measured at 530 nm.

Determination of antioxidant activities

To measure SOD activity, 50 μ L of enzyme extract and 50 mM phosphate buffer (pH 7.8) were mixed. Subsequently, the extract was mixed with 50 μ M NBT, 1.3 μ M riboflavin, 13 mM methionine, and 75 mM EDTA. This mixture was then stored in an aluminum foil-coated dark chamber. After that, the reaction mixture was exposed to fluorescent lights for 30 minutes, and absorbance was recorded at 560 nm. To determine CAT activity 0.1 mL enzyme extract was obtained and added with phosphate buffer and diluted to 3 ml by adding H_2O_2 and absorbance was taken at 240 nm. To determine POD: leaves were taken supernatant was obtained and it was added guaia-col (20 mM) and H_2O_2 (40 mM) and absorbance was taken at 470 nm.

Determination of oxidative stress markers

For determining H_2O_2 concentration, 0.1 g fresh leaves were extracted with TCA (0.1%) and centrifuged (12000 rpm). After adding 0.5 mL of 10 mM potassium phosphate buffer (pH 7.0) and 1 mL of 1 M potassium iodide to 0.5 mL of supernatant, the H_2O_2 activity was measured by measuring the absorbance at 390 nm. To determine MDA: plant leaves were extracted by using 1 ml TCS and then centrifuged (12 000 rpm) and extract was taken. Thereafter, 1 ml of extract and 1 ml thiobarbituric acid were healthy together and allowed to cool and absorbance was taken (532 nm).

Determination of nutrients

The dried plant samples were digested by using a mixture of acids (HNO_3 and HCl, 2:1). Thereafter, these digested materials were diluted to 50 ml by adding water. A series of standards for different nutrients and standard curves were prepared to obtain the final concentration of each nutrient (K^+ , P, Ca^{2+} , and Na^+). In the case of nitrate determination, 0.5 g of plant samples were taken in tubes and added with 5 ml of water as well as autoclaved for 15 minutes and filtered; afterwards, the volume was made to 50 ml. Then, 3 ml of obtained extract was taken and mixed of 7 ml of chromotropic acid (CTA) and placed at room temperature for 20 minutes and absorbance (430 nm) was measured. Moreover, for determination of $PO_4^{3-}P$ concentration 1 ml of plant extract was mixed with 2 ml HNO_3 and the volume was made to 8 ml. Later, 1 ml molybdate-vanadate reagent was added to 8 ml mixture and the volume was increased to 10 ml and absorbance was measured at 420 nm. Last to determine sulfate concentration 1 ml HCl, 1 ml gum-acacia, and 10 ml of plant extract were mixed. Then 0.5 barium chloride was added to this mixture and continuously mixed to obtain a clear solution and absorbance was noted at 340 nm.

Statistical analysis

The data of diverse traits was analyzed with the analysis of variance technique further, to ascertain the variations between treatment means, the least significant difference test was employed.

RESULTS

Growth and photosynthetic traits

All stress treatments root and shoot growth and biomass. The combined salinity + waterlogging treatment showed the most severe effect on both grasses (Figure 1). However, *C. citratus* performed better as compared to *A. officinalis* in both cases. The photosynthetic pigments of both grasses showed significant changes under the differential condition of stress in both grasses. However, combined salinity + waterlogging treatment caused a massive reduction in the Chl-a, b, and total chl contents of both varieties. However, carotenoid contents were increased with enhancement of given stress condition for both in combined salinity + waterlogging treatment.

Phenolics and osmolytes accumulation

The soluble phenolic contents of both grasses varied significantly under waterlogging and salinity stress (Fig. 2). Both grasses showed different concentration of phenolic under different growing conditions of waterlogging and salinity stress. The concentration of anthocyanin was more or less and identical in both types of grasses. The anthocyanin concentration showed a decrease under both stress conditions. Overall, *C. citratus* leaf displayed more accumulation of anthocyanin contents, as compared to *A. Official*. The soluble sugar contents under different growing conditions of waterlogging and salinity stress of both grasses varied significantly. *A. Officinalis* showed more variations for soluble sugars than *C. citratus*. Overall, *A. Officinalis* leaf displayed more accumulation of ascorbic acid contents as compared to *C. citratus*. Both grasses also have a varied

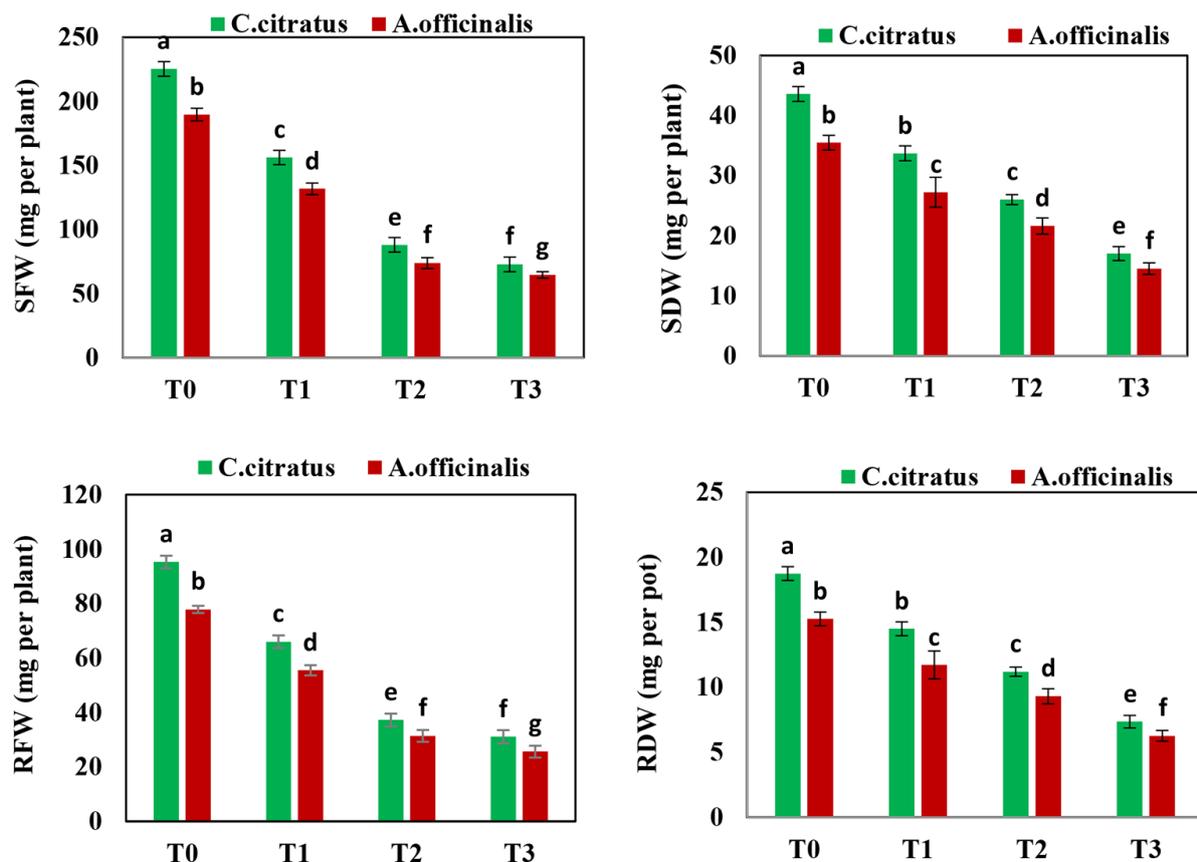


Fig. 1. Effect of variable stress condition on shoot and roots fresh and dry weights of *C. citratus* and *A. officinalis* under salinity and waterlogging conditions. T₀: control, T₁: salinity stress, T₂: water logging and T₃: salinity stress + waterlogging. The values are means of three replication and different letters on bars indicate significant difference

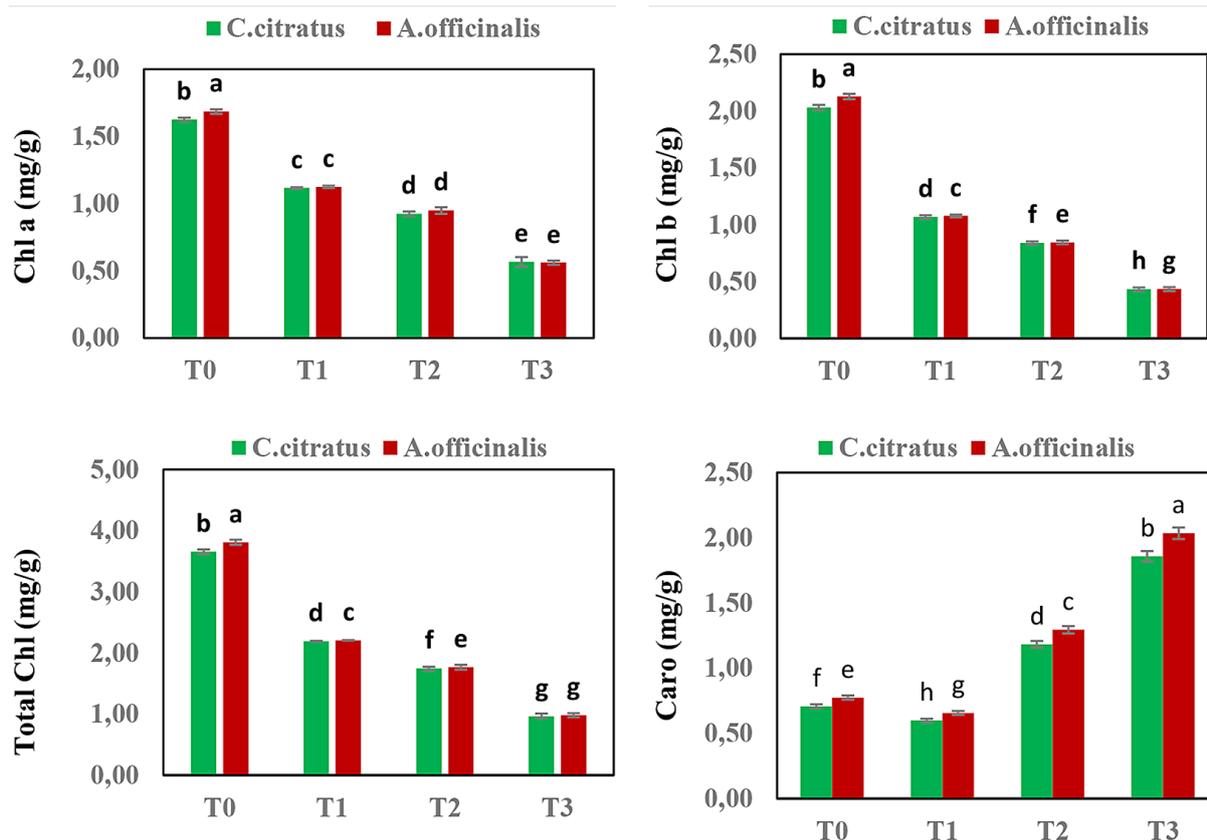


Fig. 2. Effect of variable stress condition for photosynthetic pigments *chl a*, *chl b*, *total chl* and carotenoid of *C. citratus* and *A. officinalis* under salinity and water logging conditions. T₀: control, T₁: salinity stress, T₂: water logging and T₃: salinity stress + water logging

response for TSP under different growing conditions of waterlogging and salinity stress.

Antioxidant activities and osmolytes accumulation

The SOD activity of *A. Officinalis* was the lowest under the control condition, which increased greatly in the stress condition and attained the highest value in combined treatment Salinity + waterlogging stress (Fig. 3, Fig. 4). Similar behavior was observed with *C. citratus* for the given treatment of stress condition. Both types of grass indicated individualistic behavior of CAT activity. In both types of grass, CAT activity increased from the stress condition alone and attained the maximum value under combined treatment of salinity + waterlogging stress; similar behavior was observed with *C. citratus* for the given treatment of stress condition. Significant differences observed in both grasses under given condition of stress treatment. Overall, *A. Officinalis* indicated the highest POD activity followed by *C. citratus*. There was a significant difference in both types of

grass growing under different treatments of stress for H₂O₂ concentrations. Data analysis showed that H₂O₂ production was highest under combined treatment of salinity + waterlogging pressure in *C. citratus*, while *A. Officinalis* produced maximum H₂O₂ under salinity stress alone. Overall, *A. Officinalis* indicated the highest H₂O₂ followed by *C. citratus*. The results indicate that both grasses exhibited similar MDA accumulation trend with enhanced stress conditions. In both grasses, MDA contents were lower under the control condition, and the maximum values were noted under the combined treatment of salinity + waterlogging stress. Overall, *A. Officinalis* indicated the highest MDA contents, followed by *C. citratus*.

Nutrient concentration

Both grasses showed significant differences in nitrate concentration (Fig. 5). In both grasses, nitrate-N declined under stress conditions than control conditions. They attained the lowest value in *C. citratus* under combined waterlogging

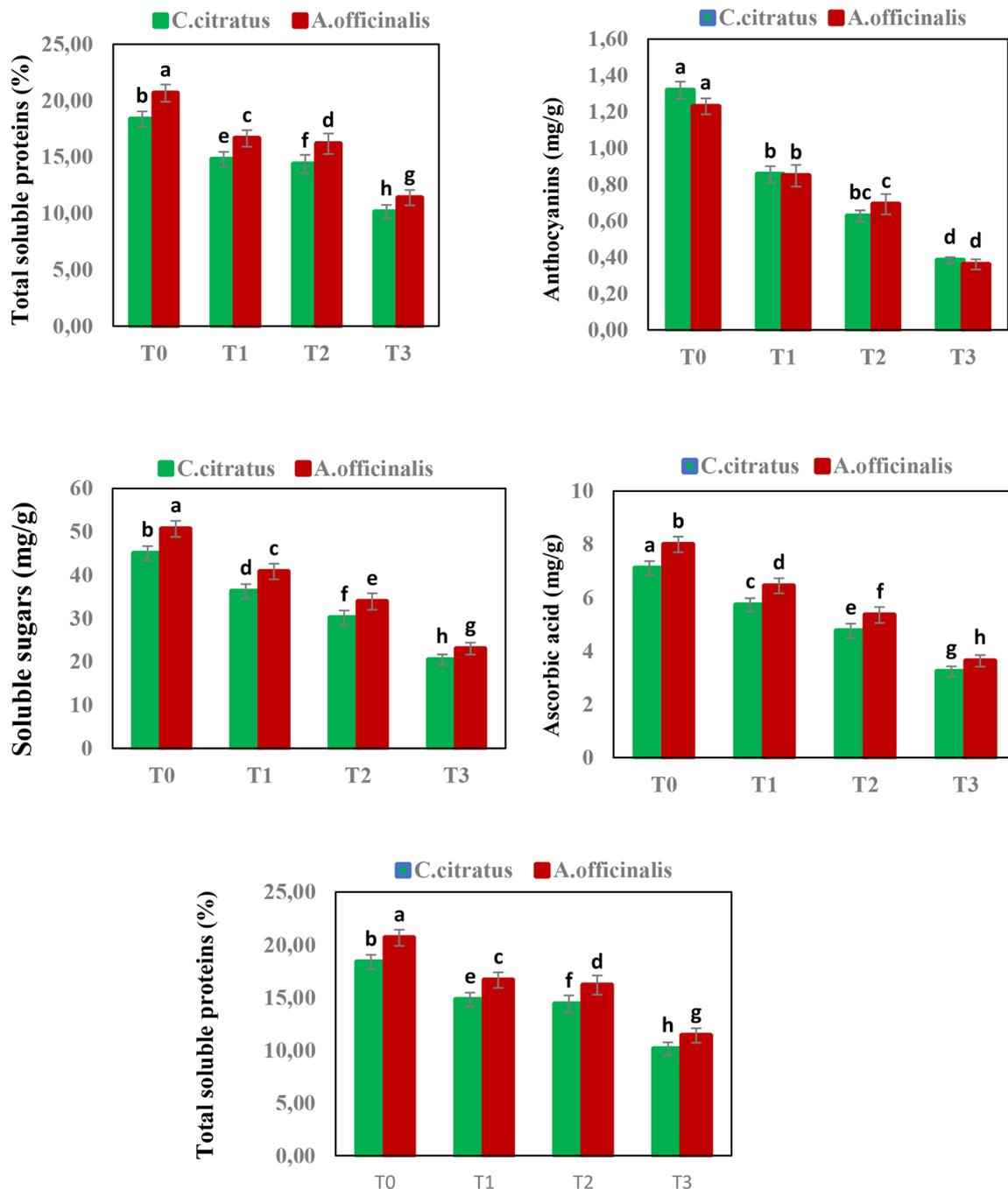


Fig. 3. Effect of variable stress condition of total soluble phenolics (*TSP*), anthocyanin (*Anth*), total soluble sugars (*TSS*), Ascorbic acid (*AsA*) and total soluble protein (*TSPro*) of *C. citratus* and *A. officinalis* under salinity and water logging conditions. T₀: control, T₁: salinity stress, T₂: water logging and T₃: salinity stress + water logging

and salinity stress treatment. Overall, the concentration of nitrate was lower in *A. Officinalis* than those observed in *C. citratus*. The concentration of soluble P was also lower in *C. citratus* than in *A. Officinalis* under the given conditions of stresses. Minimum sulfate-S contents were found in *C. citratus* under combined waterlogging and salinity stress treatment. Overall, the sulfate-S was

much lower in *C. citratus* than in *A. Officinalis* under given conditions of stresses. K concentration was also lower in *C. citratus* as compared to *A. Officinalis* under given stress conditions. The results indicate that Ca contents were lowered in treatment containing salinity stress either alone or in combination with waterlogging conditions. Further, Ca concentration was significantly lower

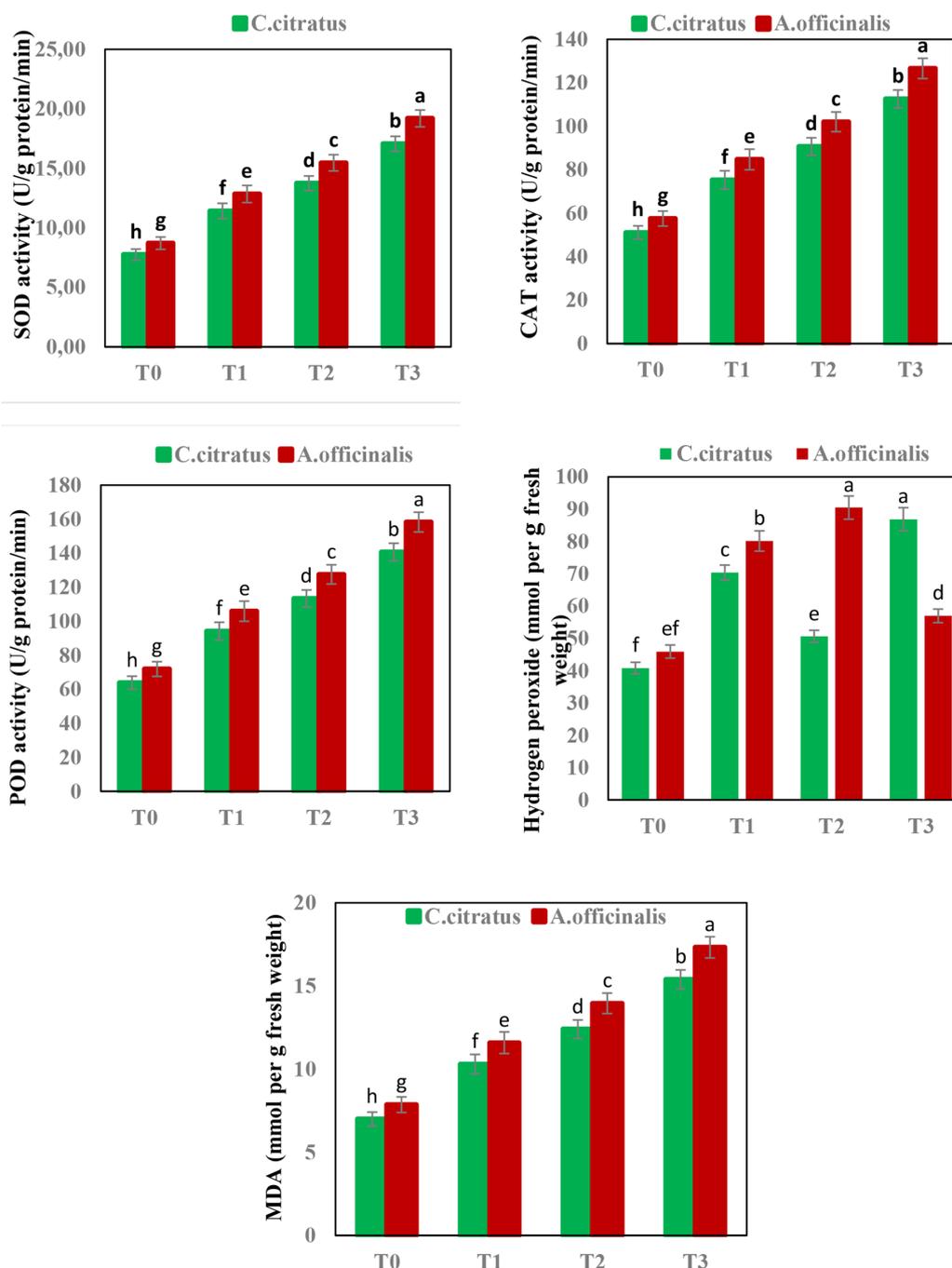


Fig. 4. Effect of variable stress condition on SOD, CAT, and POD activities and H₂O₂ and MDA) concentration *C. citratus* and *A. officinalis* under salinity and water logging conditions. T₀: control, T₁: salinity stress, T₂: water logging and T₃: salinity stress + water logging

in *C. citratus* as compared to *A. Officinalis* under given conditions of stresses.

DISCUSSION

Salt stress and waterlogging are two most important environmental issues decreasing agricultural productivity across the globe. Both stresses

have the potential to alter a plant's growth, development, and productivity by adversely affecting plant physiological and metabolic processes. The way plants respond to these stressful circumstances is incredibly complex and is impacted by other factors, including species and genotype. C₄ grasses lemongrass and asparagus can flourish under unfavorable conditions. Given that both grasses are important plants, thus,

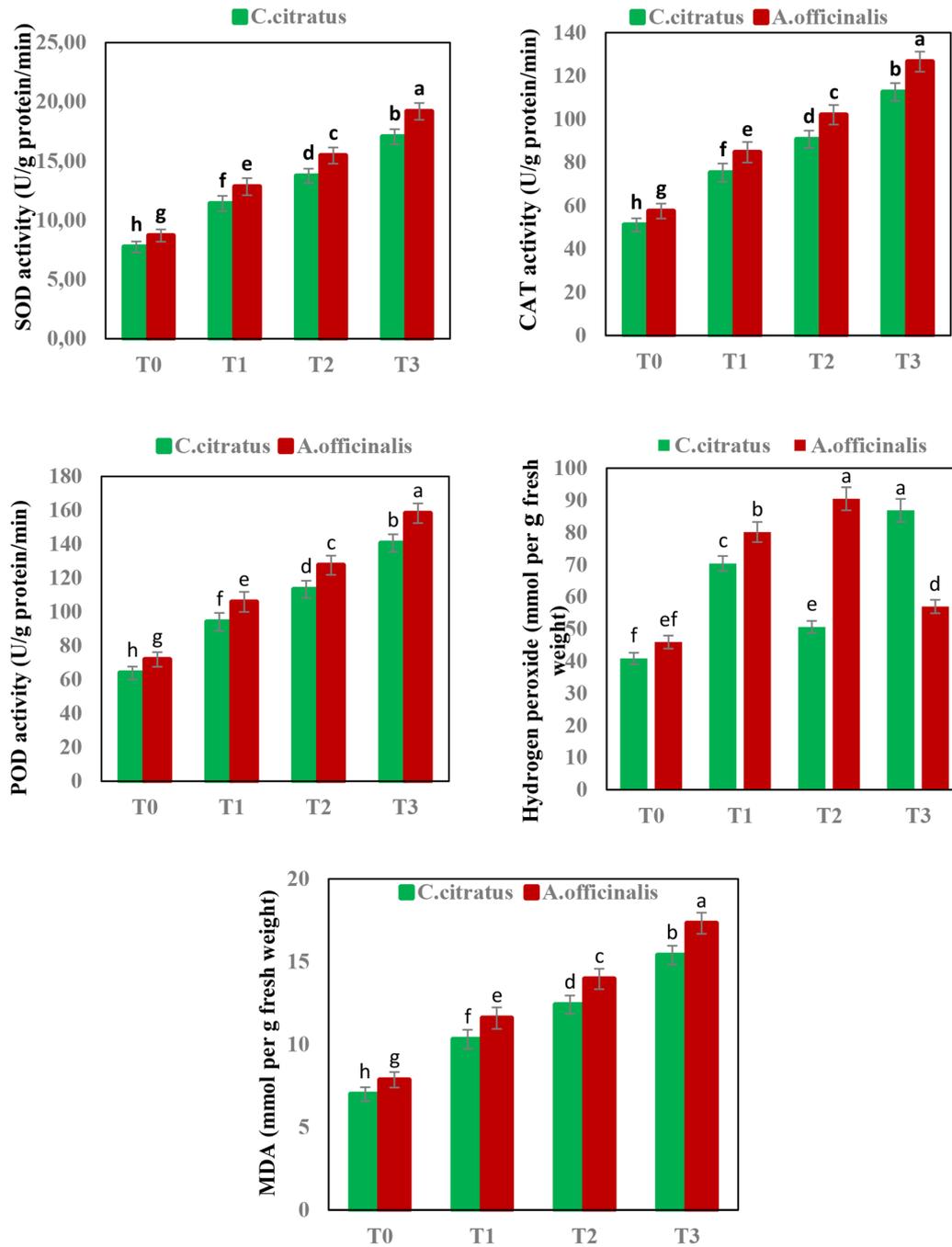


Fig. 5. Effect of variable stress condition for soluble nitrate (NO_3^- -N), soluble phosphate (PO_4^{3-} -P), sulfur (SO_4^{2-} -S), potassium (K) and calcium (Ca^{2+}) contents of *C. citratus* and *A. officinalis* under salinity and waterlogging conditions. T₀: control, T₁: salinity stress, T₂: waterlogging and T₃: salinity stress + waterlogging

it is critical to understand the physiological and biochemical characteristics that enable them to adapt to the current environmental conditions [Kaur et al., 2020]. The fresh/dry weight of the shoot and root depends upon the normal conditions; however, stress conditions reduce the plant growth and biomass production. In the present study, salinity and water logging decreased the growth and morphological traits of both grass by

increasing ROS production and decreasing photosynthetic pigments and osmolytes accumulation [Striker, 2012; Alkharabsheh et al., 2021]. The plant's photosynthetic rate depends upon the light-harvesting efficiency [Ruban, 2009]. However, in the present study, salinity and waterlogging reduced the chlorophyll synthesis by increasing the ROS production and activity of chlorophyll degrading enzymes [Mukarram et

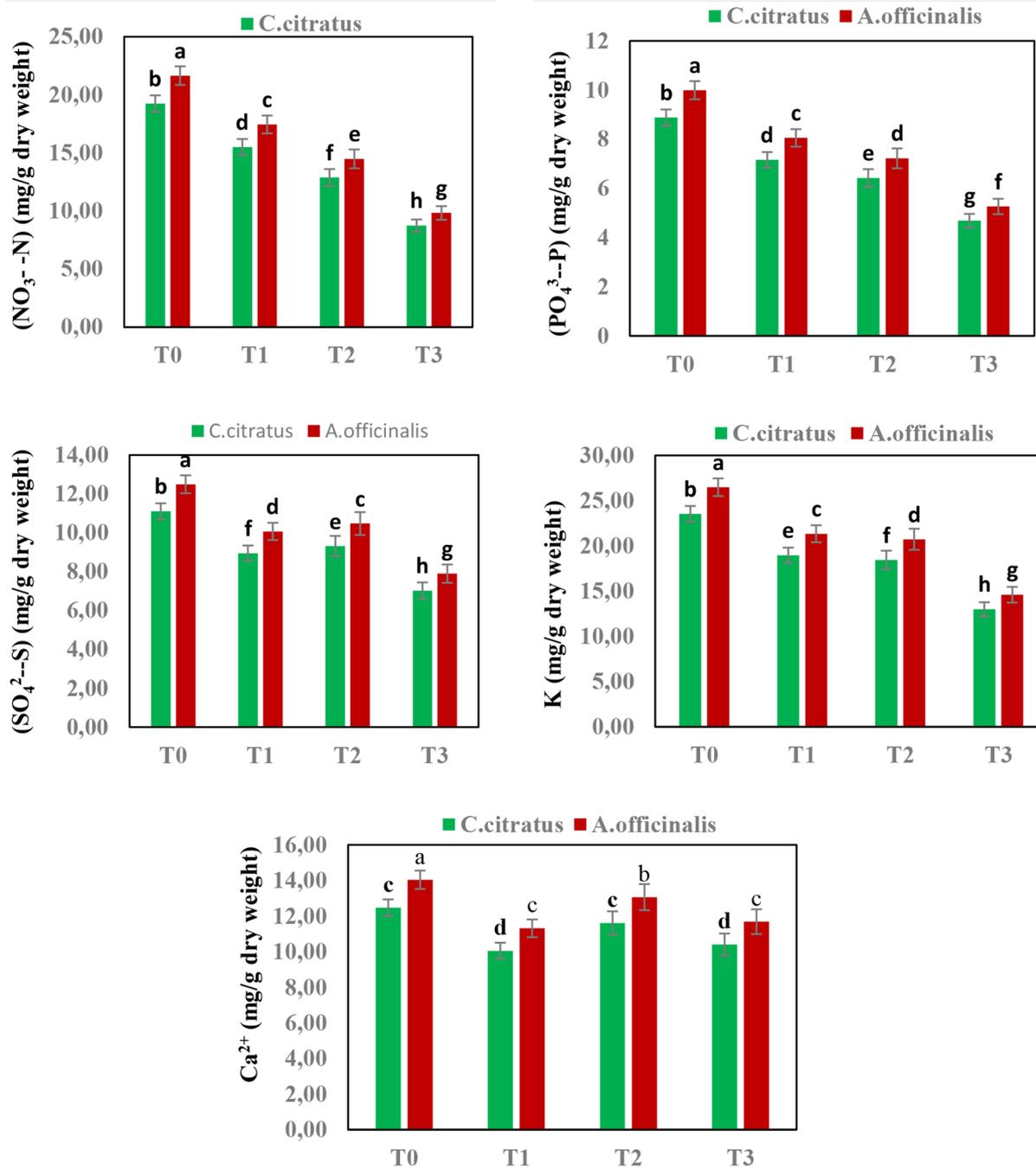


Fig. 6. Effect of variable stress condition on POD activity and concentration of hydrogen peroxide and MDA *C. citratus* and *A. officinalis* under salinity and waterlogging conditions. T0: control, T1: salinity stress, T2: waterlogging and T3: salinity stress + waterlogging.

al., 2022]. The carotenoid appreciably scavenges ROS and production of ROS can be mitigated by higher carotenoid concentration. The lemon grass had higher carotenoid concentration under stress conditions, which might reduce the MDA and H₂O₂ production in this grass species [Manvitha and Bidya, 2014]. The ratio and total amount of secondary metabolites produced by plants might vary depending on the growing

conditions. Changes in stress may cause specific secondary metabolites to be produced. The levels of “total soluble phenolics (TSP), anthocyanin (Anth), total soluble sugars (TSS), ascorbic acid (AsA), and total soluble protein (TSP_{ro})” in both grasses were examined.

Both salinity and waterlogging significantly increased H₂O₂ and MDA, which pose negative impacts on proteins, and membranes [Lu et

al., 2008]. Mukarram et al. [2022] also found a positive between H_2O_2 and increasing salt concentration. Both the grass species showed substantial variations for ascorbic acid, anthocyanins, total soluble sugars, total soluble proteins, and total soluble phenolics (TSP). Both grass species were more or less comparable under the prescribed stress conditions, with all concentrations dropping with rising occupational stress and maximum under the combined salinity+waterlogging treatment. Similar trends were seen for both grasses for SOD, CAT and POD activities. However, both plants showed a substantial increase in the aforementioned antioxidants, though, lemongrass performed appreciably well than *C. citratus*. Earlier, different authors also found significant difference among different plants growing under stress conditions [Aslam 2016; Chandio and Anwar 2017]. Mineral nutrients are a component of macromolecules and they are crucial for the growth as well as development of plants. Under unfavorable circumstances, the higher concentration of soluble nutrients in plant parts is essential for sustainable growth. Results showed that *Asparagus officinalis* offers a fantastic chance for usage in herbal and medicinal remedies due to the wide range of compounds produced in its leaves [Beacham et al., 2017].

CONCLUSIONS

In conclusion, salt and waterlogging induced significant negative impacts on the growth, physiological, and metabolic processes of both grasses. However, lemongrass exhibited better tolerance to these stresses by increasing antioxidant activities, phenolic synthesis, and accumulation of potential osmolyte. Therefore, asparagus and lemongrass can thrive under unfavorable environmental conditions. Given that both types of grass are valuable plants, thus, identifying the physiological and biochemical traits that allow them to adapt to the current environmental conditions is crucial.

Acknowledgements

This work was supported by the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia [Project No. GRANT 5392].

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