JEE Journal of Ecological Engineering

Journal of Ecological Engineering, 2025, 26(2), 221–230
https://doi.org/10.12911/22998993/196931
ISSN 2299–8993, License CC-BY 4.0

Received: 2024.11.30
Accepted: 2024.12.15
Published: 2025.01.01

Foliar feeding of citric acid mitigates artificial urine and NaCl-induced saline stress in hydroponic sugar beets

Muhammad Aamir Iqbal[1], Mohammad Tarikuzzaman[1], Muhammad Saad Atique[2], Zannatul Ferdous Tulona[1], Joan G. Lynam[1*]

[1] Department of Chemical Engineering, Louisiana Tech University, Ruston LA 71270, USA
[2] Department of Materials Engineering, National University of Sciences and Technology, Pakistan
* Corresponding author's e-mail: lynam@latech.edu

ABSTRACT

In lunar and deep-space farming, saline stress (SS) induced by human urine in hydroponically grown vegetables constitutes one of the prime concerns in its utilization for plant nutrition. Therefore, an indoor hydroponic trial was performed to assess the effectiveness of foliar feeding of citric acid (CA, 50 µM as two sprays at 26 days and 38 days after germination using a common hand-held sprayer) and the control treatment (a standardized plant nutrient with 10% synthetic urine replacement and 50 mM NaCl-induced SS) for sugar beets. The response variables included vegetative growth traits of sugar beets, such as the height of plants and stem girth along with lengths and fresh weights of leaves, roots, and whole plants. The recorded findings demonstrated that at the fourth week of germination, CA foliar feeding did not produce a statistically significant impact on the leaf width and plant height of sugar beets. However, CA produced 17%, 14%, and 35% taller plants at the six, eight, and ten weeks of germination, respectively. Likewise, the control treatment recorded 32% and 30% smaller leaf widths of sugar beets compared to the CA foliar feeding treatment at the 8th and 10th weeks of germination, respectively. In addition, it was found that CA foliar feeding was effective in enhancing the stem length and root length by 16% and 38%, respectively compared to the control under SS. Moreover, CA foliar feeding enhanced stem diameter (26%) and root fresh weight (29%) compared to the control treatment. As far as leaf length and fresh weight along with whole plant fresh weight were concerned, foliar feeding of CA demonstrated its effectiveness by producing 32%, 21%, and 42% greater values, respectively compared to the control treatment. Therefore, CA foliar feeding could serve as a potent strategy to mitigate the deleterious effects of saline stress and boost the vegetative growth of hydroponically grown sugar beets.

Keywords: lunar farming, saline stress, astro-horticulture, organic acids, artificial urine, soilless farming systems, leaf size, root size.

INTRODUCTION

Farm products are generally perishable foods that spoil quickly, particularly during extended travel to the International Space Station (ISS) and other space missions. Recently, in the United States, research has been underway by the National Aeronautics and Space Administration (NASA) to grow vegetables in the ISS using optimized hydroponic systems and LED lights, allowing astronauts to harvest fresh produce on demand (Maury et al., 2020). One of the strategic keys to achieving these future goals in space entails enhancing self-sufficiency in terms of fresh food provisions to astronauts by leveraging technical breakthroughs and technological innovations. In addition, the selection of crops for lunar and deep-space farming is critical, and resource-efficient crops like sugar beets (*Beta vulgaris* L.), having a short growth cycle (90–120 days), hold promise. Likewise, beets' high adaptability to diverse growth conditions, nutritional value, and sodium chloride tolerance (30–140 mM) (Iqbal et al., 2015) favor its growth and yield assessment for possible cultivation in space. However, supplying enormous amounts of plant nutrients for deep-space missions has been challenging owing to exorbitant transportation costs. However,

nutrient recycling can serve as a potent strategy for efficiently using scarce resources to produce fresh produce in astro-horticulture (the concept of growing vegetables in extraterrestrial environments) (Wright et al., 2023). Urine secreted by astronauts can be a potential source of plant nutrients because it contains macro and micronutrients (Sarigul et al., 2019; Bouatra et al., 2013), whose respective concentrations generally depend on the diet, body size, and age of astronauts (Simha et al., 2024). Moreover, comparable yields of cabbage in soilless culture were recorded by applying water and urine mixture in a 3:1 ratio (Alemayehu et al., 2020). Tarikuzzaman et al. (2024) reported that artificial urine (AU) prepared using a direct contact membrane distillation **(**DCMD) thermal separation procedure may serve as an energy-efficient process to concentrate mineral constituents by virtue of its microporous and hydrophobic membrane that effectively separates cold water and a hot feed solution. The underlying mechanism is the creation of a gradient of vapor pressure across the direct contact membranes induced by temperature differences, allowing water vapors to transfer to the cold water from the hot feed. Interestingly, the hydrophobic membrane tends to restrict the liquid phase, and its pores only allow the passing of water vapors through it. Moreover, in contrast to conventional desalination and other wastewater treatment protocols, the DCMD procedure is carried out at relatively low atmospheric pressure and temperatures (50-80 °C), offering convenience of use and significantly lower operating costs.

Recently, comparable economic yields and nutritional quality of lettuce supplied with enriched urine were obtained compared to that of chemical fertilizer in a hydroponic system (Jurga et al., 2021). However, several chemical constituents of urine, such as NaCl and creatinine, tend to cause saline stress (SS) in crop plants (Simha et al., 2024). The SS induces osmotic stress, resulting in reduced water uptake by roots. This leads to the closure of stomata, and ultimately a serious reduction in growth occurs owing to a restricted photosynthesis process (Shaddam et al., 2024; Yasir et al., 2021). In addition, SS induces ionic toxicity (in particular sodium Na replacing vital nutrients such as potassium K and calcium Ca) that disrupts various vital metabolic processes. Eventually, chlorosis (yellowing of leaves owing to destruction of chlorophyll) and necrosis (gradual cell death) become inevitable (Choudhary et al., 2021; Hakim et al., 2021). Moreover, reactive oxygen species (ROS) bioaccumulation (especially hydrogen peroxide and superoxide radicals) makes plants prone to oxidative stress when exposed to SS, leading to disruption of cell functions owing to damage to cell membranes and suppression of biosynthesis of vital enzymes (Ahmad et al., 2023; Sagar et al., 2023). To cope with SS, sugar beet plants may be induced through external stimuli, such as foliar feeding of chemical substances, to produce osmolytes (e.g., proline, glycine betaine), which assist plants in maintaining cell turgor and offer protection against denaturation of vital proteins (Subbarao et al., 2001).

Citric acid (CA, $C_6H_8O_7$) is a weak organic acid that is naturally synthesized in citrus fruits (e.g., oranges, lemons, limes) as part of Kreb's cycle during respiration and assists in carbohydrate conversion into ATP (Adenosine triphosphate) (Khatun et al., 2019; Sun and Hong, 2011). It serves as a precursor for the biosynthesis of several organic acids, plays a critical role in regulating pH within cells (Sadak and Orabi, 2015), and neutralizes abiotic stresses particularly SS (El-Tohamy et al., 2013). The foliage-applied CA assisted crop plants in chelating and detoxifying NaCl toxicity and triggered the essential nutrients (particularly Ca, Mg, and K) uptake, leading to improved osmotic regulation and initiating the activation of enzymes (Hossain et al., 2024). In addition, CA foliar feeding remained effective in boosting the antioxidant defense system of crop plants exposed to abiotic stresses by triggering the biosynthesis of catalase, superoxide dismutase, and peroxidase, which neutralized ROS (Aslam et al., 2022; Attia et al., 2021). Moreover, it was revealed that CA remained effective in maintaining osmotic balance in plant tissues by restricting the uptake of NaCl and activating the biosynthesis of osmoprotectants such as proline, glycine and betaine. (Behairy et al., 2017; Gao et al., 2010). Furthermore, CA exhibited its strategic role in boosting the rate of photosynthesis by preventing the degradation of chlorophyll under SS, which increased biomass accumulation (Hu et al., 2016; Jafari and Hadavi, 2012; Yang et al., 2012).

However, pronounced research gaps exist concerning the utilization of CA in alleviating synthetic urine and NaCl-induced SS in sugar beets grown in soilless farming. This has necessitated executing fresh studies to establish CA effectiveness in mitigating SS in sugar beets. Therefore, it was hypothesized that foliar feeding of CA may mitigate the SS in sugar beets exposed to synthetic

urine and NaCl-induced SS by virtue of its potential to maintain osmotic potential, detoxify ionic imbalances, enhance essential nutrient absorption, promote the biosynthesis of osmoprotectants, and trigger photosynthesis process to lead to robust vegetative growth. Thus, the ultimate goal of this hydroponic study was to assess the effectiveness of foliar feeding of citric acid in mitigating the deleterious effects of salt stress imposed through NaCl and synthetic urine in hydroponic systems.

MATERIALS AND METHODS

The hydroponic trial was executed in the Chemical Engineering Department's Biomass lab, at the College of Engineering Science, Louisiana Tech University (Ruston), United States, in 2024. The pod kit hydroponic system (Uruq-Huijujiapin, P.R. China) of 6.5 L capacity was used for growing beet plants. The seeds (Johnny's Seeds, Albion, ME, USA) of sugar beet were purchased for growing as a test crop in this hydroponic trial. The source of plant nutrients was a standard plant nutrient medium (Aero-Grow Industries, Boulder, CO, USA) composed of nitrogen, phosphorous, and potassium (4%, 3%, and 6%, respectively) along with calcium and magnesium (1% and 0.9%, respectively).

Artificial urine preparation protocol

For this hydroponic trial, artificial urine (AU) was synthesized by dissolving thirteen chemicals (creatinine, uric acid, urea, sodium chloride, sodium phosphate dihydrate, sodium citrate, sodium phosphate dibasic dihydrate, sodium sulfate, potassium chloride, potassium oxalate monohydrate, calcium chloride, ammonium chloride, and magnesium sulphate) in deionized (DI) water (1000 ml) by following the protocol and using the exact chemical constituents reported by Tarikuzzaman et al. (2024). Thereafter, the solution was poured into a flask (2000 ml) with a magnetic stirrer (200 rpm) and it was placed on a hot plate at ambient temperature. Each chemical constituent was dissolved in the DI water sequentially to ensure complete dissolution. After the addition of all chemical constituents, stirring of the mixture was done for 60 minutes to synthesize a homogeneous and transparent solution of AU. Thereafter, AU was appropriately sealed in a bottle and stored (at ambient temperature) preceding the DCMD process.

Artificial urine concentration using DCMD procedure

In this hydroponic trial, a bath system of hot water (1130A, VWR Scientific Ltd. USA) was used to set three temperatures of 80 °C, 65 °C, and 50 °C for preparing AU, while a chiller (9510, PolyScience, USA) was used to keep the DI water cold (0 °C). A PTFE membrane (0.45 microns pore size) having dimensions of 145×97 mm (STERLITECH, USA) was integrated to carry out the DCMD process. In addition, a peristaltic pump (77200-50, Masterflex, USA) was integrated into the system to facilitate smooth and uniform fluid circulation. The duration of the entire process was 8 h, while after every 2 h, samples (50 mL each) were collected from the bath system of hot water to ensure membrane functioning. Moreover, energy consumption and analyses of membrane flux, pH changes, ionic conductivity, and density were performed (Tarikuzzaman et al., 2024). DCMD was performed to concentrate the simulated urine and extract pure water for other purposes.

Hydroponic system set-up

Under a fume hood, the hydroponic pod systems, having 6.5 liters capacity containing DI water (5 liters), were set up to maintain uniform growth conditions (25 °C temperature, 16–8 hrs cycles of light-dark using the built-in light setting, airflow, etc.) for sugar beets. In the hydroponic pod systems, a 30-minute on-off cycle of a small built-in pump was set to ensure the flow of oxygen in the root network of the sugar beets. The fume hood window, along with the exhaust fan, maintained appropriate airflow for the hydroponic systems. Sugar beet seeds were subjected to hydro-priming with DI water for 8 h at room temperature to promote germination. Each pod contained a sponge that received three hydroprimed seeds. Germination commenced after five days and was completed in nine days. In each pod, the most robust seedling was retained while the rest were pulled off. Initially, each tray received a standard nutrient solution (16 ml), then the pod systems were supplied with standard nutrient feed (90%) with a 10% 84K ppm urine stream, while SS (NaCl 50 mM as a foliar spray) was imposed at the vegetative growth phase (21 days after 100% germination). The citric acid (50 µM) was applied as foliar sprays (two sprays at 26 days and 38 days after

germination) using a common hand-held sprayer, whereas the control hydroponic system did not receive foliar feeding of the CA. After 15 days of germination, standard nutrient solution (24 ml), and a 10% 84K ppm urine solution was supplied to sugar beet plants at two-week intervals. Throughout the crop growth cycle, the DI water in the hydroponic systems was maintained (5 liters) every week.

Response variables recordings

The recording of response variables started when each plant had developed 4–5 leaves in the fifth week, and it continued weekly until the control treatment wilted out. A measuring tape was used to measure the leaf length, width, and plant height. Additionally, stem and leaf diameters (by taking the average of bottom, middle, and top values) were estimated with the help of a digital caliper. For recording the roots' weight, a paper towel was used to dry the roots and then weighed using a digital balance (Abbas et al., 2024).

Statistical analyses

The response variables data were statistically analyzed by conducting a one-way ANOVA (analysis of variance) using the CRD (completely randomized design) to determine the employed treatments' significance using statistical software (Statistix, version 10.0). Subsequently, to determine the significance among the means of employed treatments, the least significant difference (LSD) test was put into practice (at a 5% probability level) as suggested by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

The control treatment could not survive the imposed saline stress and wilted completely after the 11th week of germination (owing to the severe impact of induced saline stress by artificial urine and NaCl) therefore, a comparative analysis of employed treatments was conducted up to the 10th week of germination.

Plant height and leaf width of sugar beets (at two-week intervals)

The collected data concerning plant height and width of leaves revealed the pronounced influence of foliar feeding of citric acid on these response variables of hydroponically grown sugar beet under SS (Figure 1). At the fourth week of germination, the recorded data concerning sugar beet plant height revealed that CA foliar feeding did not produce a statistically significant impact (Figure 1) as it remained at par with the control. However, CA enhanced plant height with a 17% higher increment in plant height compared to the control treatment after the sixth week. Likewise, the control treatment recorded 14% and 35% shorter plants than CA foliar-feeding treated plants after eight and ten weeks of germination, respectively. Pertaining to sugar beets' leaf width under SS, it was recorded that employing a treatment of CA foliar application imparted a pronounced effect on the leaf width (Figure 2). It was noted that the control treatment recorded 32% and 30% lower leaf widths of sugar beets compared to those with the CA foliar feeding treatment after eight and ten

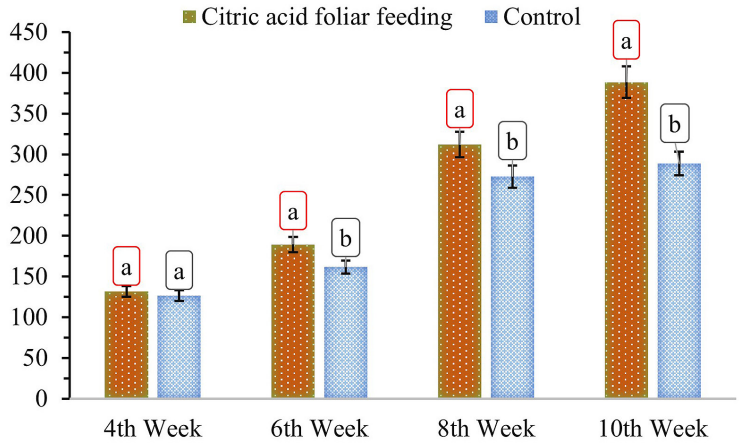

Figure1. Comparative effect of foliar feeding of citric acid and control (standard plant nutrient medium with 10% synthetic urine replacement) under synthetic urine and NaCl-induced saline stress on sugar beets plant height (mm). Atypical letters on bars present statistically significant variation at a 5% probability level

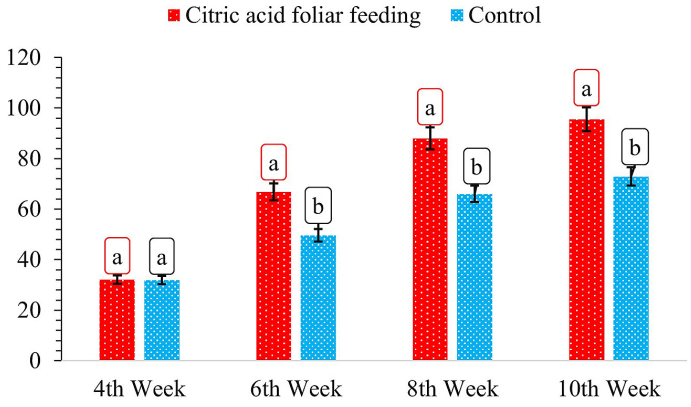

Figure 2. Comparative effect of foliar feeding of citric acid and control (standard plant nutrient medium with 10% synthetic urine replacement) under synthetic urine and NaCl-induced saline stress on sugar beets leaf width (mm). Atypical letters on bars present statistically significant variation at a 5% probability level

weeks of germination, respectively. However, following the trend of plant height in the fourth week, CA foliar feeding imparted a statistically non-significant impact as far as leaf width of sugar beets exposed to synthetic urine and NaCl-induced SS were concerned. These results endorsed the research hypothesis because CA foliar feeding and control treatment entailing 10% synthetic urine replacement and NaCl-induced SS performed differently for plant height and width of leaves in indoor hydroponic systems. The results revealed that CA foliar feeding and the control treatment remained at par with each other initially by producing statistically similar sugar beet plant height and width of leaves under SS. It may be inferred that sugar beet plants remained tolerant to the induced SS initially and therefore, no significant variations in plant height and width of leaves of soilless sugar beets were recorded. Previously, it has been reported that sugar beets hold the potential to tolerate SS by virtue of their genetic makeup and physiological mechanisms that neutralize ionic toxicity (Iqbal et al., 2015). However, at subsequent recordings, pronounced differences in plant height and leaf width might be attributed to the deleterious effects of SS in the control treatment whereas CA foliar feeding effectively mitigated the adverse effects of synthetic urine and NaCl-induced SS (at the 6th, 8th, and 10th weeks after germination). It may be ascribed to SS mitigation by the foliage-applied CA, which tends to chelate and detoxify NaCl toxicity along with enhancing the essential nutrients (e.g., K, Ca, Mg) uptake leading to improved osmotic regulation that resulted in greater vegetative growth (Hossain et al., 2024). In contrast, controlled treatment recorded dwarf plants with lower leaf width because synthetic urine and NaCl-induced SS tend to cause ionic imbalances and restrict the uptake of essential nutrients by plant roots, leading to a significant reduction in photosynthesis and ultimately restricted vegetative growth (Shaddam et al., 2024).

Stem and root lengths

It was revealed that the stem length and root length of sugar beets in hydroponic systems at the 10th week after germination had a pronounced influence in response to foliar feeding of CA under SS (Figure 3). It was exhibited that CA foliar feeding remained effective in enhancing the stem length by 16% under SS compared to the values for the control. Likewise, CA also demonstrated its potential in mitigating the deleterious impact of SS on the root length of hydroponically raised sugar beet plants by producing 38% greater root length than the control treatment. Moreover, it became evident that foliar feeding of CA had a more pronounced effect (22% higher) on root length than stem length of hydroponically grown sugar beet plants exposed to synthetic urine and NaCl-induced SS (Figure 3). The findings of this trial agree with previous studies whereby SS seriously reduced the growth of stem and roots, and it was inferred that SS induced osmotic stress resulting in reduced water uptake by roots leading to the closure of stomata and ultimately serious reduction in vegetative growth traits of crop plants (Yasir et al., 2021). Although it has also been reported that human urine (20000 L·ha^{-1}) boosted the growth of plants (Mnkeni et al., 2008), the adverse effects of SS caused by urine were also reported. Moreover, CA foliar feeding effectively

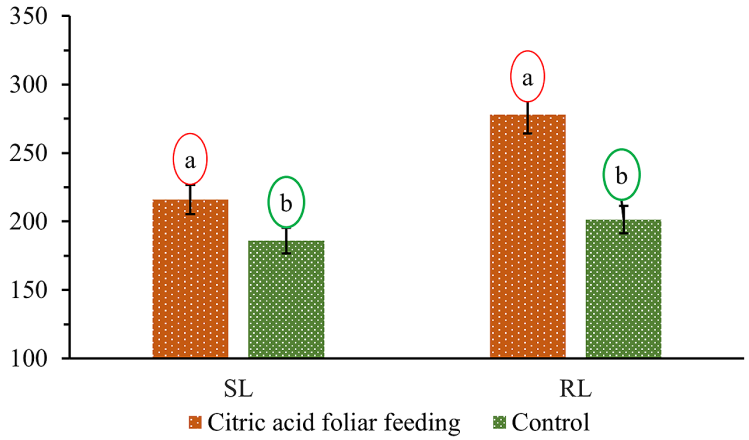

Figure 3. Comparative effect of foliar feeding of citric acid and control (standard plant nutrient medium with 10% synthetic urine replacement) under synthetic urine and NaCl-induced saline stress on sugar beets root length (RL, mm) and stem length (SL, mm) at 10th week after germination. Atypical letters on bars present statistically significant variation at a 5% probability level

triggered the antioxidant defense system of crop plants exposed to SS by activating the biosynthesis of catalase, superoxide dismutase, and peroxidase that neutralized ROS; and ultimately vegetative growth traits significantly increased (Aslam et al., 2022; Attia et al., 2021).

Stem diameter and root fresh weight

As depicted in Figure 4, foliar feeding of CA had a pronounced influence on stem diameter, and fresh root weight of hydroponically grown sugar beet subjected to synthetic urine and NaCl-induced SS. The results showed CA remained instrumental in booting the growth of stem diameters of sugar beets by producing 26% greater stem diameter than the corresponding value for the control treatment (standard nutrient solution with 10% replacement with synthetic urine and NaCl-induced SS). Moreover, it also performed superiorly by producing significantly higher fresh root weight (29%) than the control treatment. As per recorded findings, sugar beets exhibited a significant decline in stem diameter and root fresh weight in the absence of foliar feeding of CA that may be ascribed to SS-induced ionic toxicity whereby sodium replaced essential nutrients (particularly K and Ca). This would disrupt metabolic processes leading to a significant decline in the biosynthesis of vital enzymes and reduced above and below-ground growth (Choudhary et al., 2021; Hakim et al., 2021). Moreover, SS-induced

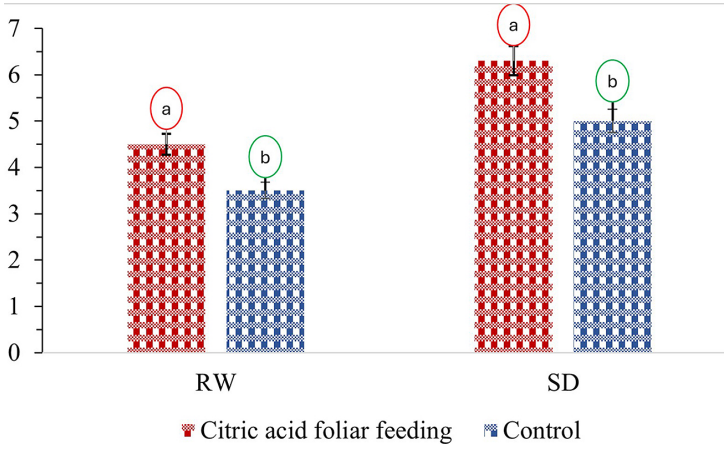

Figure 4. Comparative effect of foliar feeding of citric acid and control (standard plant nutrient medium with 10% synthetic urine replacement) under synthetic urine and NaCl-induced saline stress on sugar beets stem diameter (SD, mm), and root fresh weight (RW, mg) at 10th week after germination. Atypical letters on bars present statistically significant variation at a 5% probability level

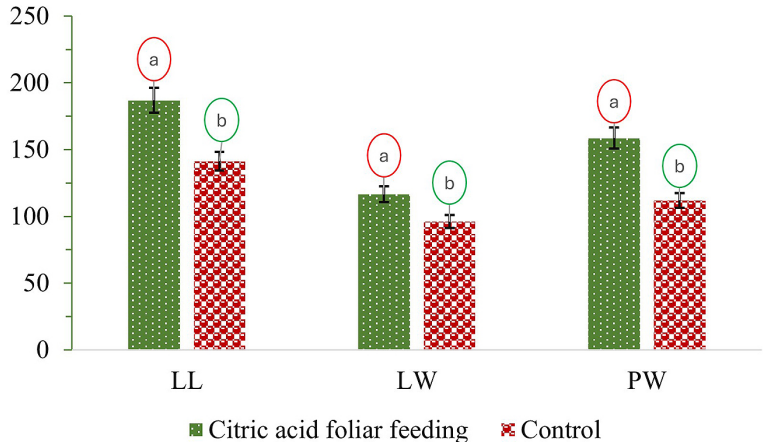

Figure 5. Comparative effect of foliar feeding of citric acid and control (standard plant nutrient medium with 10% synthetic urine replacement) under synthetic urine and NaCl-induced saline stress on sugar beets leave length (LL, mm), leaf weight (LW, g), and plant weight (PW, g) at 10th week after germination. Atypical letters on bars present statistically significant variation at a 5% probability level

bioaccumulation of ROS particularly hydrogen peroxide and superoxide radicals caused oxidative stress leading to disruption of cell functions and division, and ultimately stem and root growth were significantly hampered (Ahmad et al., 2023; Sagar et al., 2023). Previously, Barbosa et al. (2024) and Pradhan et al. (2010) opined that ionic imbalance within plat tissues owing to SS caused osmotic stress and vegetative growth traits including stem and root reduction. In contrast, Attia et al. (2021) suggested the foliar application of organic acids to activate the antioxidant defense system of tomatoes exposed to abiotic stresses. Such application may trigger the biosynthesis of catalase, superoxide dismutase, and peroxidase to neutralize ROS and ultimately increase plants' vegetative growth traits.

Leaf length and weight of leaves and whole plant

The results of this soilless trial demonstrated that vegetative growth traits (leaf length and fresh weight along with the fresh weight of whole plant fresh weight for hydroponically grown sugar beets recorded significant variation between CA foliar feeding and the control treatment (standard plant nutrients medium with 10% synthetic urine replacement and NaCl-induced saline stress) (Figure 5). As far as sugar beet leaf length and fresh weight were concerned, foliar feeding of CA demonstrated its effectiveness by producing 32% and 21% greater values than the corresponding values for the control treatment, respectively. Moreover, control treatment also performed below par compared to foliar feeding of CA by producing 42% lower whole plant fresh weight of sugar beets exposed to synthetic urine and NaCl-induced SS (Figure 5). The underlying reason for significantly lower values of leaf length and weight along with whole plant fresh weight in control treatment may be attributed to the accumulation of salt in plant tissues that developed osmotic stress, oxidative stress, and metabolic stress leading to a significant decline in vegetative growth (Hakim et al., 2021). In contrast, foliar feeding of CA assisted crop plants under SS to biosynthesize different osmolytes (e.g., proline, glycine betaine), which assisted plants in maintaining cell turgor and offered protection against denaturation of vital proteins (Sorour et al., 2021). Thus, ultimately, plants were able to accumulate more biomass (Yang et al., 2012). Furthermore, CA exhibited its strategic role in boosting the photosynthesis rate by preventing the degradation of chlorophyll under SS, which increased biomass accumulation (Hu et al., 2016; Jafari and Hadavi, 2012).

CONCLUSIONS

The results of this indoor hydroponic trial support the research hypothesis because foliar feeding of citric acid demonstrated its potential as a potent candidate to ameliorate the deleterious impacts imparted by saline stress on vegetative growth traits of sugar beets. The synthetic urine and NaCl-induced saline stress significantly

reduced plant growth traits especially plant height, stem diameter, leaf length, and fresh weight along with root length, which led to lower whole plant fresh weight. It may be inferred that saline stress-induced osmotic stress resulted in reduced water uptake by roots, which led to the closure of stomata and ultimately serious reduction in growth traits of sugar beets owing to the restricted photosynthesis process. Like belowground plant parts, SS-induced ionic toxicity disrupted various metabolic processes, and eventually, chlorosis (yellowing of leaves owing to the destruction of chlorophyll) and necrosis (gradual cell death) became evident after the 11th week of germination. At that point, plants subjected to the control treatment wilted completely. In comparison, foliar feeding of CA demonstrated its potential in mitigating the adverse effects of salinity by producing significantly greater plant growth traits under investigation. It may be inferred that foliar feeding of citric acid assisted sugar beet plants to chelate and detoxify NaCl toxicity. Foliar feeding also appeared to enhance essential nutrients (e.g., K, Ca, Mg) uptake leading to improved osmotic regulation that led to significantly higher plant height, leaf and stem growth, and root development. Based on these encouraging findings, it may be suggested that future studies should evaluate the effectiveness of other doses of citric acid in mitigating the deleterious effects of saline stress. Moreover, the co-application of citric acid and other weak organic acids may also be a potent future research direction for mitigating saline stress caused by urine used as a plant nutrient source for utilization in lunar and deep-space farming. Furthermore, these research findings hold brighter perspectives for promoting astrohorticulture ensuring the provision of fresh vegetables to astronauts on demand.

Acknowledgments

The first author extends gratitude to the Fulbright Scholar Program (United States), the United States Educational Foundation in Pakistan (USEFP), and the University of Poonch Rawalakot (AJK, Pakistan) for their respective support. This work was supported by NASA grant [Grant number: Cooperative Agreement Number: 80NSSC22M0030 (CFDA # 43.008)] and the Board of Regents of the State of Louisiana [Contact Number: LEQSF-EPS (2023)-RAP-47] with the help of LSU management.

REFERENCES

1. Abbas, R.N., Iftikhar, A., Iqbal, A., Erden, Z., Alqahtani, M.D., Almutairi, K.F., Iqbal, M. A. 2024. Counteracting heat, salinity, and osmotic stresses by reconciling seed size and sowing depth for bolstering germination and seedling growth of cluster bean (*Cyamposis tetragonoloba* L. Taub.). Polish Journal of Environmental Studies, https://doi.org/10.15244/pjoes/195278
2. Ahmad Z., Khaliq A., Waraich E.A., Artyszak A., Zaman Q., Abbasi A., Iqbal M.A. 2023. Exogenously applied silicon and zinc mitigates salt stress by improving leaf pigments and antioxidant activities in canola cultivars. Silicon, 15, 5435–5444. https://doi.org/10.1007/s12633-023-02446-y
3. Alemayehu Y.A., Demoz A.A., Degefu M.A., Gebreeyessus G.D., Demessie S.F. 2020. Effect of human urine application on cabbage production and soil characteristics. Journal of Water, Sanitation and Hygiene for Development, 10(2), 262–275.
4. Aslam A., Nawaz H., Khan A., Ghaffar R., Abbas G. 2022. Effect of exogenous application of citric acid on growth of maize (*Zea mays* L.) under sodium fluoride stress. Fluoride, 56(1), 329–350.
5. Attia M.S., Osman M.S., Mohamed A.S., Mahgoub H.A., Garada M.O., Abdelmouty E.S., Abdel Latef A.A.H. 2021. Impact of foliar application of chitosan dissolved in different organic acids on isozymes, protein patterns and physio-biochemical characteristics of tomato grown under salinity stress. Plants, 10(2), 388. https://doi.org/10.3390/plants10020388
6. Barbosa A.S., Silva A.O., Sousa G.G., Souza M.V.P., Freire M.H., Goes G.F. et al. 2024. Brackish water, phosphate fertilization and trichoderma in the agronomic performance of beet crops. Agronomy, 14(6), 1306. https://doi.org/10.3390/agronomy14061306
7. Behairy R.T., El-Hamamsy S.M.A., El-khamissi H.A.Z. 2017. Alleviation of salinity stress on Fenugreek seedling growth using salicylic acid, citric acid and proline. Middle East Journal of Agricultural Research, 6(2), 474–483.
8. Bouatra S., Aziat F., Mandal R., Guo A.C., Wilson M.R., Knox C. 2013. The human urine metabolome. PLoS ONE, 8(9), e73076. https://doi.org/10.1371/journal.pone.0073076
9. Choudhary S.K., Kumar V., Singhal R.K., Bose B., Chauhan J., Alamri S., Siddiqui M.H., Javed T., Shabbir R., Rajendran K., Iqbal M.A. 2021. Seed priming with $Mg(NO_3)_2$ and $ZnSO_4$ salts triggers the germination and growth attributes synergistically in wheat varieties. Agronomy, 11, 2110. https://doi.org/10.3390/agronomy11112110
10. Darandeh N., Hadavi E. 2012. Effect of pre-harvest foliar application of citric acid and malic acid on chlorophyll content and post-harvest vase life of Lilium

cv. Brunello. Frontier in Plant Science, 2, 106.

11. El-Tohamy W.A., El-Abagy H.M., Badr M.A., Gruda N. 2013. Drought tolerance and water status of bean plants (*Phaseolus vulgaris* L.) as affected by citric acid application. Journal of Applied Botany and Food Quality, 86, 212–216.
12. Gao Y., Miao C.Y., Mao L., Zhou P., Jin Z.G., Shi W.J. 2010. Improvement of phytoextraction and antioxidative defense in *Solanum nigrum* L. under cadmium stress by application of cadmium resistant strain and citric acid. Journal of Hazardous Materials, 18, 771–777.
13. Gomez K.A., Gomez A.A. 1984. Statistical procedures for Agricultural Research. 2nd Edition, John Wiley and Sons, New York.
14. Hakim A.R., Juraimi A.S., Rezaul Karim S.M., Khan M.S.I., Islam M.S., Choudhury M.K., Soufan W., Alharby H. 2021. Effectiveness of herbicides to control rice weeds in diverse saline environments. Sustainability, 13, 2053. https://doi.org/10.3390/su13042053
15. Hossain M.A., Khatun M.S., Hosen M., Sayed Z.I., Islam M.R., Chowdhury M.K., Iqbal M.A., Al-Ashkar I., Erden Z., Toprak C.C., Sabagh A.E., Islam M.S. 2024. Citric acid alleviated salt stress by modulating photosynthetic pigments, plant water status, yield and nutritional quality of black gram [*Vigna mungo* (L.) Hepper]. Legume Research, 10.18805/LRF-820.
16. Hu L., Zhang Z., Xiang Z., Yang Z. 2016. Exogenous application of citric acid ameliorates the adverse effect of heat stress in Tall Fescue (*Lolium arundinaceum* L.). Frontiers in Plant Science, 7, 179.
17. Hussein M.M., El-Saady A., Gobarah M., Abo El-Khier A. 2020. Nutrient content and growth responses of sugar beet plants grown under salinity condition to citric acid and algal extract. Egyptian Journal of Agronomy, 42(2), 209–224. https://doi.org/10.21608/agro.2020.38200.1223
18. Iqbal M.A., Asif I., Kashif A., Haider A, Khan R.D., Bilal A., Faisal N., Ali R. 2015. Integration of forage sorghum and by-products of sugarcane and sugar beet industries for ruminant nutrition: A review. Global Veterinaria, 14(5), 752–760.
19. Iqbal M.A., Saleem A.M. 2015. Sugar beet potential to beat sugarcane as a sugar crop in Pakistan. American-Eurasian Journal of Agricultural & Environmental Sciences, 15 (1), 36–44.
20. Jafari, N., Hadavi, E. (2012) Growth and essential oil yield of dill (*Anethum graveolens*) as affected by foliar sprays of citric acid and malic acid. Acta Hort. 955, 287–290.
21. Jurga A., Janiak K., Wizimirska A., Chochura P., Miodoński S., Muszyński-Huhajło M., Ratkiewicz K., Zięba B., Czaplicka-Pędzich M., Pilawka T. 2021. Resource recovery from synthetic nitrified urine in the hydroponic cultivation of lettuce (*Lactuca sativa* Var. capitata L.). Agronomy, 11(11), 2242. https://doi.org/10.3390/agronomy11112242
22. Khatun M.R., Mukta R.H., Islam M.A., Huda A.N. 2019. Insight into citric acid-induced chromium detoxification in rice (*Oryza sativa* L). International Journal of Phytoremediation, 21(12), 1234–1240.
23. Maury T., Loubet P., Serrano S.M., Gallice A., Sonnemann G. 2020. Application of environmental life cycle assessment (LCA) within the space sector: a state of the art. Acta Astronaut, 170, 122–135.
24. Pradhan S.K., Holopainen J.K., Weisell J., Heinonen-Tanski H. 2010. Human Urine and wood ash as plant nutrients for red beet (*Beta vulgaris*) cultivation: Impacts on yield quality. Journal of Agriculture and Food Chemistry, 58, 2034–2039.
25. Sadak M.Sh., Orabi, S.H. 2015. Improving thermo tolerance of wheat plant by foliar application of citric acid or oxalic acid. International Journal of Chemical Technology and Research, 8(1), 333–345.
26. Sagar A., Hossain M.A., Uddin M.N., Tajkia J.E., Mia M.A., Iqbal M.A. 2023. Genotypic divergence, photosynthetic efficiency, sodium extrusion, and osmoprotectant regulation conferred salt tolerance in sorghum. Phyton-International Journal of Experimental Botany, 92(8): 2349–2368. https://doi.org/10.32604/phyton.2023.028974
27. Shaddam M.O., Islam M.R., Ditta A., Ismaan H.N., Iqbal M.A., Al-Ashkar A. 2024. Genotypic divergences of important mungbean varieties in response to salt stress at germination and early seedling stage. Polish Journal of Environmental Studies, 33(5), 5857–5868. http://dx.doi.org/10.15244/pjoes/183567
28. Simha P., Courtney C., Randall D.G. 2024. An urgent call for using real human urine in decentralized sanitation research and advancing protocols for preparing synthetic urine. Frontiers in Environmental Science, 12, 1367982. https://doi.org/10.3389/fenvs.2024.1367982
29. Subbarao G.V., Wheeler R.M., Levine L.H., Stutte G.W. 2001. Glycine betaine accumulation, ionic and water relations of red-beet at contrasting levels of sodium supply. Journal of Plant Physiology, 158, 767–776. https://doi.org/10.1078/0176-1617-00309
30. Sun Y.L., Hong S.K. 2011. Effects of citric acid as an important component of the responses to saline and alkaline stress in the halophyte *Leymus Chinensi*s (Trin.). Plant Growth Regululation, 64, 129–139.
31. Sorour S., Amer M.M., El Hag D., Hasan E.A., Awad M., Kizilgeci F., Ozturk F., Iqbal M.A., El Sabagh A. 2021. Organic amendments and nano-micronutrients restore soil physico-chemical properties and boost wheat yield under saline environment. Fresenius Environmental Bulletin, 30(9), 10941–10950.
32. Tarikuzzaman M., Iqbal M.A., Lynam J.G. 2024. Direct Contact Membrane Distillation of Artificial Urine for Sugar Beet Production in a Hydroponic System.

Journal of Ecological Engineering, 25(10), 252–260. https://doi.org/10.12911/22998993/192174

33. Tyburski J., Nowakowski M., Nelke R., Żurek M. 2024. Optimizing an organic method of sugar beet cultivation and yield gap decrease in Northern Poland. Agriculture, 14(6), 937. https://doi.org/10.3390/agriculture14060937
34. Wright H.C., Fountain L., Moschopoulos A., Ryan A.J., Daniell T.J., Cullen D.C., Shaughnessy B., Cameron D.D. 2023. Space controlled environment agriculture offers pathways to improve the sustainability of controlled environmental agriculture on Earth. Nature Food, 4, 648–653. https://doi.org/10.1038/s43016-023-00819-5
35. Yang L., Ma C., Wang L., Chen S., Li H. 2012. Salt stress induced proteome and transcriptome changes in sugar beet monosomic addition line M14. Journal of Plant Physiology, 169, 839–850. https://doi.org/10.1016/j.jplph.2012.01.023
36. Yasir T.A., Khan A., Skalicky M., Wasaya A., Rehmani M.I.A., Sarwar N., Mubeen K., Aziz M., Hassan M.M., Hassan F.A.S., Iqbal M.A. 2021) Exogenous sodium nitroprusside mitigates salt stress in lentil (*Lens culinaris* Medik.) by affecting the growth, yield, and biochemical properties. Molecules, 26, 2576. https://doi.org/10.3390/molecules26092576