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Review Article

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Physiological and Biochemical Response of Water Lettuce (Pistia stratiotes) to Short-Term Mild Saline Stress

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Abstract

This current work describes the response of Pistia stratiotes (water lettuce) to moderate saline stress under controlled conditions. Plant biomass as well as the levels of aldehydes, phenolics, chlorophylls and carotenoids was evaluated after a week of stress. Following the first experiment (up to 80mM NaCl), high levels of mortality were observed. Hence, a second trial was undertaken where lower salt concentrations were tested, i.e. 0, 5, 10, 15 and 20mM NaCl. Small changes in biomass were observed with some necrosis evident. Biochemical evaluations indicated the accumulation of lipid peroxidation products in response to the saline stress, indicative of oxidative damage. Furthermore, water lettuce plants were unable to accumulate phenolics (soluble and bound) or carotenoids, both regarded as antioxidants that play an important role in mitigating against the adverse effects of damage caused by oxidative stress. Hence, water lettuce appeared to be sensitive to saline stress with adverse effects evident even at low exposure levels. One of the reasons proposed for the inability of water lettuce to tolerate saline stress might be the inefficient antioxidant systems which led to the accumulation of damage. This requires further investigation. In addition, it is recommended that future studies consider the ability of water lettuce to tolerate longer term mild stress as this might allow plants to adapt suitable protective mechanisms to promote survival and have implications on their spread and weed status.

Keywords: Invasive plants; Salt stress; Salinity; Water plants; Water salinization

Introduction

The impacts of climate change as a consequence of anthropogenic activities are being experienced across the globe. Increases in temperature are accompanied by changes in annual rainfall and extreme, unpredictable weather events such as storms, floods, hurricanes, drought, frost, etc. Rising sea levels are also a concern with levels increasing at a rate of 1 mm a-1 during the last century and up to 3 mm a^{-1} since the 1990s [1]. Archipelagos and islands are particularly at risk from rising sea levels. Cuba is one such archipelago which is comprised of approximately 1600 islands, islets and cays with a coastline stretching more than 5700 km. Fresh water systems in Cuba are also at risk as intrusion of sea water into these ecosystems have become apparent due to anthropogenic activities and the rise in the mean sea level. Such incursions can have adverse implications on the composition of the biodiversity within these systems. For example, aquatic plants inhabit these ecosystems and the sensitivity of these plants to changing conditions will have implications on the management of vulnerable ecosystems in response to climate change.

Pistia stratiotes commonly known as water lettuce belongs to the *Araceae* family. It is a monocotyledonous, perennial floating plant which grows on the surface of lakes, rivers, ponds and other water bodies. It is a stoloniferous macrophyte with rosette-like leaf morphology and long, feathery roots. Water lettuce is capable of sexual and vegetative reproduction with the latter occurring through the attachment of daughter offshoots to the mother plant via stolons. The plant can multiply rapidly vegetatively leading to the production of a dense mat of connected plants in a short period of time. Dispersal is aided when daughter plants detach and establish new colonies. The plant is adapted to grow in temperatures ranging from 15-35°C and prefers a slightly acidic environment. Water lettuce is native to Tropical America, but as a result of its adaptability, it has a widespread, cosmopolitan distribution throughout tropical and subtropical regions of the world, apart from Antarctica. The ability of this plant to rapidly propagate and overtake waterways has led to it being classified as a notorious weed and it is currently listed as an invasive species on the Global Invasive Species Database (GISD 2021) and as a quarantine pest (EPPO 2020). There are concerns surrounding the dispersal of water lettuce as climate change models have predicted that by 2070 populations could become established in Europe and the Mediterranean. In Cuba, it is not clear when this species was first introduced as an early report undertaken by Uphof (1924) surveying the plants found in lagoons and marshes of the central zone did not mention this species [2].

The impacts of water lettuce infestations include alterations in the physical-chemical environment of waterways such as a reduction in dissolved oxygen and pH and increases in carbon dioxide concentration. This affects populations of other macrophytes, macroinvertebrates and fish species leading to biotic homogenization of ecosystems. The dense mat formed by *P. stratiotes* impedes the penetration of solar radiation into the water column, thereby adversely affecting the growth of submerged plants. Furthermore, mats of *P. stratiotes* physically clog waterways having a negative impact on recreational activities and causes problems with irrigation, drainage, hydropower systems, etc. From a public health perspective, *P. stratiotes* plants serve as an ideal host for harboring larvae of several mosquito species which serve as vectors for the transmission of diseases such as malaria. While *P. stratiotes* is less aggressive than *Eichhornia crassipes* and *Salvinia molesta*, it is nonetheless an important floating aquatic weed and can have significant implications on water resource management, biodiversity conservation, public health and socio-economic impacts.

A number of studies have been undertaken in attempts to harness the positive attributes of *P. stratiotes*. For example, it has been used

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for phytoremediation purposes and as a substrate for biogas and bio-ethanol production. It has been reported that water lettuce is able to accumulate high concentrations of nitrogen and phosphorus, which makes it suitable to be used as a bio-fertilizer. The medicinal properties of this species have also been identified. Despite some of the benefits that can be derived from *P. stratiotes*, it is nevertheless still considered to be one of the 100 most harmful species in the world and control measures to prevent its spread are critical. Some methods have been recommended for its control which includes physical removal by raking or seining, chemical control using herbicides (GISD 2021) and biological control [3].

As indicated above, there are serious risks associated with infestations of *P. stratiotes*. Like with other invasive weeds, *P. stratiotes* possesses a range of competitive functional traits which allows it to adapt to local conditions. Hence, it is imperative that the response of this species to climate change is evaluated in order to ensure that the weedy nature of this plant can be controlled and that management plans can be adapted to minimize the risk of further infestations. It is known that abiotic factors, including temperature and salinity, can affect the growth and mortality of water lettuce. The biochemical constituents of water lettuce are affected by increasing salinity in water with changes becoming apparent shortly after exposure to salinity stress. Considering this, the current study investigated the response of water lettuce to short-term moderate salinity stress under controlled conditions. This scenario is intended to simulate future projections of saline water intrusion into lagoons and marshes. Examination of published literature revealed that specific studies on the salinity tolerance of water lettuce has been investigated to a lesser extent than other aquatic weeds such as water hyacinth thereby emphasizing the need for the current work [4].

Materials and Methods

Healthy *P. stratiotes* plants were collected in Ciego de Ávila, Cuba. Plants were grown in 5 L-water containers in the acclimatization area of the Bioplant Center (University of Ciego de Ávila, Cuba) with an average ambient temperature of 24.5°C. Experiments were conducted between February and March 2021. Plants were grown in 5l buckets, with one plant per bucket.

The present study considered two levels of saline exposure using NaCl. It has been shown that NaCl is a suitable substitute to simulate sea water conditions in salinization studies. In the first experiment, intermediate levels of NaCl were tested, viz. 0, 20, 40, 60 and 80 mM. In the second, lower levels were investigated, viz. 0, 5, 10, 15 and 20 mM. Plants were grown for seven days under the applied saline stress conditions. Following this period of exposure, plant biomass was evaluated (leaves and stems were pooled to assess aerial plant parts and roots were determined separately). A range of biochemical indicators were also measured in response to the saline stress, i.e. levels of malondialdehyde and other aldehydes, soluble and cell wall-linked phenolics, chlorophyll a and b and carotenoids [5].

Malondialdehyde and other aldehydes were determined using the method described by Heath and Packer (1968) where the malondialdehyde produced during oxidation of polyunsaturated fatty acids reacting with thiobarbituric acid was assessed. Plant phenolic compounds were extracted and quantified using a colorimetric method based on the reaction with Folin Ciocalteu reagent (mg chlorogenic acid equivalents per g fresh weight). Chlorophyll pigments were extracted with 750 µl methanol (100%). Samples were centrifuged (10000 rpm at 4°C for 15 min), supernatants were collected and the absorbance was read at 652.4 nm. The levels of carotenoids were also determined according to Lichtenthaler (1987) with the absorbance of samples measured at 470 nm.

Each treatment involved 20 plants and each biochemical determination started from three independent samples of 100 mg plant material. All data were statistically analyzed using SPSS (Version 8.0 for Windows, SPSS Inc., New York, NY) to perform One-Way Analysis of Variance (ANOVA) and Tukey tests (p=0.05). The Overall Coefficient of Variation (OCV) was calculated as follows: (standard deviation/average) * 100. In this formula, we considered the average values of the five NaCl levels. Therefore, the higher the difference between the five treatments, the higher the OCV. The OCVs were classified as ¨Low¨ from 10.1 to 53.8%, ¨Medium¨ from 53.8 to 97.4%, and ¨High¨ from 97.4 to 141.1% [6].

Results

The first experiment considered the response of water lettuce to intermediate levels of NaCl ranging from 0 to 80 mM. The results Figure 1 showed increasing levels of necrosis as the NaCl concentration increased from 20 to 80 mM with no green tissue observed at the higher concentrations (> 40 mM). Since it was obvious that the intermediate levels of NaCl tested were lethal to the water lettuce plants, a second experiment was conducted with lower salt concentrations, i.e. 0, 5, 10, 15 and 20 mM NaCl. Low to medium levels of necrosis were observed and plant fresh weight showed a slight (although non-significant) decline (Figure 2) [7].

Evaluation of biochemical indicators revealed that malondialdehyde levels showed an almost seven fold increase from 0.55 nmol g^{-1} FW in control plants to 3.78 nmol g^{-1} FW in plants exposed to 10 mM NaCl. At concentrations greater than 10 mM NaCl, the malondialdehyde content of plants was lower but these levels were still higher than that observed in the control (Figure 3A).

NaCl (mM)	0	20	40	60	80
Plant fresh weight (g) (average ± SE)	11.9 ± 1.2 a	10.8 ± 1.3 a	12.5 ± 1.1 a	12.2 ± 0.9 a	7.1 ± 0.6 a

Figure 1: Effect of Sodium Chloride from 0 to 80 Mm on water lettuce *(Pistia stratiotes)* at one week of growth under controlled conditions. Bars represent 5 cm. Results of plant fresh weight with same letters are not statistically different (One-Way ANOVA, Tukey, p > 0.05). High levels of necrosis were observed in plants cultivated with NaCl.

NaCl (mM)	0	5	10	15	20
Plant fresh weight (g) (average ± SE)	12.8 ± 1.8 a	10.4 ± 1.6 a	11.4 ± 1.1 a	9.6 ± 0.9 a	9.9 ± 0.8 a

Figure 2: Effect of NaCl from 0 to 20 mM on water5 lettuce *(Pistia stratiotes)* at one week of growth under controlled conditions. Bars represent 5 cm. Results of plant fresh weight with the same letters are not statistically different (One-Way ANOVA, Tukey, p > 0.05). Low to medium levels of necrosis were observed in plants cultivated with NaCl.

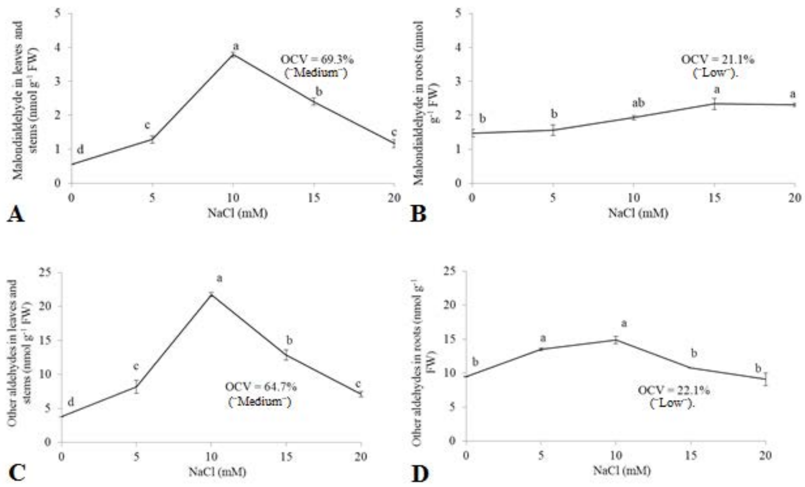

Figure 3: Effect of sodium chloride on levels of malondialdehyde and other aldehydes in water lettuce *(Pistia stratiotes)* at one week of growth under controlled conditions. Results with the same letter are not statistically different (One-Way ANOVA, Tukey, p > 0.05). Vertical bars represent ± SE. OCV were only calculated when statistically significant differences were observed. A High OCV is indicative of a large difference between the five groups compared. Classification of OCVs: ¨Low¨ from 10.1 to 53.8%, ¨Medium¨ from 53.8 to 97.4%, and ¨High¨ from 97.4 to 141.1%.

The levels of malondialdehyde in roots showed a slight increasing trend as the NaCl concentration was raised (Figure 3B). Similar to what was found in aerial parts with malondialdehyde, the levels of other aldehydes also increased following exposure to salinity with the highest levels observed at 10 mM NaCl (Figure 3C). A similar trend was observed in roots of water lettuce albeit at reduced levels compared with leaves and stems (Figure 3D) [8].

The levels of soluble phenolics in aerial plant parts and roots were reasonably stable in response to saline exposure with small, mostly non-significant fluctuations (Figure 4A and 4B). Exposure to saline stress had no effect on the level of cell wall-linked phenolics in leaves and stems (Figure 4C) while for roots, the highest levels were observed with 15 mM NaCl (Figure 4D) [8].

The levels of chlorophyll a in leaves and stems remained stable until exposure to 20 mM NaCl where the significantly highest levels were observed (Figure 5A). In contrast, chlorophyll a in roots showed an incline as the NaCl concentration was increased up to 20 mM (Figure 5B). For chlorophyll b in aerial plant parts, there was first a decline in levels upon exposure to 5 mM NaCl followed up a sharp increase at 10 mM and a subsequent decline at higher concentrations (Figure 5C). Chlorophyll b was not detectable in roots up to 10 mM NaCl but was present when plants were exposed to 15 and 20 mM NaCl (Figure 5D). The level of carotenoids in leaves and stems were significantly lowest when plants were supplied with 10 mM NaCl (Figure 6A) and 15 mM in roots (Figure 6B). In all plant parts analyzed, the level of carotenoids was lower in response to saline stress than in the control [9].

Discussion

The current study considered the effect of imposition of short-term salinity stress on water lettuce. Stress caused by salinity is leads to adverse conditions for plant growth as a consequence of ionic stress due to the accumulation of ions (such as sodium) to toxic levels, reduced water uptake as a result of osmotic stress and oxidative stress through increased production of Reactive Oxygen Species (ROS). The first range of salt concentrations tested were 0-80 mM NaCl. However, this level of stress was too high and the water lettuce plants did not survive (Figure 1) as plants became necrotic and died within the seven day trial period. Similarly, Haller (1974) also noted the salt sensitivity of water lettuce and reported that plant deterioration in response to salt stress was characterized by initial chlorosis of leaf margins followed by twisting of leaves and eventual complete necrosis. This progression of events was also observed in the current

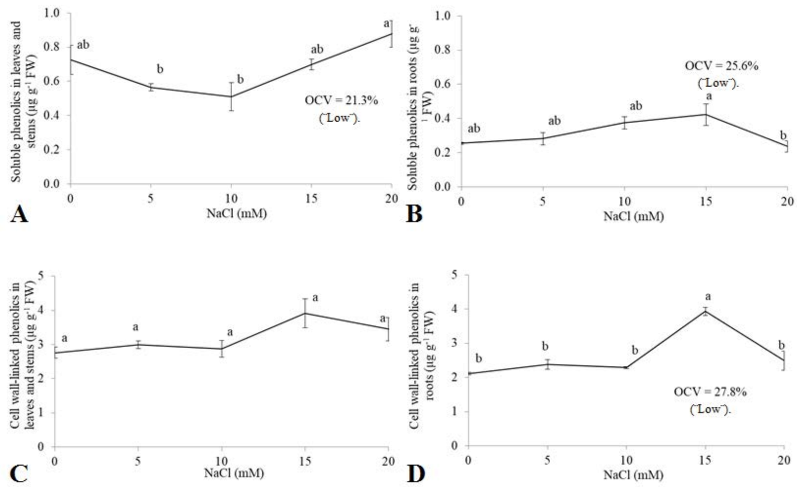

Figure 4: Effect of sodium chloride on levels of soluble and cell wall-linked phenolics in water lettuce *(Pistia stratiotes)* at one week of growth under controlled conditions. Results with the same letter are not statistically different (One-Way ANOVA, Tukey, p > 0.05). Vertical bars represent ± SE. OCV were only calculated when statistically significant differences were observed. A High OCV is indicative of a large difference between the five groups compared. Classification of OCVs: ¨Low¨ from 10.1 to 53.8%, ¨Medium¨ from 53.8 to 97.4%, and ¨High¨ from 97.4 to 141.1%.

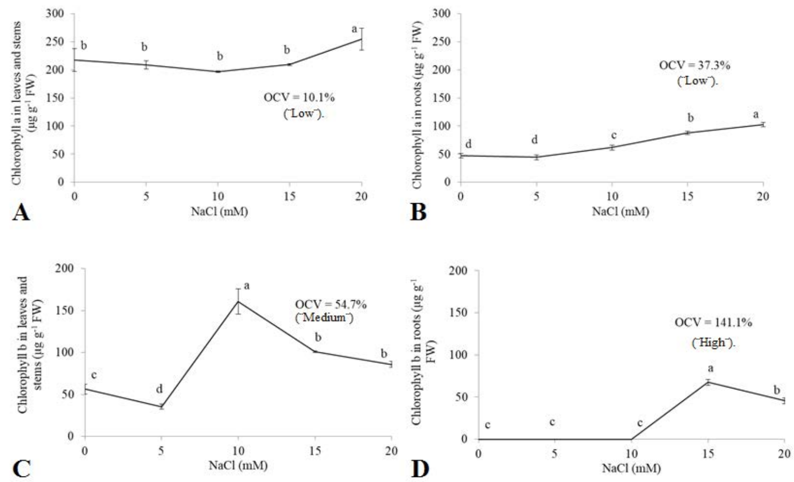

Figure 5: Effect of sodium chloride on levels of chlorophyll in water lettuce *(Pistia stratiotes)* at one week of growth under controlled conditions. Results with the same letter are not statistically different (One-Way ANOVA, Tukey, p>0.05). Vertical bars represent ± SE. OCV were only calculated when statistically significant differences were observed. A High OCV is indicative of a large difference between the five groups compared. Classification of OCVs: ¨Low¨ from 10.1 to 53.8%, ¨Medium¨ from 53.8 to 97.4%, and ¨High¨ from 97.4 to 141.1%.

study (Figure 1). The sensitivity of water lettuce to saline conditions was also reported by Galal (2019) and Khedr (1998). For this reason, lower concentrations of NaCl were evaluated, i.e. 0-20 mM (Figure 2). At this lower stress level, plant biomass declined slightly and there was evidence of leaf chlorosis as the salt concentration was increased, which is indicative of salt stress [10].

The OCV was determined using the data collected in order to identify factors that suggest significant relationships between the saline stresses imposed and measured parameters. The OCV is calculated as the ratio between the standard deviation and mean and describes dispersion around the treatment means. A high OCV is indicative of a large effect. A ¨High¨ OCV was found for the levels of chlorophyll b in roots in response to the saline stress but this is likely due to the fact that no chlorophyll b was detected at lower NaCl concentrations (Figure 5D). ¨Medium¨ OCVs were calculated for malondialdehyde, other aldehydes and chlorophyll b in leaves and stems (Figure 3A, 3C and Figure 5C). These results agree with previous experiments conducted in different culture systems and plant species. Malondialdehyde is the product of the peroxidation of polyunsaturated fatty acids of membranes and is indicative of the extent of oxidative damage under stress conditions. Overall, the results indicated that when plants were exposed to salt stress even at the lowest level (5 mM NaCl), oxidative stress was apparent as malondialdehyde levels were higher than the control. This was

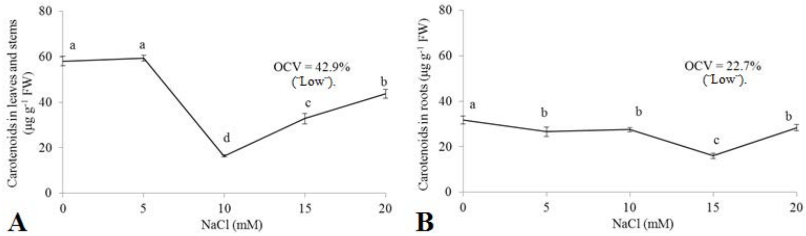

Figure 6A and Figure 6B: Effect of sodium chloride on levels of carotenoids in water lettuce *(Pistia stratiotes)* at one week of growth under controlled conditions. Results with the same letter are not statistically different (One-Way ANOVA, Tukey, p>0.05). Vertical bars represent ± SE. OCV were only calculated when statistically significant differences were observed. A High OCV is indicative of a large difference between the five groups compared. Classification of OCVs: ¨Low¨ from 10.1 to 53.8%, ¨Medium¨ from 53.8 to 97.4%, and ¨High¨ from 97.4 to 141.1%.

accompanied by a reduction in chlorophyll b and a non-significant decline in plant fresh mass. At a higher salt concentration (10 mM NaCl), higher levels of oxidative damage evident as increased malondialdehyde levels were apparent, the chlorophyll b content of aerial plant parts was still high and plant biomass was maintained at a level similar to the control. When the salt stress was increased further, malondialdehyde levels remained higher than the control; chlorophyll b levels were lower than for 10 mM and biomass decline was evident. This shows a pattern of damage accumulation in response to saline stress that is ultimately manifested as a reduction in biomass [11].

Similar to the observation made above, Du (2021) and Gil (2020) also reported an increase in malondialdehyde in other floating macrophytes exposed to saline stress and in terrestrial plants e.g. such as wheat and barley, common purslane, etc. In water lettuce, malondialdehyde has also been found to accumulate in response to copper stress. Lipid peroxidation can cause damage to DNA, denature proteins, lead to carbohydrate oxidation, pigment breakdown and impair enzyme activity. Chlorophyll b is one of the pigments involved in photosynthesis and it is known that photosynthesis is affected by salinity stress. For example, the functioning of photosystem II is recognized as being particularly sensitive to salt stress with a reduction in the efficiency of this system being reported. Limitations in photosynthesis due to stress can lead to the inefficient dissipation of excess energy, which can damage photosystem II as a result of over reduction of reaction centers. The above can result in the generation of ROS in chloroplasts which could be quenched under normal conditions but accumulate to cause damage under stress conditions. Furthermore, it is known that abiotic stresses destabilize membrane systems including those that contain chlorophylls [12].

In the present study, the levels phenolics (soluble and insoluble, i.e. cell wall- linked) and carotenoids were also determined in response to salt stress. Phenolics are known to be strong antioxidants that play a role in the scavenging of ROS. There were no major changes in the phenolic profile of water lettuce plants in response to saline stress (with the only significant difference observed in cell wall-bound phenolics in roots at 15 mM NaCl). This is indicative that despite the saline stress imposed; water lettuce plants were not able to mount a strong defense against the resulting oxidative stress (as indicated by lipid peroxidation described above). It is generally accepted that biotic and abiotic stresses trigger the production of phenolic compounds via activation of the phenyl propanoid pathway, and the endogenous plant hormones methyl jasmonate and jasmonic acid also play a role, ultimately activating plant defense mechanisms. However, this was not observed in the present study and others have also reported the lack of accumulation of phenolic compounds in response to saline stress, e.g. in sugarcane, small flower brugiera, lettuce and broccoli. Kim (2008) have further suggested that the ability of a plant to modify its phenolic content is dependent on the level of salt sensitivity of plants.

Carotenoids play a role as non-enzymatic antioxidants that protect plants against oxidative damage. For aerial plant parts and roots, water lettuce displayed a reduction in carotenoid levels (less than the control) following exposure to salt stress. This suggests an inability of water lettuce to mobilize protective mechanisms to prevent or minimize photo-oxidative damage in chloroplasts following salt stress [13].

The present study considered the effect of short-term mild water stress on water lettuce as a simulation of projected climate change predictions where lagoons and marshes will become increasingly salinized (either intermittently or permanently). The results from the present study indicated that water lettuce plants could not tolerate the higher NaCl concentrations tested (up to 80 mM) but survived exposure to lower levels (up to 20 mM). Evidence of damage was apparent even at the lower NaCl concentrations tested as revealed by increasing levels of necrosis and a slight reduction in biomass. Plants also showed evidence for the accumulation of oxidative damage in the form of lipid peroxidation. It is postulated that water lettuce plants are unable to mobilize an efficient defense to mitigate against the adverse effects of oxidative damage as shown by their inability to accumulate phenolics and carotenoids. It is recommended that future studies should investigate the effects of imposition of a longer term mild salt stress to determine if the plant is able to adapt to such conditions to develop protective strategies to enable its survival. This will have implications on the spread and distribution of this noxious weed as well as associated management strategies [14,15].

Author's Contribution

JGV, LHF, LH, LPB, RC, YE, JM, RGDZ, EH and JCL designed the research; JGV, LHF, LH, LPB, RC, YE and JM conducted the experiments; LHF, RGDZ, EH and JCL analyzed the data and wrote the paper; and JCL had primary responsibility for the final content. All authors have read and approved the final manuscript.

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Compliance with Ethical Standards

Conflict of Interest

Authors do not have any conflict of interests.

Human and Animal Rights

This research did not involve experiments with human or animal participants.

Informed Consent

Informed consent was obtained from all individual participants included in the study. Additional informed consent was obtained from all individual participants for whom identifying information is included in this article.

Data Availability Statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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