Effect of Treatment with Selected Plant Extracts on the Physiological and Biochemical Parameters of Rice Plants under Drought and Salt Stress

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Article

Effect of Treatment with Selected Plant Extracts on the Physiological and Biochemical Parameters of Rice Plants under Drought and Salt Stress

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Abstract: Stresses due to drought and high soil salt content are the most severe threats to global rice production, causing a significant decline in rice yield. Therefore, this study examined the effects of treatment with various plant extracts on the stresses on rice plants associated with drought and high salinity. Additionally, this study examined various physiological and biochemical parameters such as growth, photosynthetic activity, chlorophyll content, and lipid peroxidation in rice plants after treatment with selected plant extracts under drought and salt stress conditions. Out of the eleven extracts tested, four extracts, namely soybean leaf, soybean stem, Chinese chive, and onion extracts, were found to effectively reduce drought stress. A reduction of only 24-37% in shoot fresh weight was observed in rice plants under drought stress that were treated with these extracts compared to the 78% reduction observed in control plants. In addition, rice plants under salt stress which were treated with soybean leaf, soybean stem, moringa (*Moringa oleifera*), and *Undaria pinnatifida* extracts were observed to have lower reductions in shoot fresh weight (3-23%) compared to the control plants (43%). The effectiveness varied with the concentrations of the plant extracts. Water content was higher in the rice plants treated with extracts than in the control plants after 6 days of drought and salt stress, but not after 4 days of drought and salt stress. Although photosynthetic efficiency (Fv/Fm) and electron transport rate (ETR), and the content of pigments (chlorophyll and carotenoid) varied based on the types and levels of stress and the extracts that the rice plants were treated with, generally photosynthetic efficiency and pigment contents were higher in the treated rice compared to control plants than in the control plants. Reactive oxygen species (ROS) such as the superoxide radical, hydrogen peroxide (H_2O_2), and malondialdehyde (MDA) levels increased as the duration of the period of stress increased. ROS and MDA levels were lower in the treated rice compared to control plants. Proline and soluble sugar accumulation also increased as the duration of the stressperiod increased. However, proline and soluble sugar accumulation were lower in the treated rice compared to control plants. Generally, all the parameters investigated in this study were similar regardless of the plant extract used for treating the rice plants. Thus, extracts found to be effective in this study can be used to alleviate the adverse effects of stress on rice crops associated with drought and high salinity soil.

Keywords: biostimulant; drought stress; plant extract; rice; salt stress

1. Introduction

Rice (*Oryza sativa* L.) is the most common staple food of 50% of the world's population [1,2], and provides 80% of the daily calorie needs in many populations, predominately in Asia [3,4].

Crops are often exposed to unfavorable environmental conditions, such as abiotic stresses, which limits the yield. Among these, stress due to drought is one of the most severe threats to global rice production, causing a significant decline in rice yield [5,6]. The scale of damage caused by drought is dependent on the duration of the drought and the plant growth stage at which the drought occurs. Increases in drought-related stress as a result of climate change are causing many problems

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in Asian countries such as China, Bangladesh, and South Korea [7]. During stress conditions caused by the drought, oxidative stress, directly or indirectly generated in plants, is one of the main drivers of damage to plant structure and function. Oxidative stress results in damage of the cell membrane, alters membrane integrity and causes physiological and biochemical changes, which leads to acute metabolic disorders and eventually reduce crop productivity [8,9].

Salinity is also one of the most important environmental factors limiting the productivity of crops. Most crops are sensitive to high concentrations of salts in the soil, and almost 30% of the world's potentially cultivable land is high salinity soil. This is a result of saline water irrigation and has been responsible for quantitative economic losses [10,11]. High salinity concentration in the soil or the irrigation water can have a devastating effect on plant metabolism, disrupting cellular homeostasis and uncoupling major physiological and biochemical processes [12,13]. The negative impact of the stress associated with both salinity and drought on agriculture is expected to increase as a result of the projected global climate changes [14–17].

There are different approaches to mitigate drought and salt-related stress on plants. Among them, traditional breeding and modern biotechnological tools are being used to prevent yield losses. Another approach includes the use of plant growth hormones, gibberellic acid (GA), cytokinin, and salicylic acid (SA), antioxidants (ascorbic acid), and osmoprotectants in foliar applications and seed treatments [18,19]. In addition, applications of biostimulants, such as seaweed extracts, are currently being studied to improve plant performance [20–22]. Treatments based on natural sources, such as plant extracts, are eco-friendly and safe for sustainable agriculture and do not produce any adverse effects on the soil ecosystems.

The effect of biostimulants on the growth and quality of various plant species has been assessed [23–27]. Further, various seaweed extracts have been reported to enhance plant tolerance against a wide range of abiotic stresses [21,28–30]. There are also numerous reports on the benefits of applying seaweed extracts which have been shown to increase plant growth and yield parameters [31,32]. Until recently, only extracts of seaweed or related species have been used as treatments to potentially increase crop growth and reduce the damage associated with stress. There have only been a few studies on how extracts from natural plant sources such as leaves can be used to reduce stress damage.

The extracts of soybean (*Glycine max* (L.) Merr.) and Chinese chive (*Allium tuberosum* Rottler) leaves contain antioxidant compounds such as flavonoids, phenolic acids, and minerals and therefore may be effective in increasing crop yields by helping the crops cope better with environmental stress [33–36]. In addition, Morsy et al. [37] found that treatment with garlic or onion extracts significantly improved all the plant growth characteristics of the cucumber plant, i.e., the number of leaves, number of flowers, shoot and root length, and fresh and dry weight of the shoot and root system compared with untreated plants. However, few studies conducted to date have established whether other plant extracts or their chemical components could protect plants against abiotic stresses. Therefore, this study examined various plant extracts and their ability to reduce the stresses associated with drought and salt in rice plants. Additionally, this study examined various physiological and biochemical parameters such as growth parameters, photosynthetic activity, and proline accumulation in rice plants after treatment with the selected plant extracts under drought and salt stress conditions.

2. Materials and Methods

2.1. Plant Materials and Extracts

For this study, tomato, onion, and Chinese chive leaf powder were purchased from Shinyoung Mall Co., Ltd., Chamduri Co., and Healthy Chotori Co., Ltd., respectively. Dried moringa (*Moringa oleifera*) leaves were purchased from Dooson Herb Co., Ltd. and then made into a powder using a coffee grinder (Proctor Silex E160B, Southern Pines, NC). Soybean leaves, soybean stems, and green tea (*Camellia sinensis*) leaves were collected from the Sunchon National University's farm. *Undaria pinnatifida*, *Saccharina japonica*, *Hizikia fusiforme*, and *Gracilaria verrucosa* were collected from Goheung,

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Jeollanam-do, South Korea. These materials were dried in a drying oven at 45 °C for 3 days and made into a powder using a coffee grinder (Proctor Silex E160B, Southern Pines, NC).

To make the plant extracts, 50 g of the ground plant materials were mixed with 1000 mL of distilled water for 24 h. Thereafter, the extract was filtered through a Miracloth and completely evaporated using a vacuum dryer (Hanbaek Scientific Co. South Korea). Afterward, the extract was adjusted by distilled water to ensure that the final concentration was 50% (w/v), which was then further diluted with distilled water as needed to attain the proper concentrations for each experiment.

2.2. Drought and Salt Stress Treatments

Rice (cv. Hopumbyeo) plants were germinated in seed trays, and grown until the 2nd–3rd true leaf stage. At this stage, three rice plants were transplanted to plastic pots (200 mL) filled with a mixture of commercial paddy soil (SUNGHWA. Co., Ltd., Beolgyo, South Korea). Each treatment was applied to three plastic pots containing three plants each, and all experiments were replicated three times. After transplanting, the pots were placed in growth chambers (25 ºC/22 ºC under a 14/10 h day/night regime, 150 µmol $m^{-2}s^{-1}$ photosynthetically active radiation and relative humidity of 60%). For soil drench treatments, 5 mL of plant extracts were applied on the soil surface at 1, 3, and 5% concentrations 2 weeks after transplanting. In contrast, the control plants were treated with only 5 mL of tap water. The soil moisture content was set at 75% of field capacity. Subsequently, the plants were subjected to drought stress by withholding water for six days in the growth chamber to mimic drought conditions. One group of plants was maintained under optimal irrigation and received no extract application which served as the control. Among the various concentrations of plant extracts tested, treatments of extracts at 3% concentrations were selected for further study as they produced significant reduction of drought stress on shoot fresh weight.

For studies on salt stress, after 30 days of seed germination, roots of the seedlings were washed and seedlings were placed in conical tubes (15 mL). Each treatment was applied to conical tubes containing five plants each, and all experiments were replicated three times. Each tube was subjected to root treatments of either 100 mM NaCl or plant extracts at concentrations of 0.1%, 0.5%, 1%, or 3% combined with 100 mM NaCl. We chose a concentration of 100 mM NaCl after preliminary experiments showed that lower or higher concentrations caused either not enough or too much damage to plants. Seedlings were then placed in growth chambers (25 ºC/22 ºC under a 14/10 h day/night regime, 150 µmol $m^{-2}s^{-1}$ photosynthetically active radiation and relative humidity of 60%) for six days. Plant extracts with a 1% concentration were selected for further study due to their significant reduction of salt stress on shoot fresh weight.

2.3. Rice Growth and Relative Water Contents After Drought and Salt Stress

Plant injury was investigated at 1, 2, 4, and 6 days after drought and salt stress. Plant height and shoot fresh weight were measured after 6 days of drought and salt stress. For relative water content, after harvesting, the samples were immediately weighed (Wf). The samples were then oven-dried at 105 °C for 24 hours and the dry weight was calculated (Wd). Then, their average was computed (Wt). Relative water content was calculated using the following formula:

$$\text{Relative water content} = (Wf - Wd)/Wt \times 100 \text{ [38]}.$$

2.4. Photosynthetic Efficiency, Chlorophyll and Carotenoid Contents After Drought and Salt Stress

Chlorophyll fluorescence analysis involves a non-invasive measurement of photosystem II (PSII). The quantum yield (Fv/Fm) and electron transport rate (ETR) of rice plants were measured after 4 and 6 days of drought or salt stress. *In vivo* chlorophyll fluorescence of PSII was determined by using a portable pulse modulation fluorometer (PAM 2500, Walz, Effeltrich, Germany). Prior to measurements, the fronds were dark adapted for 15 min to expose all the antennae pigments.

Chlorophyll and carotenoid contents were assayed according to the procedure laid down by Hiscox and Israelstam [39]. The leaves (0.2 g) from each treatment were ground in N_2 with a mortar and pestle, re-suspended with 5 mL of 100% methanol, and then centrifuged at 10,000 × g for 3 min.

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Spectrophotometric measurements of the absorbance of the resultant supernatant were made at 652.4, 665.2, and 470.0 nm. The chlorophyll and carotenoid contents were calculated using the following equations:

$$\text{Chlorophyll a } (C_a) = 16.72\ A_{665.2} - 9.16\ A_{652.4}$$

$$\text{Chlorophyll b } (C_b) = 34.09\ A_{652.4} - 15.28\ A_{665.2}$$

$$\text{Total chlorophylls } (C_{a+b}) = 1.44\ A_{665.2} + 24.93\ A_{652.4}$$

$$\text{Total carotenoids } (C_{x+c}) = \frac{1{,}000A_{470.0} - 1.63C_a - 104.96C_b}{221}$$

2.5. Determination of Superoxide Radical (O_2^-) and H_2O_2 Contents After Drought and Salt Stress

The superoxide radical contents in the leaves were determined using the modified method of Elstner and Heupel [40]. A quantity of 200 mg of the leaves was homogenized with 1 mL of 50 mM phosphate buffer solution (pH 7.8) containing 2 mM hydroxylamine hydrochloride. After centrifugation at 12,000 x g for 30 min, 600 µl of the supernatants were mixed with 400 µl of phosphate buffer solution and incubated at 25°C for 30 min. Then 1ml of 17mM L-sulfanilic acid and 1 mL of 7 mM L-1-*α*-naphthylamine were added, and the mixture was shaken using an incubator (VS-1203PFC-L, LabTech. Namyang, South Korea) at room temperature for 30 min. The absorbance was determined colorimetrically at 530 nm.

The hydrogen peroxide (H_2O_2) level was measured colorimetrically as described by Jana and Choudhuri [41] Hydrogen peroxide was extracted by homogenizing 0.2 g of leaves with 3 mL phosphate buffer (50 mM; pH, 6.8). The homogenate was centrifuged at 6000 × g, for 25 min. To determine the H_2O_2 levels, 3 mL of the extracted solution was mixed with 1 mL of 0.1% titanium chloride (Aldrich, St. Louis) in 20% (v/v) H_2SO_4, and the mixture was centrifuged, at 6000 × g for 15 min. The intensity of the yellow supernatant was measured at 410 nm. The H_2O_2 level was calculated using the extinction coefficient 0.28 $mm^{-1}cm^{-1}$.

2.6. Lipid Peroxidation After Drought and Salt Stress

Lipid peroxidation was estimated by quantifying the amount of MDA production using a slight modification of the thiobarbituric acid (TBA) method, as previously described by Buege and Aust [42]. Each leaf (0.5 g) was ground using a mortar and pestle with 5 mL 0.5% TBA in 20% trichloroacetic acid. The homogenate was then centrifuged at 20,000 × g for 15 min, and the supernatant was collected. The supernatant was heated in a boiling water bath for 25 min and allowed to cool in an ice bath. After additional centrifugation at 20,000 × g for 15 min, the resulting supernatant was used for the spectrophotometric determination of MDA. The absorbance at 532 nm for each sample was recorded and subtracted for nonspecific turbidity at 600 nm. MDA concentrations were calculated using a molar extinction coefficient of 156 $mm^{-1}cm^{-1}$ and the following formula: MDA (micromoles per gram dry weight) = $[(A_{532} - A_{600})/156] \times 10^3 \times$ dilution factor [43].

2.7. Proline and Sugar Accumulation After Drought and Salt Stress

Proline was extracted by grinding 1 g of frozen plant material using a mortar and pestle. The grinding material was homogenized with 5 mL of 3% sulfosalicylic acid and the debris was removed by centrifugation at 2000 × g for 10 minutes. 3 mL of the extract was reacted with 3 mL of glacial acetic acid and 3 mL color-developing solution (125 mg ninhydrin warmed to be dissolved in 3 mL glacial acetic acid and 2 mL 6 M phosphoric acid) for 30 minutes at 100 °C, and the reaction was terminated in an ice bath. The reaction mixtures were mixed with 5 mL of toluene. The chromophore containing toluene was aspirated from the aqueous phase and warmed up to room temperature for 24 hours. The amount of proline was determined with a spectrophotometer at 520 nm.

The soluble sugar of the leaves was extracted and quantified by a modified method of Xu et al. [44]. 50 mg of ground sample was extracted with 1 mL of 80% (v/v) ethanol at 80°C for 30 min, followed by centrifugation at 14,000 × g for 10 min. The residue was extracted two more times using 80% ethanol. The three supernatants were combined and 80% ethanol was added to bring the total

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volume to 5 mL. The soluble sugar content was determined spectrophotometrically at A_{620} nm wavelength. The sucrose content was determined spectrophotometrically at A_{480} nm wavelength.

2.8. Statistical Analysis

The experiments were conducted in a completely randomized design with three replications. Some data were expressed as percentages of the untreated control for easy comparisons between the treatments. Data were analyzed using the analysis of variance (ANOVA) procedure using the Statistical Analysis Systems software 7 (SAS 2000). There were significant treatments x concentrations by factorial interaction. The means were separated using Duncan's Multiple Range Test ($p = 0.05$).

3. Results and Discussion

3.1. Effects of Plant Extracts on Rice Injury and Growth Under Drought and Salt Stress

To select the plant extracts that were effective in reducing drought associated stress damage, we treated rice plants with various plant extracts (Table 1). Injuries to the rice plant, identified based on visual symptoms, increased with time under drought stress regardless of the plant extract used for treatment. However, all the extracts tested here except for *Undaria pinnatifida* reduced the injuries caused by drought stress. Injuries were observed to affect 70% of the control plants subjected to drought stress within 5 days. On the other hand, most treated plants had fewer injuries than the control plants. However, the levels of injuries to the rice plants varied depending on the concentrations of the plant extracts used for treatment. Specifically, treatments with soybean leaf, soybean stem, Chinese chive, onion, green tea (*Camellia sinensis*), *Saccharina japonica, Hizikia fusiforme,* and *Gracilaria verrucose* extracts were the most effective in reducing plant injuries caused by drought stress. Plant height was also significantly lower among the control plants subjected to drought stress. However, there was no significant difference in plant heights between the control plants subjected to drought stress and those treated with the plant extracts. Further, the rice plants that were treated with soybean leaf, soybean stem, Chinese chive, onion, green tea, *Saccharina japonica, Hizikia fusiforme,* and *Gracilaria verrucose* extracts fared better under drought stress with a higher shoot fresh weight than the control plants. The shoot fresh weight after the extract treatments mentioned above was only reduced by 25-50% compared to the 78% reduction in the shoot fresh weight of the control plants. There was significant interaction between various plant extract treatments and concentrations in terms of drought stress reduction. Four plant extracts (soybean leaf, soybean stem, Chinese chive and onion) that effectively reduced drought stress were selected for further studies.

Table 1. Effects of various plant extracts on rice injury, plant height, and shoot fresh weight under drought stress.

Extract	Con.* (%)	Leaf injury (%)*				Plant height (cm)	Shoot F.W* (g/plant)
		1 DAT	2 DAT	4 DAT	6 DAT		
Non-stress		0.0^{a}	0.0^{a}	0.0^{g}	0.0^{k}	35.9(100)^{a}	0.339(100)^{a}
Drought stress		0.0^{a}	0.0^{a}	40^{a}	70^{a}	29.6(82.2)^{b}	0.076(22.4)^{m}
Soybean leaf	1	0.0^{a}	0.0^{a}	0.0^{g}	21.3^{g-i}	31.4(87.2)^{ab}	0.238(70.0)^{bcd}
	3	0.0^{a}	0.0^{a}	0.0^{g}	0.0^{k}	29.6(82.2)^{b}	0.228(67.4)^{de}
	5	0.0^{a}	0.0^{a}	0.0^{g}	3.8^{jk}	30.9(85.8)^{ab}	0.246(72.4)^{bcd}
Soybean stem	1	0.0^{a}	0.0^{a}	28.8^{a-c}	32.5^{fg}	31.1(86.4)^{ab}	0.159(46.8)^{ij}
	3	0.0^{a}	0.0^{a}	15.0^{c-f}	18.8^{g-j}	31.6(87.8)^{ab}	0.213(62.7)^{ef}
	5	0.0^{a}	0.0^{a}	7.5^{e-g}	11.3^{h-k}	30.9(85.8)^{ab}	0.199(58.5)^{fg}
Chinese chive	1	0.0^{a}	0.0^{a}	3.8^{fg}	17.5^{g-j}	29.6(80.8)^{b}	0.257(75.6)^{bc}

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	3	0.0^{a}	0.0^{a}	0.0^{g}	0.0^{k}	30.1(83.6)^{b}	0.244(71.8)^{bcd}
	5	0.0^{a}	0.0^{a}	5.0^{fg}	8.8^{h-k}	31.4(87.5)^{ab}	0.246(72.4)^{bcd}
Onion	1	0.0^{a}	0.0^{a}	25.0^{b-d}	42.5^{ef}	30.1(83.6)^{b}	0.148(43.5)^{jk}
	3	0.0^{a}	0.0^{a}	0.0^{g}	7.5^{h-k}	29.7(82.8)^{b}	0.236(69.4)^{cd}
	5	0.0^{a}	0.0^{a}	0.0^{g}	7.5^{h-k}	31.9(88.6)^{ab}	0.244(71.8)^{bcd}
Tomato	1	0.0^{a}	0.0^{a}	17.5^{b-f}	41.3^{ef}	29.3(81.7)^{b}	0.131(38.5)^{kl}
	3	0.0^{a}	0.0^{a}	25.0^{b-f}	57.3^{a-e}	21.3(85.0)^{c}	0.115(33.8)^{l}
	5	0.0^{a}	0.0^{a}	38.8^{b-f}	65.0^{ab}	29.8(82.8)^{b}	0.075(22.1)^{m}
Camellia sinensis	1	0.0^{a}	0.0^{a}	30.0^{ab}	57.5^{a-e}	31.5(87.5)^{ab}	0.129(37.9)^{kl}
	3	0.0^{a}	0.0^{a}	20.0^{b-e}	52.5^{b-e}	31.6(87.8)^{ab}	0.167(49.4)^{hij}
	5	0.0^{a}	0.0^{a}	26.3^{a-c}	46.3^{c-f}	32.3(89.7)^{ab}	0.173(50.9)^{hi}
Moringa oleifera	1	0.0^{a}	0.0^{a}	16.3^{b-f}	45.0^{d-f}	30.9(86.1)^{ab}	0.157(46.2)^{ij}
	3	0.0^{a}	0.0^{a}	20.0^{b-e}	57.5 $^{a-e}$	29.9(83.3)^{b}	0.123(36.2)^{l}
	5	0.0^{a}	0.0^{a}	20.0^{b-e}	62.5^{a-c}	31.8(86.9)^{ab}	0.117(34.4)^{l}
Undaria pinnatifida	1	0.0^{a}	0.0^{a}	30.0^{ab}	67.5^{ab}	30.1(83.6)^{b}	0.085(25.0)^{m}
	3	0.0^{a}	0.0^{a}	11.3^{d-g}	53.8^{a-e}	29.7(82.5)^{b}	0.121(35.6)^{l}
	5	0.0^{a}	0.0^{a}	22.5^{b-d}	60.0^{a-d}	29.4(81.7)^{b}	0.095(27.9)^{m}
Saccharina japonica	1	0.0^{a}	0.0^{a}	28.8^{a-c}	32.5^{fg}	31.1(86.4)^{ab}	0.159(46.8)^{ij}
	3	0.0^{a}	0.0^{a}	15.0^{c-f}	18.8^{g-j}	31.6(87.8)^{ab}	0.213(62.7)^{ef}
	5	0.0^{a}	0.0^{a}	7.5^{e-g}	11.3^{h-k}	30.8(85.8)^{ab}	0.199(58.5)^{fg}
Hizikia fusiforme	1	0.0^{a}	0.0^{a}	3.8^{fg}	6.3^{h-k}	29.1(80.8)^{b}	0.187(35.6)^{gh}
	3	0.0^{a}	0.0^{a}	0.0^{g}	5.0^{i-k}	30.6(85.0)^{b}	0.233(68.5)^{de}
	5	0.0^{a}	0.0^{a}	5.0^{fg}	15.0^{h-k}	32.9(91.4)^{ab}	0.260(72.1)^{b}
Gracilaria verrucosa	1	0.0^{a}	0.0^{a}	20.0^{b-e}	22.5^{gh}	32.1(89.2)^{ab}	0.238(62.4)^{bcd}
	3	0.0^{a}	0.0^{a}	16.3^{b-f}	20.0^{g-j}	31.2(88.9)^{ab}	0.229(60.6)^{de}
	5	0.0^{a}	0.0^{a}	25.0^{b-d}	32.5^{fg}	29.6(82.5)^{b}	0.159(46.8)^{ij}
Treatment						***	***
Concentration						0.12	***
Treatment × Concentration						0.28	***

*Con., concentration; DAT, days after treatment; Leaf injury based on visual rate (0~100%, 100; complete death); F.W, Fresh weight; Parentheses are % of control. Means with different letters are significantly different by Duncan's Multiple Range Test at 5% level. Significance. ***= *p* value<0.001.

Drought is one of the most widespread abiotic stresses that negatively affect both crop growth and yield [45]. Visible symptoms in plants exposed to drought stress are the wilting of leaves, decline in fresh shoot weight, and interruption in budding and flowering [46]. Drought conditions also limit the uptake of nutrients by the plants due to limited soil moisture, leading to decreased plant growth parameters [47]. To select the plant extracts that are effective in reducing the damage associated with salt stress, we treated rice plants with various plant extracts (Table 2). Injuries to the rice plant identified based on visual symptoms increased with time under salt stress regardless of the type of plant extract used. Chinese chive, onion, and tomato extracts at certain concentrations reduced injuries due to salt stress, but other concentrations were not effective. However, most extracts, such as soybean leaf, soybean stem, moringa, and *Undaria pinnatifida,* effectively reduced injuries caused due to salt stress. Plant height was not significantly reduced by salt stress in both the control plants and the treated plants. The shoot fresh weight of rice plants was higher when treated with Chinese chive, tomato, and moringa extracts at some concentrations when compared with the shoot fresh

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weight of the control plants under salt stress. The shoot fresh weight of the rice plants after the onion and green tea extract treatments was similar to the shoot fresh weight of the control plants. However, the shoot fresh weight of plants treated with other extracts such as soybean leaf, soybean stem, moringa, and *Undaria pinnatifida* was higher than the shoot fresh weight of the control plants under salt stress. Hence, we selected the soybean leaf, soybean stem, moringa, and *Undaria pinnatifida* extracts for further studies.

Table 2. Effects of various plant extracts on rice injury, plant height, and shoot fresh weight under salt stress.

Extract	Con.* (%)	Leaf injury (%)*				Plant height (cm)	Shoot F.W* (g/plant)
		1 DAT	2 DAT	4 DAT	6 DAT		
Non-stress		0^{d}	0^{g}	0^{e}	0^{k}	24.3(100)$^{a-f}$	0.230(100)^{a}
Salt stress	100 mM	10^{c}	30^{a-c}	40^{a-c}	65^{a-d}	23.6(97.1)$^{b-f}$	0.130(56.5)$^{k-o}$
Soybean leaf	0.1	0^{d}	10^{e-g}	20^{c-e}	20^{i-k}	24.8(102.1)$^{a-f}$	0.195(84.8)^{b}
	0.5	0^{d}	10^{e-g}	20^{c-e}	20^{i-k}	25.7(105.8)$^{a-e}$	0.224(97.4)^{a}
	1	10^{c}	10^{e-g}	20^{c-e}	30^{g-j}	26.6(109.5)^{ab}	0.178(77.4)$^{b-d}$
	3	10^{c}	25^{b-d}	45^{ab}	60^{a-e}	24.6(101.2)$^{a-f}$	0.139(60.4)$^{h-n}$
Soybean stem	0.1	10^{c}	10^{e-g}	20^{c-e}	30^{g-j}	25.9(106.6)$^{a-e}$	0.152(66.1)$^{e-k}$
	0.5	10^{c}	10^{e-g}	20^{c-e}	25^{h-j}	25.3(104.1)$^{a-f}$	0.188(81.7)^{b}
	1	10^{c}	10^{e-g}	20^{c-e}	25^{h-j}	25.9(106.6)$^{a-e}$	0.217(94.3)^{a}
	3	0^{d}	10^{e-g}	15de	20^{i-k}	26.9(110.7)^{a}	0.221(96.1)^{a}
Chinese chive	0.1	0^{d}	10^{e-g}	20^{c-e}	50^{c-g}	23.4(96.6)$^{c-f}$	0.154(67.0)$^{e-j}$
	0.5	10^{c}	20^{c-e}	40^{a-c}	50^{c-g}	25.0(102.9)$^{a-f}$	0.174(75.6)$^{b-e}$
	1	10^{c}	30^{a-c}	45^{a-e}	70^{a-c}	25.1(103.3)$^{a-f}$	0.149(64.8)$^{f-l}$
	3	30^{a}	40^{a}	50^{a}	80^{a}	26.1(107.4)$^{a-c}$	0.133(57.8)$^{j-o}$
Onion	0.1	0^{d}	0^{g}	30^{a-d}	45^{d-h}	22.9(94.2)$^{d-f}$	0.125(53.4)$^{m-p}$
	0.5	0^{d}	0^{g}	30^{a-d}	50^{c-g}	23.4(96.3)$^{c-f}$	0.130(56.5)$^{k-o}$
	1	10^{c}	20^{c-e}	40^{a-c}	60^{a-e}	23.7(97.5)$^{b-f}$	0.135(58.7)$^{i-o}$
	3	10^{c}	20^{c-e}	50^{a}	80^{a}	26.3(108.2)$^{a-c}$	0.134(58.2)$^{i-o}$
Tomato	0.1	0^{d}	10^{e-g}	20^{c-e}	50^{c-g}	24.1(99.2)$^{a-f}$	0.134(58.3)$^{i-o}$
	0.5	0^{d}	10^{e-g}	30^{a-d}	60^{a-e}	25.5(104.9)$^{a-f}$	0.166(71.2)$^{c-f}$
	1	10^{c}	25^{b-d}	40^{a-c}	60^{a-e}	23.8(97.9)$^{a-f}$	0.146(63.5)$^{f-m}$
	3	25^{ab}	35^{ab}	45^{ab}	75^{ab}	24.0(98.8)$^{a-f}$	0.144(62.6)$^{f-m}$
Camellia sinensis	0.1	0^{d}	10^{e-g}	20^{c-e}	40^{e-i}	22.8(93.8)^{ef}	0.107(46.5)^{p}
	0.5	0^{d}	10^{e-g}	20^{c-e}	47^{c-h}	22.5(92.5)^{f}	0.142(61.7)$^{g-n}$
	1	10^{c}	10^{e-g}	20^{c-e}	47^{c-h}	25.1(103.3)$^{a-f}$	0.129(56.1)$^{l-o}$
	3	5^{cd}	10^{e-g}	30^{a-d}	55^{b-f}	23.5(96.7)$^{b-f}$	0.116(50.4)^{op}
Moringa oleifera	0.1	0^{d}	5^{fg}	10de	30^{g-j}	23.5(96.7)$^{b-f}$	0.149(64.8)$^{f-l}$
	0.5	0^{d}	10^{e-g}	20^{c-e}	30^{g-j}	26.1(107.4)$^{a-d}$	0.178(77.4)$^{b-d}$
	1	0^{d}	15^{d-f}	25^{b-d}	40^{e-i}	24.0(98.8)$^{a-f}$	0.121(52.6)$^{n-p}$
	3	20^{b}	30^{a-c}	50^{a}	80^{a}	25.3(104.1)$^{a-f}$	0.108(47.0)^{p}
Undaria pinnatifida	0.1	5^{cd}	10^{e-g}	20^{c-e}	30^{g-j}	26.0(107.0)$^{a-d}$	0.161(70.0)$^{c-h}$
	0.5	5^{cd}	10^{e-g}	20^{c-e}	30^{g-j}	25.6(105.3)$^{a-f}$	0.155(67.4)$^{e-j}$
	1	5^{cd}	10^{e-g}	15de	20^{i-k}	25.8(106.2)$^{a-e}$	0.182(79.1)^{bc}

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	3	5^{cd}	10^{e-g}	15de	20^{i-k}	24.0(98.8)$^{a-f}$	0.175(76.1)$^{b-e}$
Saccharina japonica	0.1	0^{d}	10^{e-g}	30^{a-d}	50^{c-g}	25.4(104.5)$^{a-f}$	0.150(65.2)$^{f-l}$
	0.5	0^{d}	10^{e-g}	30^{a-d}	50^{c-g}	24.6(101.2)$^{a-f}$	0.161(70.0)$^{c-h}$
	1	10^{c}	20^{c-e}	40^{a-c}	45^{d-h}	25.3(104.1 $^{a-f}$	0.162(70.4)$^{c-g}$
	3	10^{c}	15^{d-f}	25^{b-d}	40^{e-i}	25.3(104.1)$^{a-f}$	0.163(70.9)$^{c-g}$
Hizikia fusiforme	0.1	0^{d}	10^{e-g}	20^{c-e}	40^{e-i}	23.9(96.7)$^{a-f}$	0.139(60.4)$^{h-n}$
	0.5	5^{cd}	10^{e-g}	30^{a-d}	47^{c-h}	24.4(100.4)$^{a-f}$	0.153(66.5)$^{e-j}$
	1	5^{cd}	10^{e-g}	30^{a-d}	50^{c-g}	24.7(101.6)$^{a-f}$	0.173(75.2)$^{b-e}$
	3	0^{d}	10^{e-g}	30^{a-d}	45^{d-h}	24.7(101.6)$^{a-f}$	0.146(63.5)$^{f-m}$
Gracilaria verrucosa	0.1	0^{d}	10^{e-g}	15de	25^{h-j}	24.4(100.4)$^{a-f}$	0.143(62.2)$^{f-n}$
	0.5	0^{d}	5^{fg}	20^{c-e}	30^{g-j}	24.2(99.6)$^{a-f}$	0.164(71.3)$^{c-g}$
	1	0^{d}	5^{fg}	10de	15^{jk}	25.0(102.9)$^{a-f}$	0.174(75.7)$^{b-e}$
	3	10^{c}	20^{c-e}	20^{c-e}	35^{f-j}	25.8(106.2)$^{a-e}$	0.156(67.8)$^{d-i}$
Treatment						**	***
Concentration						0.13	***
Treatment × Concentration						0.44	***

*Con., concentration; DAT, days after treatment; Leaf injury based on visual rate (0~100%, 100; complete death); F.W, Fresh weight; Parentheses are % of control. Means with different letters are significantly different by Duncan's Multiple Range Test at 5% level. Significance. ***= *p* value<0.001.

Out of the eleven extracts used, only the tomato and *Undaria pinnatifida* extract treatment of crops under drought stress, and onion and green tea extract treatment of crops under salt stress did not reduce rice damage. All other extracts, including *Undaria pinnatifida, Saccharina japonica, Hizikia fusiforme,* and *Gracilaria verrucose* extracts which originated from sea plants, were effective in reducing either drought or salt stress. To date, extracts made from seaweed species have been studied extensively and have been found to be effective in alleviating a variety of abiotic stresses, including drought, salinity, and temperature [22]. Seaweed extract also increases the endogenous concentrations of stress-related molecules, such as cytokinins, proline, and antioxidants in treated plants [48].

Pretreatment with the seaweed extract of *Sargassum latifolium* (1.5%) or *Ulva lactuca* (1%) resulted in alleviation of the damaging effects of drought on *Triticum aestivum* during the vegetative stage, by enhancing growth, improving metabolic activities, and increasing the yield [49]. Another effective extract used in this study came from moringa, which ameliorated drought and salt stress. However, the effectiveness was lower when compared to other plant extracts. In another similar study, foliar-applied moringa leaf extract could ameliorate salinity-induced adverse effects by activating the antioxidant defense system and decreasing the accumulation of Na^+ and Cl^- in the shoots under moderate saline conditions [50]. *Chaetomorpha antennina* aqueous extracts were also found to be capable of alleviating salt stress in rice plants in an efficient, eco-friendly, and economical manner [51]. *Acacia dealbata* bark extract significantly increased the height as well as the leaves, roots, and total biomass of plants in soils irrigated with NaCl solution (120 mmol L^{-1}) [52]. However, the effect of this bark extract was negligible on plants that grew under drought stress. Also, the application of a foliar spray of garlic extract caused significant increases in growth, physiological aspects, anatomical structure as well as the yield components of pea plants under drought stress [53]. The foliage application of sorghum, brassica, sunflower, and moringa extracts also improved wheat production and yield under terminal heat and drought stresses [54]. Although many plant extracts have been studied for their ability to reduce stress damage, the extracts made from soybean leaf, soybean stems and Chinese chive used in this study, have not been studied in detail earlier.

3.2. Effects of Selected Plant Extracts on Relative Water Content Under Drought and Salt Stress

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Relative water content is an integrated measurement of plant water status. It is an indicator of the physiological consequences of cellular water deficit and represents the variations in the water potential and turgor potential of the plants [55]. Water stress is one of several factors that negatively affect the relative water content, turgor pressure, and transpiration in many crops [56]. Thus, to determine the water content of leaves under drought stress, rice plants were treated with the selected plant extracts (Figure 1). After 4 days of drought stress, the water content in the leaves was similar regardless of the type of plant extract used in the treatments. However, after 6 days of drought stress, the water content in the leaves was higher in the plants that had received plant extract treatments than in the control plants. Additionally, there was little variation in the leaf water content, regardless of the type of extract used in treatments.

To determine the water content of the leaves under salt stress, rice plants were treated with the selected plant extracts. After 4 days of salt stress, the water content in the leaves did not vary regardless of the type of plant extract treatments used. However, after 6 days of salt stress, the water content in the leaves of the treated plants was significantly higher than that in the control plants. All the selected extracts except *Gracilaria verrucose* helped plants retain the water content in their leaves.

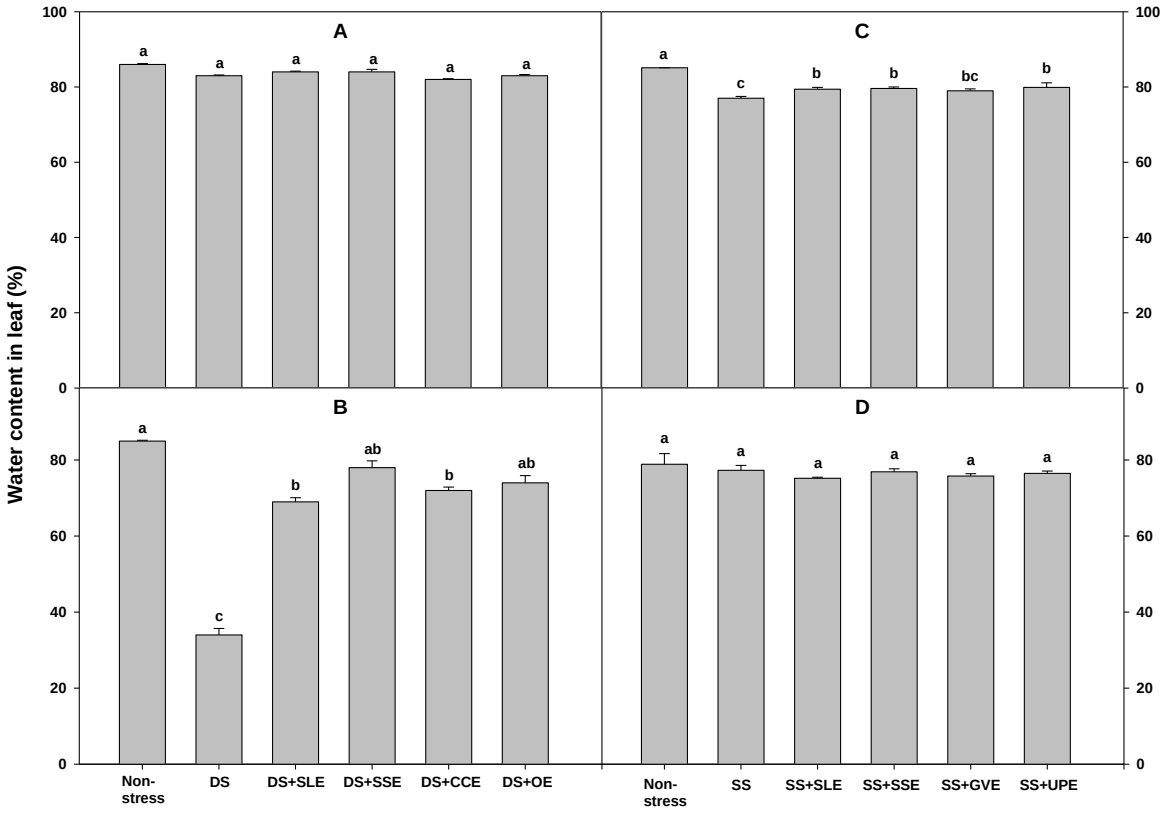

Figure 1. Effects of selected extracts on leaf water content at 4 (A and C) and 6 (B and D) days after treatments under a 3% concentration of drought stress (A and B) or 1% concentration of salt stress (C and D). (DS, Drought stress; DS+SLE, Drought stress+Soybean leaf extract; DS+SSE, Drought+Soybean stem extract; DS+CCE, Drought stress+Chinese chive extract; DS+OE, Drought stress+Onion extract; SS, Salt stress; SS+SLE, Salt stress+Soybean leaf extract; SS+SSE, Salt stress+Soybean stem extract; SS+GVE, Salt stress+*G. verrucosa* extract; SS+UPE, Salt stress+*U. pinnatifida* extract). Means with different letters are significantly different by Duncan's Multiple Range Test at 5% level. Means expressed error bar (±SE) with three replicates.

3.3. Effects of Selected Plant Extracts on Photosynthetic Efficiency and Pigments Under Drought and Salt Stress

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One of the major consequences of water deficit in higher plants is the decrease or suppression of photosynthesis [57]. Fv/Fm and ETR can be used as indicators to evaluate the photosynthetic activity of plant leaves [58]. The photosynthetic efficiency (Fv/Fm and ETR) of the rice plants under drought stress was measured after treatment with the selected plant extracts (Figure 2). Fv/Fm was significantly higher in all the treated plants compared to the control plants at 4 and 6 days of drought stress. However, there was no significant difference in the Fv/Fm between the treatments with the different types of plant extracts. After 4 days of drought stress, ETR did not vary between the plants treated with plant extracts and the control plants. However, after 6 days of drought stress, ETR was higher in the plants that received the extract treatments compared to the control plants.

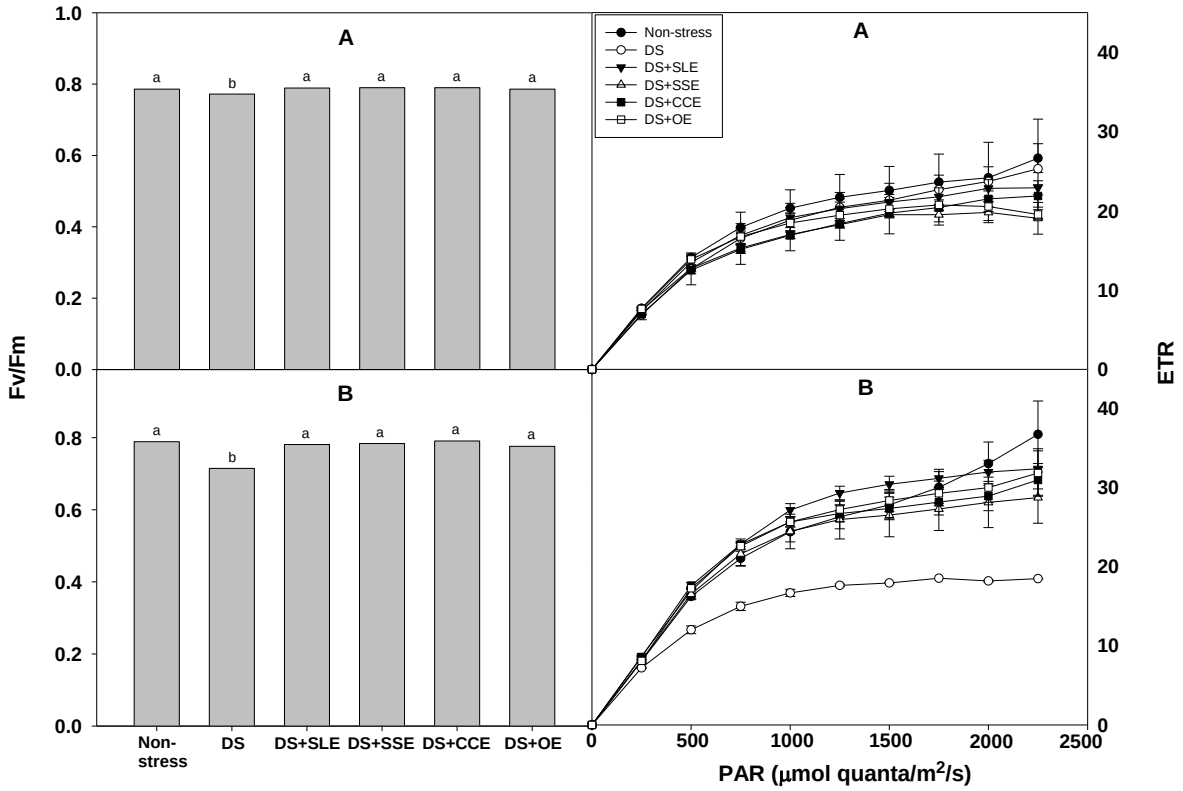

Figure 2. Effects of selected plant extracts at 3% concentrations on chlorophyll a fluorescence (Fv/Fm) and ETR at 4 (A) and 6 (B) days after treatments under drought stress (DS, Drought stress; DS+SLE, Drought stress+Soybean leaf extract; DS+SSE, Drought+Soybean stem extract; DS+CCE, Drought stress+Chinese chive extract; DS+OE, Drought stress+Onion extract). Means with different letters are significantly different by Duncan's Multiple Range Test at 5% level. Means expressed error bar (±SE) with three replicates.

We also determined the photosynthetic efficiency (Fv/Fm and ETR) in plants under salt stress (Figure 3). Similar to the drought stress, Fv/Fm was significantly higher in all the treated plants compared with the control plants after 4 and 6 days of salt stress. ETR was also significantly higher in all the treated plants than in the control plants after 6 days of salt stress, but showed no difference after 4 days. The photosynthetic capacities of leaves are important factors that reduce yield in susceptible rice genotypes under drought stress conditions [59]. In addition, drought or salt stress lowers the rate of photosynthesis, and alters the distribution and metabolism of carbon in plants, leading to depleted energy and decreased yield [60]. This study, too, suggests that the reduction of the water content and reduced photosynthetic efficiency resulting in lower shoot fresh weight may be caused by drought and salt stress.

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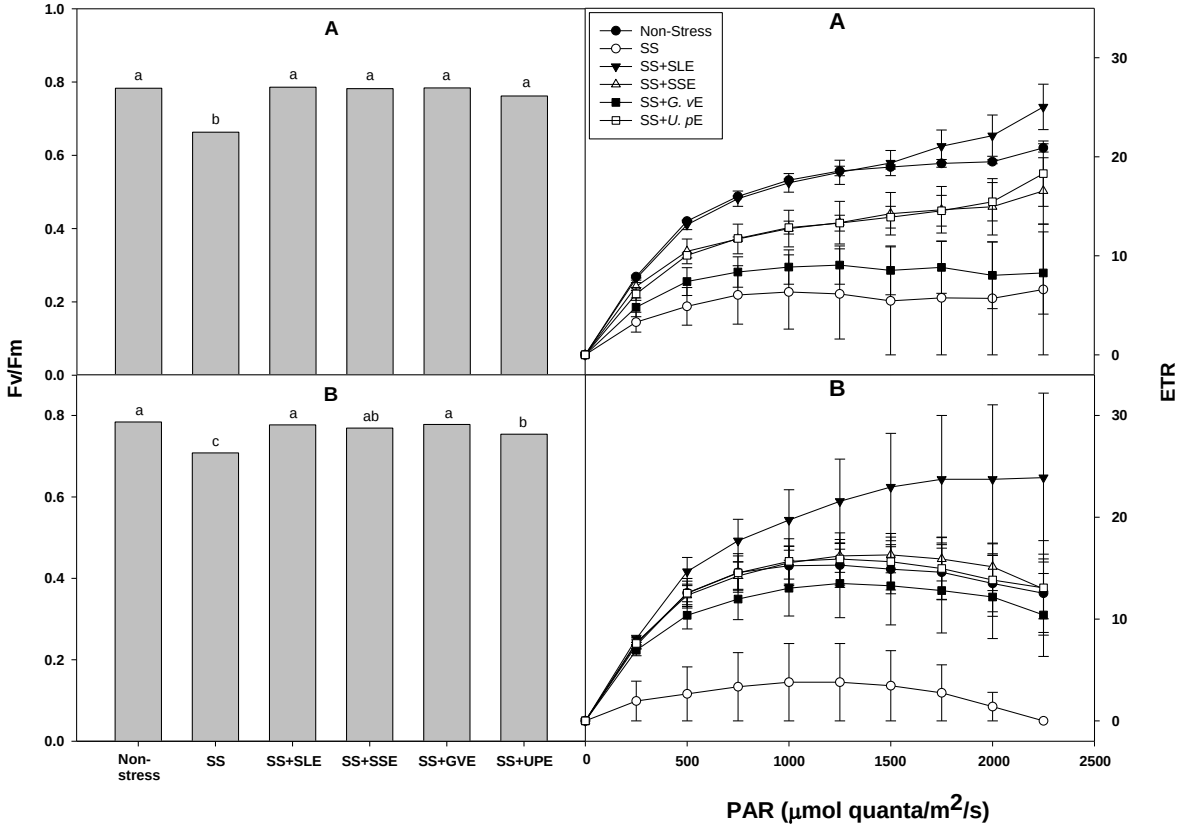

Figure 3. Effects of selected plant extracts at 1% concentrations on chlorophyll a fluorescence (Fv/Fm) and ETR at 4 (A) and 6 (B) days after treatment under salt stress (SS, Salt stress; SS+SLE, Salt stress+Soybean leaf extract; SS+SSE, Salt stress+Soybean stem extract; SS+GVE, Salt stress+*G. verrucosa* extract; SS+UPE, Salt stress+*U. pinnatifida* extract). Means with different letters are significantly different by Duncan's Multiple Range Test at 5% level. Means expressed error bar (±SE) with three replicates.

Chlorophyll, the primary pigment in photosynthesis and is found in the chloroplasts. [61]. The decrease in chlorophyll content under drought stress has been considered a typical symptom of pigment photooxidation and chlorophyll degradation [62]. In addition, carotenoids are the essential components for photoprotection and act as precursors in directing the signals for the growth of plants under stress conditions. After treatment with plant extracts, the chlorophyll and carotenoid content of the rice plants under drought stress were measured (Figure 4). Compared to the control plants, the chlorophyll content after 4 days of drought stress was lower in all the treated plants except for those treated with onion extracts. However, the chlorophyll content after 6 days of drought stress was higher in all the treated plants except for those treated with soybean leaf extract. The carotenoid content in the control plants after 4 days of drought stress was also not significantly reduced compared with the control plants which were not subjected to stress. However, the carotenoid content in all treated plants after 4 days of drought stress was lower than that in the control plants. After 6 days of drought stress, the chlorophyll content was lower in control plants. Additionally, when compared to the control plants, the chlorophyll content was significantly higher in all the treated plants except for those treated with soybean leaf extract. After 6 days of drought stress, the carotenoid content was also lower in the control plants and significantly higher in all the treated plants compared to the control plants.

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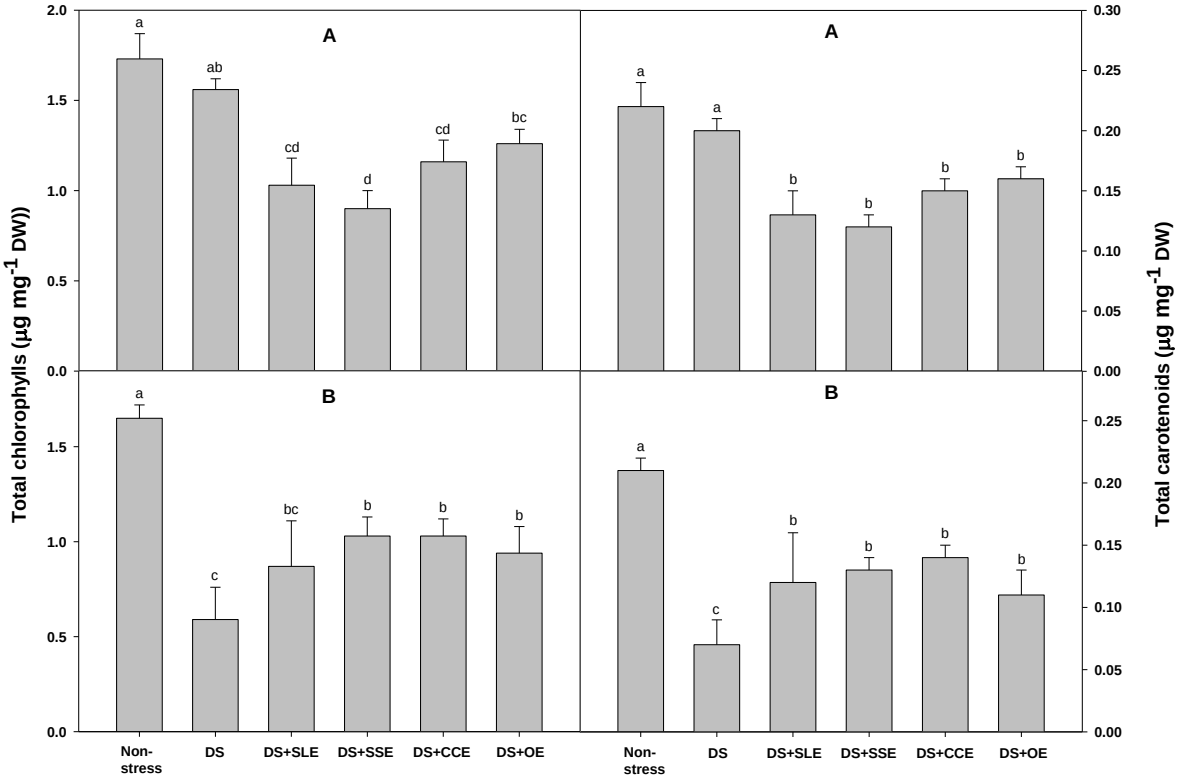

Figure 4. Effects of selected plant extracts at 3% concentrations on total chlorophyll and carotenoid contents at 4 (A) and 6 (B) days after treatments under drought stress (DS, Drought stress; DS+SLE, Drought stress+Soybean leaf extract; DS+SSE, Drought+Soybean stem extract; DS+CCE, Drought stress+Chinese chive extract; DS+OE, Drought stress+Onion extract). Means with different letters are significantly different by Duncan's Multiple Range Test at 5% level. Means expressed error bar (±SE) with three replicates.

The chlorophyll and carotenoid contents of the plants under salt stress were measured after treatment with the selected plant extracts (Figure 5). The chlorophyll content after 4 days of salt stress was higher in all the treated plants when compared with the control plants. When compared with the control plants, the chlorophyll content after 6 days of salt stress was also higher in all the treated plants except for those treated with soybean leaf extract. The carotenoid content after 4 days of salt stress was significantly higher in all the treated plants except for those treated with *Gracilaria verrucose* extract. However, there were no significant differences between all the treated plants and the control plants after 6 days of salt stress. Overall, the chlorophyll and carotenoid contents varied based on the types and levels of stress that the plants were subjected to. Another similar study showed that the degree to which chlorophyll content decreases varies with the duration and severity of drought [63]. Among others, a major cause for the decline in the amount of chlorophyll due to drought stress is the drought-promoted O_2^- and H_2O_2 production, which results in lipid peroxidation and ultimately chlorophyll degradation [64]. In another plant extract study, seaweed extract treatments produced an increase in chlorophyll content in the treated plants. This effect has been observed in a wide range of crops, including grapevine and strawberry [65–70].

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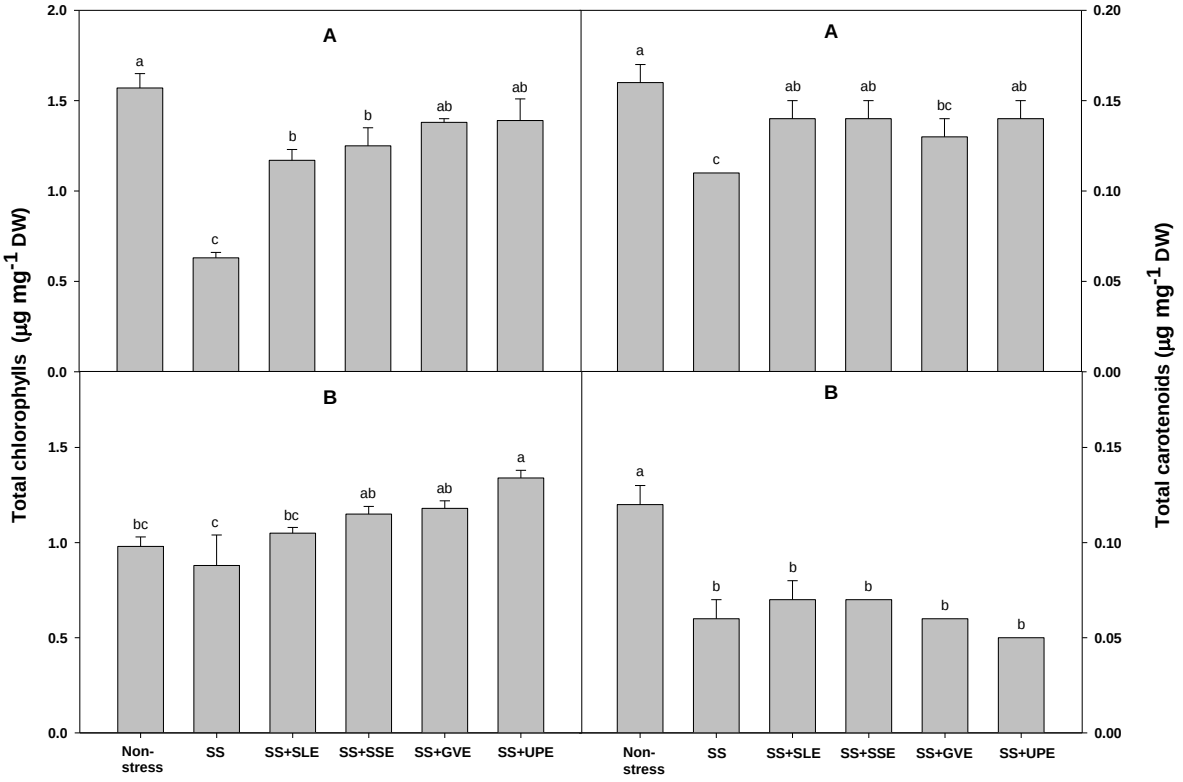

Figure 5. Effects of selected plant extracts at 1% concentrations on total chlorophyll and carotenoid contents at 4 (A) and 6 (B) days after treatments under salt stress (SS, Salt stress; SS+SLE, Salt stress+Soybean leaf extract; SS+SSE, Salt stress+Soybean stem extract; SS+GVE, Salt stress+*G. verrucosa* extract; SS+UPE, Salt stress+*U. pinnatifida* extract). Means with different letters are significantly different by Duncan's Multiple Range Test at 5% level. Means expressed error bar (±SE) with three replicates.

3.4. Effects of Selected Plant Extracts on Reactive Oxygen Species and Lipid Peroxidation Under Drought and Salt Stress

Excess amounts of reactive oxygen species (ROS) such as O_2^-, and H_2O_2 are produced during times of drought stress due to the adverse effects of the photo-respiratory pathways [71]. ROS are highly toxic radicals that damage various cellular components such as proteins and membranes and disrupt cellular processes like lipid peroxidation, ultimately leading to cellular death [71]. Hence, we measured the O_2^- and H_2O_2 levels to confirm the effectiveness of treatment with plant extracts given to rice plants that were subjected to drought stress (Figure 6). The O_2^- levels after 4 days of drought stress were lower in plants treated with soybean stem and onion extracts when compared with the control plants. However, the O_2^-content did not vary significantly between the control plants and those treated with extracts of soybean leaf and Chinese chive. After 6 days of drought stress, the O_2^- content was lower in all the treated plants when compared with the control plants. Similar to studies measuring the O_2^- levels, the H_2O_2 levels after 4 days of drought stress were also lower in the plants treated with soybean stem and onion extracts when compared with the control plants. However, there was no significant difference in the H_2O_2 levels between the control plants and the plants treated with extracts from soybean leaf and Chinese chive. After 6 days of drought stress, the H_2O_2 levels were lower in all the treated plants compared to the control plants.

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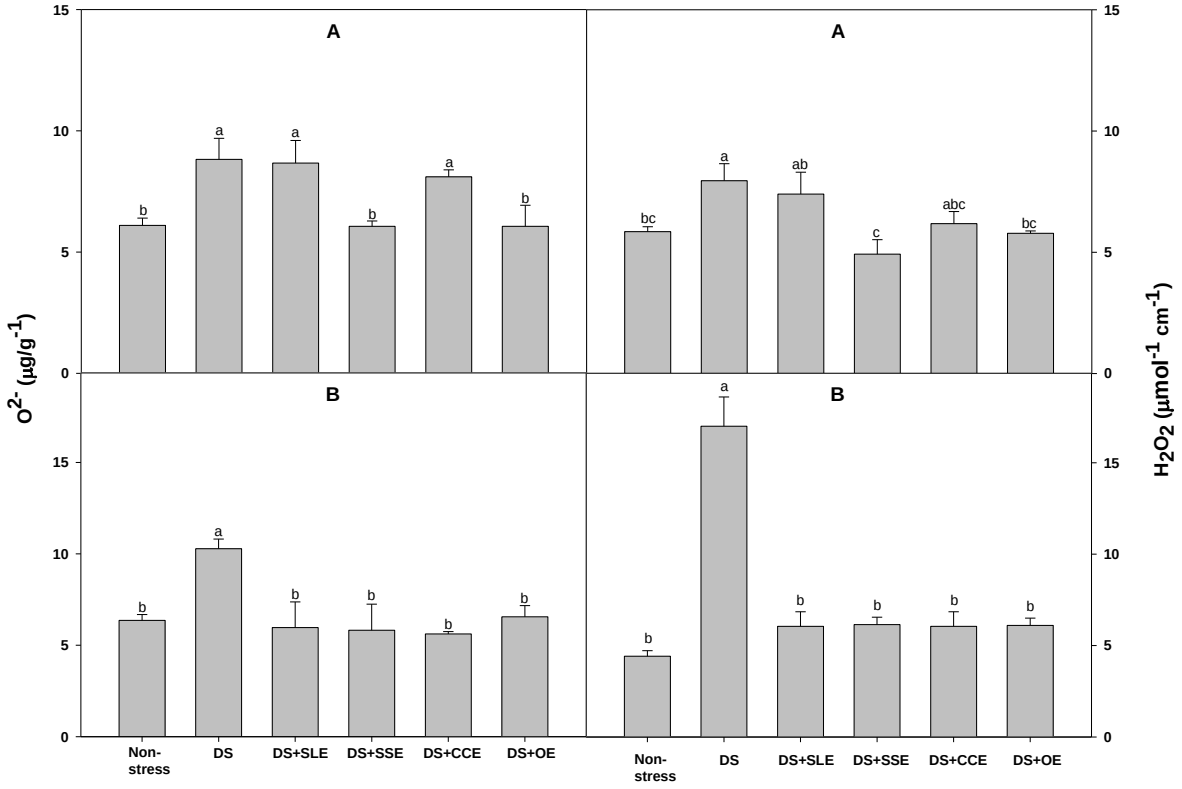

Figure 6. Effects of selected plant extracts at 3% concentrations on O_2^- and H_2O_2 contents at 4 (A) and 6 (B) days after treatments under drought stress (DS, Drought stress; DS+SLE, Drought stress+Soybean leaf extract; DS+SSE, Drought+Soybean stem extract; DS+CCE, Drought stress+Chinese chive extract; DS+OE, Drought stress+Onion extract). Means with different letters are significantly different by Duncan's Multiple Range Test at 5% level. Means expressed error bar (±SE) with three replicates.

$O_2^-$and H_2O_2 levels were also determined under salt stress (Figure 7). The levels of O_2^- of all the treated plants and control plants after 4 days of salt stress were similar. At 6 days of salt stress, the levels of O_2^- were lower only in the plants treated with the *Undaria pinnatifida* extract. Plants treated with the extracts of soybean stem and *Gracilaria verrucose* had a lower H_2O_2 level after both 4 and 6 days of salt stress when compared with the control plants.

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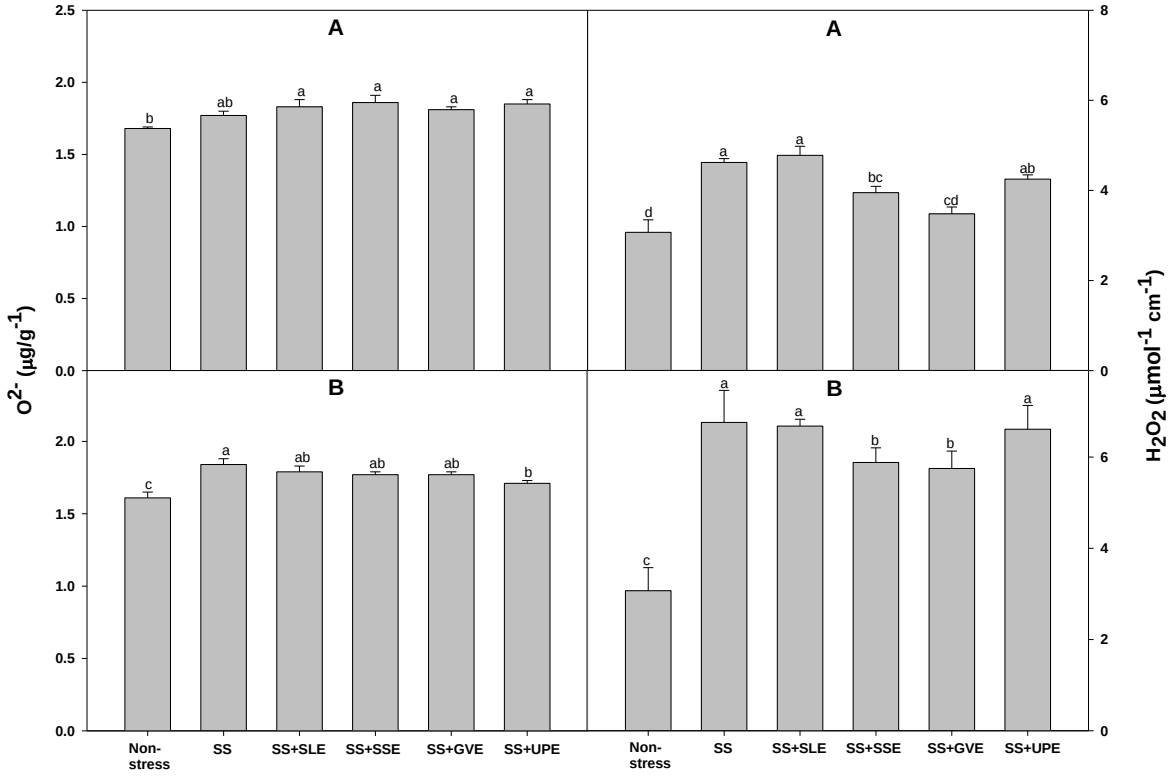

Figure 7. Effects of selected plant extracts at 1% concentrations on O_2^- and H_2O_2 contents at 4 (A) and 6 (B) days after treatments under salt stress (SS, Salt stress; SS+SLE, Salt stress+Soybean leaf extract; SS+SSE, Salt stress+Soybean stem extract; SS+GVE, Salt stress+*G. verrucosa* extract; SS+UPE, Salt stress+*U. pinnatifida* extract). Means with different letters are significantly different by Duncan's Multiple Range Test at 5% level. Means expressed error bar (±SE) with three replicates.

Lipid peroxidation is measured as the amount of MDA produced when polyunsaturated fatty acids in the membrane undergo oxidative degradation due to the accumulation of reactive oxygen species [72]. We determined the amount of MDA after drought stress (Figure 8). The amount of MDA after 4 days of drought stress was significantly lower in all the treated plants when compared with the control plants. However, after 6 days of drought stress, the MDA levels were much higher in all the treated plants than it was after 4 days of drought stress. Additionally, the MDA levels after 6 days of drought stress were lower in the plants treated with soybean leaf, soybean stem, and onion extracts. The levels of MDA after 4 days of salt stress were lower in the plants treated with soybean leaf and soybean stem extracts than in the control plants . However, the levels of MDA after 6 days of salt stress were lower in all the treated plants when compared with the control plants.

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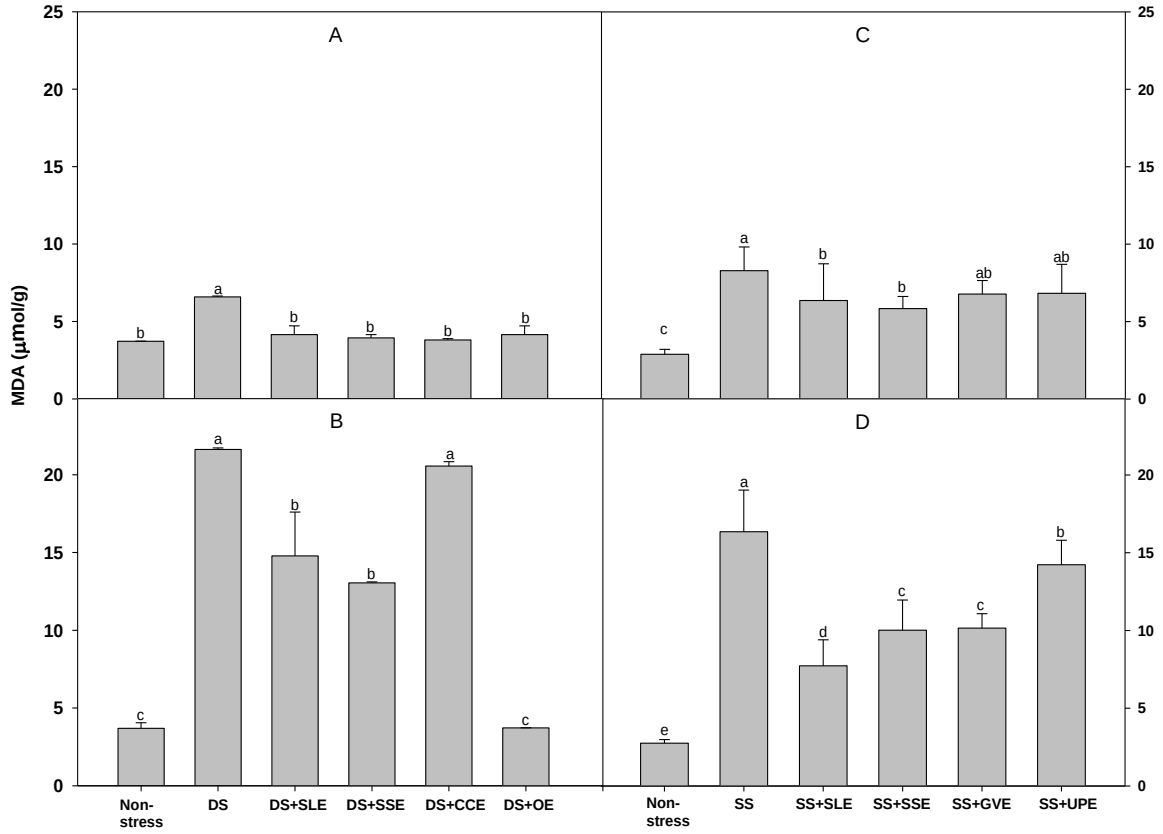

Figure 8. Effects of selected extracts on MDA production at 4 (A and C) and 6 (B and D) days after treatments under a 3% concentration of drought stress (A and B) or 1% concentration of salt stress (C and D). (DS, Drought stress; DS+SLE, Drought stress+Soybean leaf extract; DS+SSE, Drought+Soybean stem extract; DS+CCE, Drought stress+Chinese chive extract; DS+OE, Drought stress+Onion extract; SS, Salt stress; SS+SLE, Salt stress+Soybean leaf extract; SS+SSE, Salt stress+Soybean stem extract; SS+GVE, Salt stress+*G. verrucosa* extract; SS+UPE, Salt stress+*U. pinnatifida* extract). Means with different letters are significantly different by Duncan's Multiple Range Test at 5% level. Means expressed error bar (±SE) with three replicates.

In an earlier study, the MDA levels in rice seedlings increased significantly with increasing stress levels [73]. Rice and rapeseed seedlings under stress conditions also showed significant increases in H_2O_2 and MDA content [73,74]. In addition, Saruhan et al. [75] reported that drought stress increased the MDA content in two maize genotypes. However, a foliar spray with garlic extract significantly reduced the H_2O_2 and MDA levels in the shoots of soybean plants under drought stress when compared with the control plants. In another study, *Lessonia nigrescens* treatments decreased lipid peroxidation and alleviated the salt-induced loss of chlorophyll content [76].

3.5. Effects of Selected Plant Extracts on Proline and Sugar Accumulation Under Drought and Salt Stress

Proline accumulation is a well-known metabolic response of plants to drought and other stresses [77]. Hence, to confirm the effectiveness of the plant extract treatments on rice plants that experienced drought stress, we measured the levels of proline (Figure 9).

Compared to the control plants, the levels of proline content after 4 days of drought stress were significantly lower in all the treated plants except for those treated with onion extract. However, after 6 days of drought stress, the proline levels were significantly lower in all the treated plants compared with the control plants.

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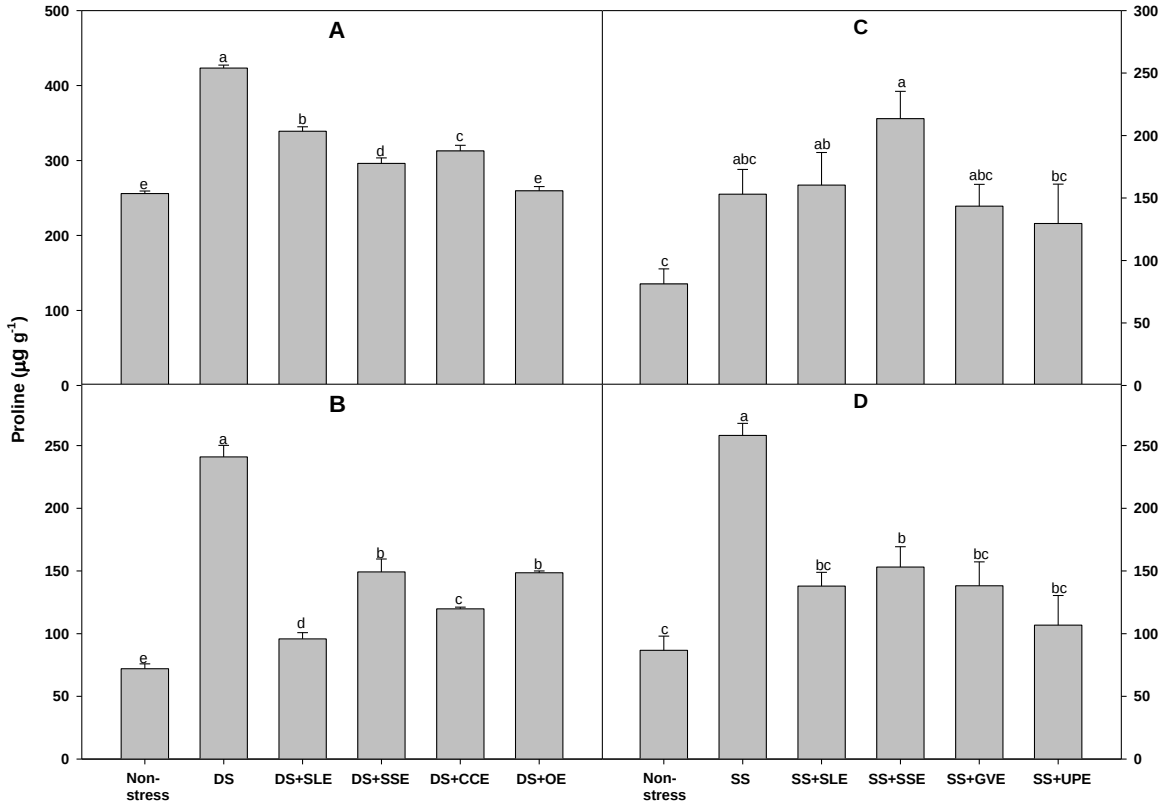

Figure 9. Effects of selected extracts on proline contents at 4 (A and C) and 6 (B and D) days after treatments under a 3% concentration of drought stress (A and B) or 1% concentration of salt stress (C and D). (DS, Drought stress; DS+SLE, Drought stress+Soybean leaf extract; DS+SSE, Drought+Soybean stem extract; DS+CCE, Drought stress+Chinese chive extract; DS+OE, Drought stress+Onion extract; SS, Salt stress; SS+SLE, Salt stress+Soybean leaf extract; SS+SSE, Salt stress+Soybean stem extract; SS+GVE, Salt stress+*G. verrucosa* extract; SS+UPE, Salt stress+*U. pinnatifida* extract). Means with different letters are significantly different by Duncan's Multiple Range Test at 5% level. Means expressed error bar (±SE) with three replicates.

There was no significant difference in the proline levels after 4 days of salt stress between the treated plants and the control plants. However, after 6 days of salt stress, the proline levels were significantly higher in all the treated plants compared to those of the control plants. Based on the results of this study, we believe that the accumulation of proline is a key indicator of the response of rice plants to drought and salt stress. Some studies have shown that proline accumulation is generally an indicator of stress and is associated with stress susceptibility [78]. In an earlier study, proline was found to accumulate in drought-susceptible potato genotypes but not in drought-resistant potato genotypes [79,80]. Additionally, earlier studies have shown that the proline concentration in rice increased significantly during drought and salt stress [81,82]. However, Bing-Sheng et al. [83] have suggested that proline accumulation is not correlated with salt, alkaline, and osmotic stresses in rice.

Soluble sugars (sucrose, glucose, and fructose) play an important role in maintaining the overall structure and growth of plants [84]. Hence, we measured the levels of soluble sugars in plants treated with the extracts and the control plants under drought stress (Figure 10). The soluble sugar content after 4 days of drought stress was significantly lower only in the plants treated with the onion extract when compared with the control plants. However, after 6 days of drought stress, the soluble sugar content was significantly lower in all the treated plants compared with that in the control plants.

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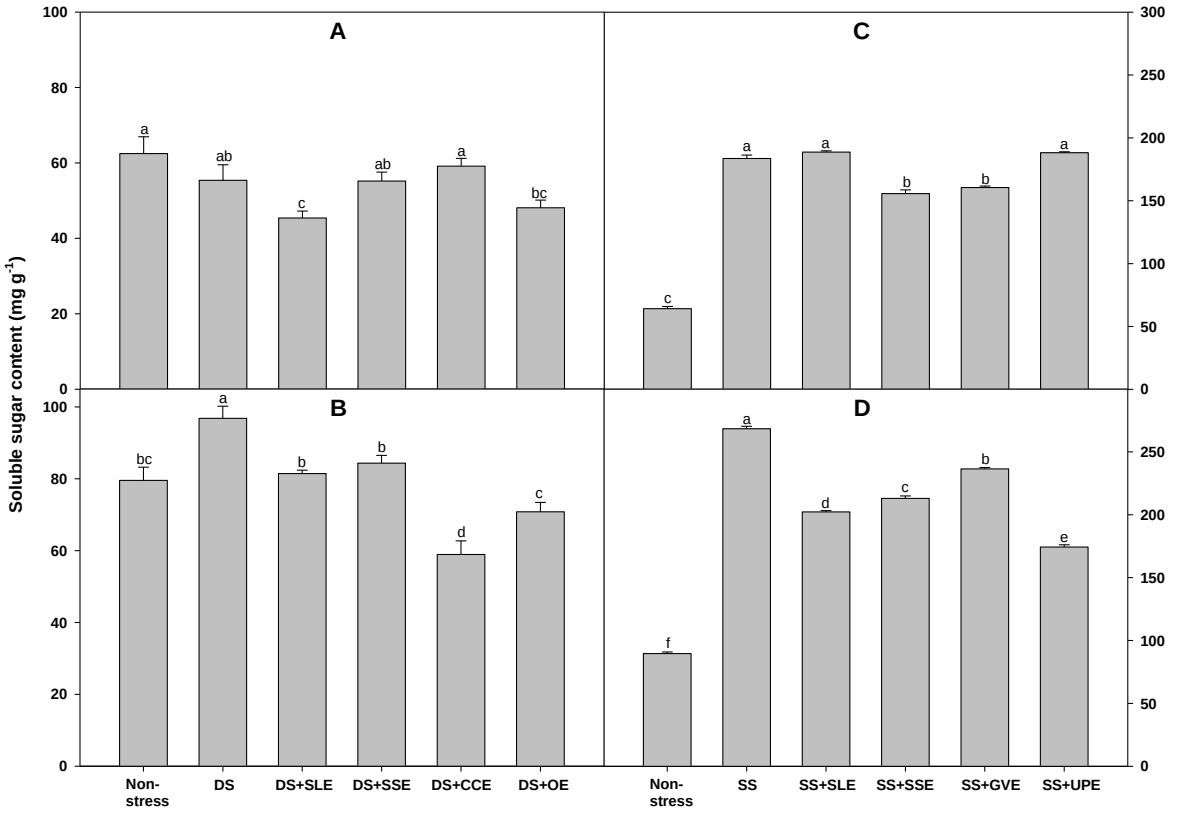

Figure 10. Effects of selected extracts on soluble sugar contents at 4 (A and C) and 6 (B and D) days after treatments under a 3% concentration of drought stress (A and B) or 1% concentration of salt stress (C and D). (DS, Drought stress; DS+SLE, Drought stress+Soybean leaf extract; DS+SSE, Drought+Soybean stem extract; DS+CCE, Drought stress+Chinese chive extract; DS+OE, Drought stress+Onion extract; SS, Salt stress; SS+SLE, Salt stress+Soybean leaf extract; SS+SSE, Salt stress+Soybean stem extract; SS+GVE, Salt stress+*G. verrucosa* extract; SS+UPE, Salt stress+*U. pinnatifida* extract). Means with different letters are significantly different by Duncan's Multiple Range Test at 5% level. Means expressed error bar (±SE) with three replicates.

The soluble sugar content after 4 days of salt stress was significantly lower in plants treated with soybean stem and the *Gracilaria verrucosa* extracts than in the control plants. However, after 6 days of drought stress, the soluble sugar content was significantly lower in all the treated plants when compared with that in the control plants.

Soluble sugars maintain the leaf water content and ensure the osmotic adjustments of plants facing drought stress conditions [85,86]. Xu et al. [87] found that drought stress conditions significantly increased the soluble sugar concentrations in the roots and leaves of susceptible rice varieties but not in the resistant ones. In this study, the soluble sugar content also increased with increasing drought and salt stress levels. In addition, the soluble sugar content in the shoots of soybean plants significantly increased under drought stress. These results are consistent with those of Abass and Mohamed [88] who reported that drought conditions caused a significant increase in the proline and soluble sugar content in the shoots of common bean plants. Our study also showed increased proline and soluble sugar content in rice plants under drought and salt stress. In addition, in this study, the proline and soluble sugar accumulation content was lower in the plants treated with extracts than in the control plants.

4. Conclusions

Out of the eleven extracts used, we selected four plant extracts (soybean leaf, soybean stem, Chinese chive, and onion) which effectively reduced drought stress. Compared to control plants,

plants that received extract treatments showed a 41-54% reduction in drought stress. In addition, soybean leaf, soybean stem, moringa, and *Undaria pinnatifida* extracts reduced salt stress by 20-40% compared to the control. Although the effectiveness of the extracts varied depending on the types and levels of stress and the extract concentrations, the overall levels of effectiveness were similar across all the plant extracts used. Generally, most parameters such as water content, photosynthetic efficiency (Fv/Fm and ETR), and pigments (chlorophyll and carotenoid) were higher in the plants treated with extracts when compared with the control plants. However, the levels of reactive oxygen species, MDA, proline, and soluble sugars were lower in the plants treated with extracts than in the control plants. Thus, the selected plant extracts can be used to alleviate the adverse effects of drought and salt stress. In addition, the application of selected plant extracts could be beneficial for sustainable production, due to several advantages, such as low toxicity to humans and the environment, enhanced resistance of cultivated plants to abiotic stress, as well as the reduction in the use of mineral fertilizers and pesticides. Nevertheless, the substantial initial costs and challenges in scaling up have posed significant hurdles for food technologists and biochemists.

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