

THE POTENTIAL OF *Enhalus acoroides* AS A BIOSTIMULANT TO ENHANCE MAIZE GROWTH AND DROUGHT TOLERANCE

Made Pharmawati^{1*}, Luh Putu Wrasiaty², I Made Anom Sutrisna Wijaya³ and Kadambot H.M. Siddique⁴

¹Biology Study Program, Faculty of Mathematics and Natural Sciences, Udayana University, Jimbaran 80361, Bali, Indonesia

²Agroindustrial Technology Study Program, Faculty of Agricultural Technology, Udayana University, Jimbaran, 80361, Bali, Indonesia

³Agricultural Engineering and Biosystems Study Program, Udayana University, Jimbaran, 80361, Bali, Indonesia

⁴The UWA Institute of Agriculture, University of Western Australia, WA 6001 Perth, Australia

HIGHLIGHTS

- Biostimulant Potential of *Enhalus acoroides*
- Positive Effects of *E. acoroides* on Maize Growth
- Reduction in H₂O₂ (Oxidative Stress) by *Enhalus acoroides* extract

ABSTRACT

Drought poses a significant challenge to crop productivity, with maize particularly vulnerable. Enhancing maize tolerance to drought stress is crucial, and one promising approach involves using biostimulants derived from natural sources. The seagrass *Enhalus acoroides* is a potential biostimulant due to its rich phytochemical composition, including phenols, tannins, flavonoids, and pigments, such as chlorophyll, lutein, pheophytin, and beta-carotene. These compounds exhibit antioxidant activity, suggesting their potential role in enhancing plant resilience to drought stress. This study evaluated the effects of *E. acoroides* extract on maize growth under drought conditions during the seedling phase and analyzed biochemical changes in maize plants treated with the extract. The extract was prepared using 10% dried *E. acoroides* leaves with a chloroform-to-ethanol solvent ratio of 9 : 1 (v/v) and subsequently dissolved in distilled water for final concentrations of 0.05%, 0.1%, 0.15%, 0.2%, and 0.25%. The results demonstrated that *E. acoroides* extract enhanced plant height, increased shoot and root fresh and dry weights. Additionally, plants sprayed with *E. acoroides* extract exhibited higher total sugars and protein content in the shoots as compared to non-sprayed plants. Under 20% polyethylene glycol (PEG)-induced drought stress, control plants showed severe leaf wilting, whereas extract-treated plants only had mild wilting. The chlorophyll, reducing sugars, total N, and tocopherol contents were also higher in extract-treated plants under PEG stress than in untreated controls. These findings indicate the potential of *E. acoroides* extract as a biostimulant for improving drought tolerance in maize.

Article Information

Received : 10 March 2025

Revised : 8 April 2025

Accepted : 8 April 2025

*Corresponding author, e-mail:

made_pharmawati@unud.ac.id

Reviewers:

Prof Ketut Budaraga & Anonymous

Keywords: antioxidant, drought stress, maize

INTRODUCTION

Maize (*Zea mays* L.) is a vital cereal crop belonging to the Poaceae family, serving as a major source of nutrients and phytochemicals with significant health benefits (Shah *et al.* 2016). In regions such as Africa, Latin America, and Asia, maize contributes more than 20% of the daily caloric intake (Shiferaw *et al.* 2011). Compared to wheat and rice, maize is highly versatile, with applications in food, animal feed, industrial processing, and bioenergy production (Grote *et al.* 2021). Maintaining stable maize production is essential, given its diverse roles in agricultural and food systems.

In Indonesia, maize production has fluctuated, with dried maize kernel production (14% moisture content) reaching 14.77 million tonnes in 2023, representing a 10.61% decline from 16.53 million tonnes in 2022 (Badan Pusat Statistik 2023). Climate change, particularly prolonged drought periods linked to global warming, is a major factor affecting maize productivity (Herlina & Prasetyorini 2020). Drought stress significantly hampers maize growth, primarily due to inadequate water availability in the root zone and excessive transpiration from leaves, where water loss outpaces absorption (Nieves-Cordones *et al.* 2019). Physiologically, drought stress disrupts cell division and differentiation, reduces turgor pressure, alters enzymatic activity, impairs photosynthetic energy production, and leads to the accumulation of reactive oxygen species (ROS) (Jothimani & Arulbalachandran 2020). The seedling and flowering stages are particularly sensitive to drought stress.

Research has shown that photosynthesis rates in maize significantly decrease when soil moisture levels drop to 50% of field capacity. Furthermore, soil moisture measurements have indicated that levels close to the wilting threshold point (22.2% soil moisture) can severely stress the plant (Shao *et al.* 2021).

Sustainable agricultural technologies are needed to mitigate the adverse effects of drought. Biostimulants, organic substances applied to plants, seeds, or soil, have emerged as a promising solution to enhance nutrient efficiency, improve stress tolerance, and promote plant growth (Bulgari 2019; Melo *et al.* 2020). Biostimulants are derived from various sources, including microorganisms, such as bacteria, yeasts, and fungi, either as live organisms or as metabolites (Rouphael 2020).

Additionally, plant-derived biostimulants can be extracted from different plant parts, such as seeds, leaves, and roots, across various botanical families, including Amaryllidaceae, Brassicaceae, Ericaceae, Fabaceae, Fagaceae, Moringaceae, Plantaginaceae, Poaceae, Rosaceae, Solanaceae, Theaceae, and Vitaceae (Yakhin *et al.* 2017).

Marine-derived biostimulants, particularly macroalgae extracts, have also shown promising results. For example, water extracts from *Ulva rigida* enhance wheat growth when applied as a foliar spray (Latique *et al.* 2021). Likewise, extracts from *Ascophyllum nodosum* improve drought tolerance by increasing proline levels, a key non-enzymatic antioxidant (Rasul *et al.* 2021). Polyphenols, fucoidans, and alginates found in macroalgae exhibit strong antioxidant activity, which helps mitigate ROS damage under drought conditions (Wang *et al.* 2020). Using biostimulants also promotes sustainable agriculture by enhancing crop productivity while reducing reliance on chemical fertilizers (Xu & Geelen 2018).

Despite the growing interest in plant and algal biostimulants, seagrasses remain an underexplored resource. Seagrasses are marine flowering plants that contribute to primary productivity, carbon sequestration, and habitat formation for marine life (Jalaludin *et al.* 2020). Among them, *Enhalus acoroides* is widely distributed in Indonesia and is characterized by its long leaf blades. Seagrasses thrive in intertidal zones, providing ecological benefits, such as carbon sequestration and nutrient cycling, which help mitigate the effects of global warming (Stankovic *et al.* 2021).

A recent study highlighted the bioactive potential of *E. acoroides*. Extracts obtained from its leaves using a chloroform-to-ethanol solvent (9 :1) contain phenolic compounds, tannins, flavonoids, and pigments such as chlorophyll, lutein, pheophytin, and beta-carotene (Pharmawati & Wrasati 2020). These compounds possess strong antioxidant properties, suggesting their possible role in plant stress tolerance. The potential of *E. acoroides* extract as a biostimulant for drought tolerance in maize remains unexplored.

This study assessed the effects of *E. acoroides* extract on maize growth under drought stress during the seedling phase and analyzed biochemical changes induced by extract application, providing insights into its potential role as a drought-tolerance biostimulant.

MATERIALS AND METHODS

Sample Collection and Extraction

Leaves of *E. acoroides* were collected from Semawang Beach, Bali. They were washed, air-dried, and then oven-dried at 50 °C for 24 hours. The dried leaves were ground and sieved through a 60-mesh sieve. A 20 g sample of the powdered leaves was extracted using a Soxhlet extractor (Pyrex®) with 200 mL of chloroform : ethanol (9 : 1) for 3 hours. The extract was filtered, and the filtrate was evaporated using a rotary evaporator (IKA®RV10). Soxhlet extraction was performed following the method of Abubakar and Haque (2020) with modifications, using chloroform : ethanol as the solvent, as chloroform extracts from various plant species have been reported to exhibit significant antioxidant activities (Uddin *et al.* 2015; Syamkumar *et al.* 2023). The resulting crude extract (100%) was diluted in distilled water to prepare concentrations of 0.05%, 0.1%, 0.15%, 0.2%, and 0.25%. The extracts were stored in a refrigerator until use.

The effectiveness of *E. acoroides* extract was evaluated in two separate experiments. The first experiment assessed its effect on maize growth at the vegetative stage, while the second examined its potential as a drought mitigation agent. Both experiments were conducted in the greenhouse of the Biology Study Program, Faculty of Mathematics and Natural Sciences, Udayana University, Bali, Indonesia from June to September 2024.

Experiment 1: *Enhalus acoroides* Treatment

Commercial hybrid maize seeds (Pertiwi variety) were purchased from an agricultural store in Denpasar, Bali. The seeds were sterilized by soaking in 10% sodium hypochlorite for 10 minutes, then rinsed with water and soaked for 12 hours. Germination was carried out in trays containing rockwool media and commercial hydroponic A and B liquid nutrients. After 7 days, uniform seedlings were selected and transferred to a hydroponic system with perlite media and AB mix nutrients for an additional 7-day acclimatization period.

Following acclimatization, seedlings were sprayed with *E. acoroides* extract at the specified concentrations (5 mL per plant), while control plants received distilled water. Treatments were applied twice weekly for four weeks. At the end of the experiment, plants were harvested. The experiment was conducted using a randomized block design with four replications.

Data Collection

Plant height was measured at harvest (four weeks after the first *E. acoroides* treatment). Fresh and dry weights of both aboveground and belowground plant parts were recorded. Reducing sugars content was determined by preparing a glucose standard curve using 0, 20, 40, 60, 80, and 100 ppm anhydrous glucose. For each concentration, 1 mL of glucose was mixed with 1 mL of Nelson's reagent, heated for 10 minutes, cooled, and treated with 1 mL of arsenomolybdate solution. After adding 7 mL of distilled water, absorbance was measured at 540 nm. For plant samples, 1 g of tissue was dissolved in 100 mL of distilled water, filtered, and processed similarly. Reducing sugars content was calculated based on Apriyantono *et al.* (1989).

Nitrogen content was measured using the Kjeldahl method. A 0.1 g sample was hydrolyzed with 0.5 g of Kjeldahl tablet and 5 mL of H₂SO₄ in a heat block. After cooling, 25 mL of distilled water, 25 mL of 50% NaOH, and three drops of phenolphthalein were added. The mixture was distilled, and the distillate was collected in 10 mL of 3% boric acid. Titration was performed using 0.1 N HCl until the solution changed from blue to light yellow. The volume of HCl used was recorded, and nitrogen content was calculated following Sudarmadji *et al.* (1989).

Experiment 2: PEG treatment

Two weeks after acclimatization, seedlings were sprayed with 5 mL of *E. acoroides* extract at concentrations of 0.05%, 0.1%, 0.15%, 0.2%, and 0.25%. Treatments were applied twice weekly for three weeks. Subsequently, the nutrient solution was replaced with a 20% PEG6000 solution to induce drought stress, while spraying with *E. acoroides* extract continued twice weekly for an additional week.

Data Collection

At the end of the experiment, plants were harvested, and chlorophyll content was analyzed using spectrophotometry. Leaves were ground in 100% acetone, and chlorophyll *a*, chlorophyll *b*, and total chlorophyll concentrations were measured at 647 nm and 664 nm, following the method of Lichtenthaler & Buschmann (1987).

Reducing sugars and total protein contents of the shoots were analyzed using previously described

methods. Tocopherol content was determined using the method described by Wong *et al.* (1988). A 0.5 g sample was dissolved in 5 mL of toluene and filtered. To 2 mL of the filtrate, 0.5 mL of toluene, 1.75 mL of 2,2-bipyridine (0.07% w/v in 95% ethanol), and 0.25 mL of FeCl₃·6H₂O (0.2% w/v in 96% ethanol) were added. Ethanol was added to bring the total volume to 5 mL. Absorbance was measured at 520 nm using a spectrophotometer. A tocopherol standard curve was prepared using concentrations ranging from 100 ppm to 1,500 ppm in toluene.

Antioxidant activity (IC₅₀) was determined following the method of Amin & Lee (2005). A stock solution of 4 g 1,1-Diphenyl-2-picrylhydrazyl (DPPH) in 100 mL methanol was prepared. A 1 mL aliquot was mixed with 1 mL of ethanol and incubated for 30 minutes, after which absorbance was measured at 517 nm. For plant extracts, a 0.03 g sample was dissolved in 5 mL of methanol. Serial dilutions (10, 20, 30, 40, and 50 µL) were prepared in test tubes and adjusted to 1,000 µL with methanol. Each solution was mixed with 1,000 µL of DPPH, vortexed, and incubated for 30 minutes. Absorbance was measured at 517 nm, and the inhibition percentage was calculated as described by Amin & Lee (2005).

In vivo detection of H₂O₂ in roots was performed using histochemical 3,3'-diaminobenzidine (DAB) staining, following Daudi & O'Brien (2012). Roots were immersed in a 1 mg/mL DAB solution (pH 3.8) and incubated under light at 25 °C for 8

hours before decolorization by immersion in 96% ethanol. The presence of H₂O₂ was indicated by deep brown coloration resulting from the reaction of DAB with H₂O₂ catalyzed by plant peroxidases.

Data Analysis

All data were statistically analyzed using analysis of variance (ANOVA) in MINITAB 20. Mean differences were evaluated using Tukey's comparison test at a significance level of P < 0.05.

RESULTS AND DISCUSSION

Spraying with *E. acoroides* extract promoted significant increases in maize plant height. As shown in Figure 1, differences in plant appearance are evident between control plants and those treated with various extract concentrations after four weeks of application (at 5 weeks old).

Quantitative analysis revealed that *E. acoroides* extract significantly influenced plant height difference (P < 0.01). Heights were significantly greater (P < 0.05) in plants treated with 0.1%, 0.15%, 0.2%, and 0.25% compared to the control, with the 0.15% concentration producing the tallest plants (Fig. 2).

Foliar application of *E. acoroides* extract also significantly increased shoot and root fresh and dry weights. Figure 3 shows that fresh shoot weight was higher in plants treated with as little as 0.05% extract, while fresh root weight significantly increased at 0.15%, 0.2%, and 0.25%



Figure 1 Visual comparison of five-week-old maize plants after four weeks of treatment with varying concentrations of *E. acoroides* extract

Notes: Treatments: a = Control (no spray); b = 0.05%; c = 0.1%; d = 0.15%; e = 0.2%; and f = 0.25% extract. Bar = 10 cm

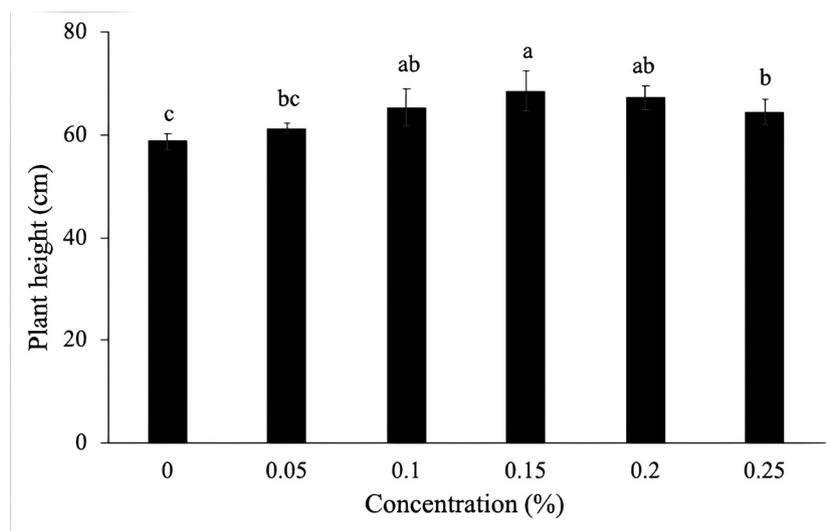


Figure 2 Plant height of five-week-old maize plants after four weeks of treatment with varying concentrations of *E. acoroides* extract

Notes: Different letters above the bars indicate significant differences among treatments ($P < 0.05$)

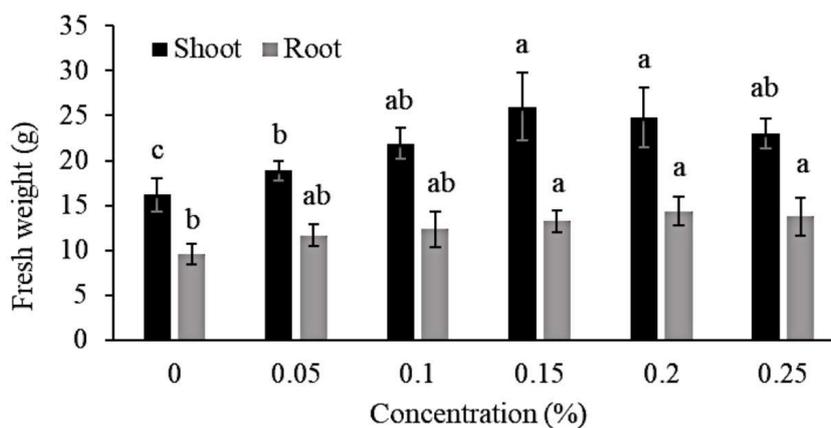


Figure 3 Shoot and root fresh weights of five-week-old maize plants after four weeks of treatment with varying concentrations of *E. acoroides* extract

Notes: Different letters above the bars indicate significant differences among treatments ($P < 0.05$)

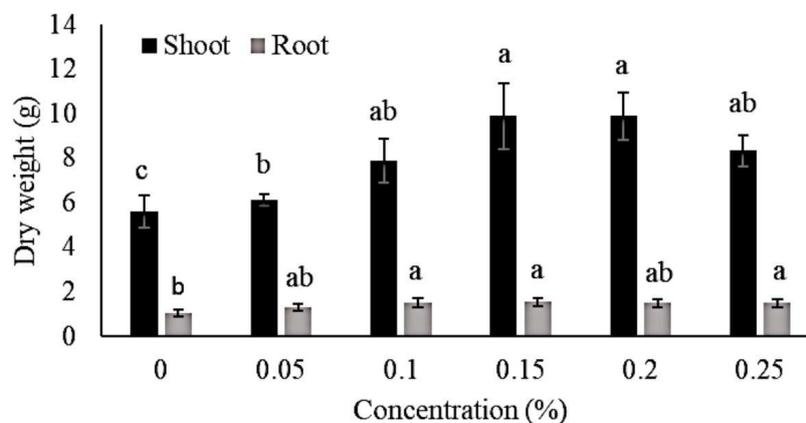


Figure 4 Shoot and root dry weights of five-week-old maize plants after four weeks of treatment with varying concentrations of *E. acoroides* extract

Notes: Different letters above the bars indicate significant differences among treatments ($P < 0.05$)

Table 1 Reducing sugars content and total N content in five-week-old maize shoots after four weeks of treatment with varying concentrations of *E. acoroides* extract

Concentration (%)	Reducing sugars (%)	N (%)
0	14.40 ± 0.386 ^b	15.86 ± 0.180 ^c
0.05	15.14 ± 0.239 ^{ab}	16.37 ± 0.259 ^{bc}
0.1	15.65 ± 0.307 ^a	17.00 ± 0.233 ^{ab}
0.15	15.75 ± 0.269 ^a	17.82 ± 0.612 ^a
0.2	15.78 ± 0.164 ^a	17.45 ± 0.476 ^{ab}
0.25	15.55 ± 0.239 ^a	16.78 ± 0.228 ^b

Notes: Data presented as mean ± standard deviation. Different letters in the same column indicate significant differences ($P < 0.05$).

concentrations ($P < 0.05$). A similar trend was observed for dry shoot weight (Fig. 4), while dry root weight was significantly greater in plants treated with 0.1%, 0.15%, and 0.25% extract compared to dry root weight in control plants.

The observed improvements on plant height and biomass may be attributed to bioactive compounds and nutrients in the seagrass extract. Like seaweed extracts, *E. acoroides* likely promotes growth by releasing plant growth hormones. Previous studies have shown that extracts from various seaweed species contain auxins, cytokinins, and abscisic acid (Yalçın *et al.* 2019) and have even been shown to upregulate genes involved in auxin biosynthesis in *Beta vulgaris*, thereby enhancing lateral root development and nutrient uptake (Bertoldo *et al.* 2023).

Table 1 summarizes the effects of *E. acoroides* extract on reducing sugars and total N content in maize shoots. Both parameters began to increase at the 0.1% extract concentration, indicating enhanced metabolic activity and nutrient assimilation in treated plants. Reducing sugars are key products of photosynthesis and serve as the primary energy source for plant cells. Glucose has been reported to influence the expression of genes that promote the activity of nitrate reductase, a key enzyme in the nitrogen assimilation pathway (Ma *et al.* 2023). Increased nitrogen availability supports the synthesis of enzymes involved in carbohydrate metabolism, thereby promoting sugar utilization and energy generation (Zhang *et al.* 2021).



Figure 5 Visual comparison of six-week-old maize plants sprayed after three weeks of twice-weekly treatment with varying concentrations of *E. acoroides* extract followed by one week of PEG-induced drought stress

Notes: a = Control (no extract, no PEG); b = PEG only; c = 0.05% extract+PEG; d = 0.1% extract+PEG; e = 0.15% extract+PEG; f = 0.2% extract+PEG; g = 0.25% extract+PEG. PEG = Polyethylene glycol. Bar = 10 cm.

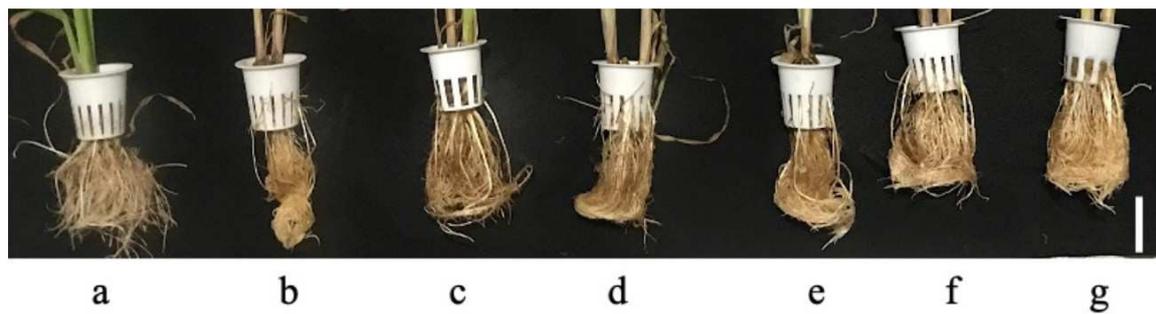


Figure 6 Maize roots of six-week-old maize plants sprayed after three weeks of twice-weekly treatment with varying concentrations of *E. acoroides* extract followed by one week of PEG-induced drought stress

Notes: a = Control (no extract, no PEG); b = PEG only; c = 0.05% extract+PEG; d = 0.1% extract+PEG; e = 0.15% extract+PEG; f = 0.2% extract+PEG; g = 0.25% extract+PEG. PEG = Polyethylene glycol. Bar = 5 cm



Figure 7 H_2O_2 accumulation in roots and leaves of six-week-old maize plants sprayed after three weeks of twice-weekly treatment with varying concentrations of *E. acoroides* extract followed by one week of PEG-induced drought stress

Notes: a = Control (no extract, no PEG); b = PEG only; c = 0.05% extract+PEG; d = 0.1% extract+PEG; e = 0.15% extract+PEG; f = 0.2% extract+PEG; g = 0.25% extract+PEG. PEG = Polyethylene glycol. Bar = 2 cm

The increase in reducing sugars and nitrogen content is comparable to effects observed with seaweed extracts. For example, an aqueous extract of *Sargassum johnstonii* increased reducing sugars levels in *Lycopersicon esculentum* when applied at concentrations above 0.1% (Kumari *et al.* 2011). Although research on seagrass-derived

biostimulants is limited, seaweed extracts have been studied extensively and are known to enhance seed germination, biomass yield, and overall plant quality (Ali *et al.* 2016; Kumar *et al.* 2020). The phenolic compounds, tannins, and flavonoids present in *E. acoroides* likely contribute to these observed benefits.

Table 2 Chlorophyll *a*, chlorophyll *b*, and total chlorophyll concentrations in six-week-old maize plants sprayed after three weeks of twice-weekly treatment with varying concentrations of *E. acoroides* extract (0.05%, 0.1%, 0.15%, 0.2%, and 0.25%) followed by one week of PEG-induced drought stress

Treatment	Chlorophyll a ($\mu\text{g}/\text{cm}^2$)	Chlorophyll b ($\mu\text{g}/\text{cm}^2$)	Total chlorophyll ($\mu\text{g}/\text{cm}^2$)
Control	28.25 \pm 0.821 ^{cd}	19.83 \pm 1.844 ^{ab}	48.08 \pm 2.448 ^{bc}
PEG only	23.21 \pm 1.575 ^d	14.95 \pm 0.431 ^b	38.15 \pm 1.754 ^d
0.05%+PEG	28.02 \pm 2.408 ^{cd}	14.16 \pm 0.840 ^b	42.18 \pm 1.966 ^{cd}
0.1%+PEG	35.28 \pm 2.278 ^{ab}	26.05 \pm 2.032 ^a	61.34 \pm 3.050 ^a
0.15%+PEG	30.36 \pm 0.909 ^{bc}	24.45 \pm 4.816 ^a	54.80 \pm 3.911 ^{ab}
0.2%+PEG	30.08 \pm 2.244 ^{bc}	19.14 \pm 2.798 ^{ab}	49.22 \pm 1.389 ^{bc}
0.25%+PEG	39.44 \pm 2.964 ^a	20.26 \pm 1.382 ^{ab}	59.69 \pm 3.508 ^a

Notes: Data presented as mean \pm standard deviation. Different letters in the same column indicate significant differences ($P < 0.05$). PEG = Polyethylene glycol.

Polyethylene glycol (PEG) induces drought stress by reducing root water uptake. In the PEG treatment, plants pre-treated with *E. acoroides* extract for three weeks exhibited faster growth and less severe drought symptoms. During the one-week PEG treatment (with continued extract application twice weekly), treated plants maintained less desiccation compared to the controls, which leaves became dry and curled (Fig. 5).

The PEG treatment reduced lateral root development, resulting in smaller root systems and root browning (Fig. 6). This damage is likely due to tissue dehydration and osmotic stress-induced cell death (Saepudin *et al.* 2017).

In vivo H_2O_2 detection revealed higher oxidative stress in PEG-only-treated plants than the control and the *E. acoroides*-treated plants (Fig. 7). The extract may enhance root development, thus improving nutrient uptake and reducing oxidative stress by increasing tocopherol levels and internal antioxidant activity. This antioxidant effect has also been observed with *Sargassum wightii* extract applications to *Abelmoschus esculentus* under salt stress (Khan *et al.* 2022).

Table 2 presents the chlorophyll content in control plants (no extract, no PEG) and PEG-treated plants (sprayed and non-sprayed with *E. acoroides* extract). Maize plants treated with PEG alone exhibited 17.8%, 24.61%, and 20.65% declines in chlorophyll *a*, chlorophyll *b*, and total chlorophyll, respectively. Studies have reported that PEG-induced osmotic stress significantly

decreases chlorophyll levels, negatively affecting photosynthesis and overall plant growth (Nio *et al.* 2019; Rao *et al.* 2017). Spraying PEG-treated plants with *E. acoroides* extract increased chlorophyll content, with significant effects starting with the 0.1% extract.

Table 3 presents the levels of reducing sugars, total N, tocopherol, and IC50 in control and PEG-treated maize plants, with and without *E. acoroides* extract application. The PEG-only treatment decreased reducing sugars content, consistent with the findings in *Phaseolus vulgaris*, where PEG-induced osmotic stress decreased reducing sugars levels (Torres-Hernandez *et al.* 2022). This reduction may be attributed to the inactivation of α -amylase, the enzyme responsible for starch hydrolysis (Torres-Hernandez *et al.* 2022). However, spraying *E. acoroides* extract three weeks before and during PEG treatment increased reducing sugars levels. Significant increases ($P < 0.05$) started at the 0.1% extract concentration, suggesting that *E. acoroides* extract helps counteract the adverse effects of PEG-induced stress.

Total N levels were the highest in plants treated with 0.2% and 0.25% *E. acoroides* extract under PEG-induced drought stress. Tocopherol content significantly increased in plants sprayed with the highest extract concentration (0.25%+PEG). The increase in tocopherol content in plants sprayed with *E. acoroides* extract can be driven by the bioactive compounds present in the extract. Bioactive compounds, such as flavonoids and

Table 3 The levels of reducing sugars, total nitrogen (N), tocopherol, and antioxidant activity (IC50) in six-week-old maize plants sprayed after three weeks of twice-weekly treatment with varying concentrations of *E. acoroides* extract (0.05%, 0.1%, 0.15%, 0.2%, and 0.25%) followed by one week of PEG-induced drought stress

Treatment	Reducing sugars (%)	N (%)	Tocopherol (%)	IC50
Control	12.08 ± 0.067 ^f	1.03 ± 0.002 ^c	608.67 ± 60.762 ^b	8344.80 ± 64.614 ^{ab}
PEG only	11.60 ± 0.059 ^e	0.97 ± 0.020 ^c	615.32 ± 29.237 ^b	8398.81 ± 9.354 ^a
0.05%+PEG	12.32 ± 0.032 ^{de}	1.03 ± 0.003 ^c	640.89 ± 5.7207 ^{ab}	8303.19 ± 31.677 ^{bc}
0.1%+PEG	12.46 ± 0.017 ^{cd}	1.04 ± 0.002 ^c	681.54 ± 36.270 ^{ab}	8270.85 ± 10.106 ^{bc}
0.15%+PEG	12.69 ± 0.187 ^{bc}	1.06 ± 0.006 ^c	671.03 ± 17.083 ^{ab}	8246.06 ± 24.023 ^c
0.2%+PEG	12.80 ± 0.126 ^b	1.14 ± 0.076 ^b	713.11 ± 37.127 ^{ab}	8031.64 ± 9.440 ^d
0.25%+PEG	13.91 ± 0.068 ^a	1.31 ± 0.139 ^a	724.02 ± 37.754 ^a	7939.72 ± 30.218 ^e

Notes: Data are presented as mean ± standard deviation. Different letters in the same column indicate significant differences ($P < 0.05$). PEG = Polyethylene glycol.

polyphenols, exhibit strong antioxidant properties, which can protect tocopherols from oxidative degradation, thereby increasing their stability and bioavailability (Domínguez-Valencia *et al.* 2023). Plant extracts can enhance tocopherol production through elicitation, which stimulates the plant's defense mechanisms, leading to increased biosynthesis of tocopherols (Almagro *et al.* 2021).

The IC50 value, which represents the concentration required to reduce oxidative stress by 50%, significantly decreased with increasing extract concentration from 0.15% to 0.25% *E. acoroides* extract, suggesting that *E. acoroides* extract helps mitigate abiotic stress by enhancing antioxidant activity. This reduced stress lowers ROS (reactive oxygen species) accumulation, as indicated by lower H₂O₂ levels (Fig. 7). A previous study on *Zostera marina*, another seagrass species, found that its aqueous extract increased the activity of antioxidant enzymes in salt-stressed tomato plants (Vinoth *et al.* 2017). The bioactive compounds in *E. acoroides*, phenolics, flavonoids, and pigments, likely increased plant growth by exerting hormonal effects similar to those of seaweed extracts.

CONCLUSION

Spraying *Enhalus acoroides* extract significantly enhanced maize growth by increasing plant height and shoot and root biomass, as well as by improving reducing sugars and total N contents. Under PEG-induced drought stress, *E. acoroides* extract alleviated stress-related damage. *Enhalus acoroides*-treated plants exhibited higher chlorophyll levels and reduced oxidative stress, as indicated by

lower H₂O₂ accumulation. Additionally, spraying with *E. acoroides* extract increased reducing sugars, total nitrogen, and tocopherol levels, suggesting improved stress tolerance. These findings highlight the potential of *E. acoroides* extract as a biostimulant for enhancing plant growth and drought resilience in maize cultivation. Further research is needed to explore its applicability across different crops and environmental conditions.

ACKNOWLEDGMENTS

The authors acknowledge the Directorate General of Higher Education for funding this research through the Fundamental Research Scheme, No. 102/E5/PG.02.00.PL/2024, dated 11 June 2024, and No. B/519-32/UN14.14.4.A/PT.01.03/2024, dated 12 June 2024.

REFERENCES

- Abubakar AR, Haque M. 2020. Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. *J Pharm Bioallied Sci* 12(1):1-10. DOI: 10.4103/jpbs.JPBS_175_19
- Ali N, Farrell A, Ramsubhag A, Jayaraman, J. 2016. The effect of *Ascophyllum nodosum* extract on the growth, yield and fruit quality of tomato grown under tropical conditions. *J Appl Psychol* 28:1353-62. DOI: 10.1007/s10811-015-0608-3
- Almagro L, Sabater-Jara AB, Belchí-Navarro S, Pedrenó MA. 2021. Recent trends in the biotechnological production of tocopherols using in vitro cultures. *Phytochem Rev* 20:1193-1207. DOI: 10.1007/s11101-021-09742-8

- Amin I, Lee WY. 2005. Effect of different blanching times on antioxidant properties in selected cruciferous vegetables. *J Sci Food Agr* 85:2314-20. DOI: 10.1002/jsfa.2261
- Apriyantono A, Fardiaz D, Puspitasari NL, Yasni S, Budijanto S. 1989. Petunjuk laboratorium analisis pangan. [Guidelines for food analysis laboratory]. Bogor (ID): Pusat Antar Universitas Pangan dan Gizi, Institut Pertanian Bogor.
- Badan Pusat Statistik. 2023. Luas panen dan produksi jagung di Indonesia 2023. [Harvest area and maize production in Indonesia]. *Berita Resmi Statistik*. No. 69/10/Th. XXVI. [Internet]. Available from: <https://www.bps.go.id/id/pressrelease/2023/10/16/2049/luas-panen-dan-produksi-jagung-di-indonesia-2023--angka-sementara.html>
- Bertoldo G, Chiodi C, Della Lucia MC, Borella M, Ravi S, Baglieri A, ..., Nardi S. 2023. Brown seaweed extract (BSE) application influences auxin- and ABA-related gene expression, root development, and sugar yield in *Beta vulgaris* L. *Plants* 12(4):843. DOI: 10.3390/plants12040843
- Bulgari R. 2019. Effects of two doses of organic extract-based biostimulant on greenhouse lettuce grown under increasing NaCl concentrations. *Front Plant Sci* 9:1870. DOI: 10.3389/fpls.2018.01870
- Daudi A, O'Brien JA. 2012. Detection of hydrogen peroxide by DAB staining in *Arabidopsis* leaves. *Bio-Protocol* 2(18):e263. Available from: <https://pmc.ncbi.nlm.nih.gov/articles/PMC4932902/>
- Domínguez-Valencia R, Cittadini A, Pateiro M, Munekata PES, Lorenzo JM. 2023. Elderberry lipophilic and hydrophilic bioactive compounds: Characterization and extract encapsulation. *Foods* 12:4233. DOI: 10.3390/foods12234233
- Grote U, Fasse A, Nguyen TT, Erenstein O. 2021. Food security and the dynamics of wheat and maize value chains in Africa and Asia. *Front Sustain Food Syst* 4:617009. DOI: 10.3389/fsufs.2020.617009
- Herlina N, Prasetyorini A. 2020. Effect of climate change on planting season and productivity of maize (*Zea mays* L.) in Malang Regency. *J Ilmu Pertanian Indonesia* 25(1):118-28. DOI: 10.18343/jipi.25.1.118
- Jalaludin M, Octaviani IN, Putri ANP, Octaviani W, Aldiansyah I. 2020. Padang lamun sebagai ekosistem penunjang kehidupan biota laut di Pulau Pramuka, Kepulauan Seribu, Indonesia. [Seagrass beds as supporting ecosystems for marine life in Pramuka Island, Thousand Islands, Indonesia]. *J Geografi Gea* 20(1):44–53. Available from: <https://ejournal.upi.edu/index.php/gea/article/view/22749/11823>
- Jothimani K, Arulbalachandran D. 2020. Physiological and biochemical studies of black gram (*Vigna mungo* (L.) Hepper) under polyethylene glycol induced drought stress. *Biocatal Agric Biotechnol* 29:101777. DOI:10.1016/j.bcab.2020.101777
- Khan Z, Gul H, Rauf M, Arif M, Hamayun M, Ud-Din A, ..., Lee I-J. 2022. Sargassum wightii aqueous extract improved salt stress tolerance in *Abelmoschus esculentus* by mediating metabolic and ionic rebalance. *Front Mar Sci* 9:853272. DOI:10.3389/fmars.2022.853272
- Kumar Y, Singhal S, Tarafdar A, Pharande A, Ganesan, M., Badgular P.C. 2020. Ultrasound assisted extraction of selected edible macroalgae: Effect on antioxidant activity and quantitative assessment of polyphenols by liquid chromatography with tandem mass spectrometry (LC-MS/MS) *Algal Res* 52:102114. DOI: 10.1016/j.algal.2020.102114.
- Kumari R, Kaur I., Bhatnagar AK. 2011. Effect of aqueous extract of *Sargassum johnstonii* Setchell & Gardner on growth, yield and quality of *Lycopersicon esculentum* Mill. *J Appl Phycol* 23(3):623-33. DOI:10.1007/s10811-011-9651-x
- Latique S, Mrid RB, Kabach I, Yasri A, Kchikich A, Nhiri M. 2021. The effect of foliar application of *Ulva rigida* extract on the growth and biochemical parameters of wheat plants. *E3S Web of Conferences* 234:00103. DOI: 10.1051/e3sconf/202123400103
- Lichtenthaler KH, Buschmann C. 2001. Chlorophylls and carotenoids: Measurement and characterization by UV-VIS spectroscopy. *In: Wrolstad RE, Acree TE, An H, Decker EA, Penner MH, Reid DS, Schwartz SJ, Shoemaker CF, Sporns P* (Editors). *Current protocols in food analytical chemistry*. 1st edition. New York (US): John Wiley & Sons Inc. p. F4.3.1-F4.3.8.

- Ma X, Nian J, Yu H, Zhang F, Feng T, Kou L, ..., Zuo J. 2023. Linking glucose signaling to nitrogen utilization by the OsHXK7-ARE4 complex in rice. *Dev Cell* 58(16):1489-1501. DOI: 10.1016/j.devcel.2023.06.003
- Melo P, Abreu C, Bahcevandziev K, Araujo G, Pereira L. 2020. Biostimulant effect of marine macroalgae bioextract on pepper grown in greenhouse. *Appl Sci* 10(11):4052. DOI: 10.3390/app10114052
- Nieves-Cordones M, García-Sánchez F, Pérez-Pérez JG, Colmenero-Flores JM, Rubio F, Rosales MA. 2019. Coping with water shortage: an update on the role of K⁺, Cl⁻, and water membrane transport mechanisms on drought resistance. *Front Plant Sci* 10:1619. DOI: 10.3389/fpls.2019.01619
- Nio SA, Pirade M, Ludong DPM. 2019. Leaf chlorophyll content in North Sulawesi (Indonesia) local rice cultivars subjected to polyethylene glycol (PEG) 8000-induced water deficit at the vegetative phase. *Biodiversitas* 20(9):2462-67. DOI:10.13057/biodiv/d200905
- Pharmawati M, Wrasati LP. 2020. Phytochemical screening and ftir spectroscopy on crude extract from, *Enhalus acoroides*. *MJAS* 24(1):70-77. Available from: https://mjas.analis.com.my/mjas/v24_n1/pdf/Pharmawati_24_1_8.pdf
- Rao S, Groach R, Singh S, Singh N 2017. Efficacy of polyethylene glycol (PEG) induced drought on germination indices and photosynthetic pigments of sweet corn var. NSC-901B. *Asian J Biosci* 12(2):185-88. DOI : 10.15740/HAS/AJBS/12.2/185-188
- Rasul F, Gupta S, Olas JJ, Gechev T, Sujeeth N, Mueller-Roeber B. 2021. Priming with a seaweed extract strongly improves drought tolerance in *Arabidopsis*. *Int J Mol Sci* 22(3):1469. DOI: 10.3390/ijms22031469
- Rouphael Y. 2020. Metabolomic responses of maize shoots and roots elicited by combinatorial seed treatments with microbial and non-microbial biostimulants. *Front Microbiol* 11:664. DOI: 10.3389/fmicb.2020.0066
- Saepudin A, Khumaida N, Sopandie D, Ardi SW. 2017. In vitro selection of four soybean genotypes using PEG for drought tolerance. *J Agron Indonesia* 45(1):14-22. DOI: 10.24831/jai.v45i1.13749
- Shah TR, Prasad K, Kumar P. 2016. Maize—A potential source of human nutrition and health: A review. *Cogent Food and Agric* 2:1166995. DOI:10.1080/23311932.2016.1166995
- Shao R, Jia S, Tang Y, Zhang J, Li H, Li L, ..., Zhao X. 2021. Soil water deficit suppresses development of maize ear by altering metabolism and photosynthesis. *Environ Exp Bot* 192: 104651. DOI: 10.1016/j.envexpbot.2021.104651
- Shiferaw B, Prasanna BM, Hellin J, Bänziger M. 2011. Crops that feed the world 6. Past successes and future challenges to the role played by maize in global food security. *Food Secur* 3:307-27. DOI: doi.org/10.1007/s12571-011-0140-5
- Stankovic M, Carli F, Galon F. 2021. Quantification of blue carbon in seagrass ecosystems of Southeast Asia and their potential for climate change mitigation. *Sci Total Environ* 783:146858. DOI: 10.1016/j.scitotenv.2021.146858
- Sudarmadji, Haryono B, Suhardi. 1989. Analisa bahan makanan dan pertanian [Food and agriculture analyses]. Yogyakarta (ID): Liberty. 172 p.
- Syamkumar TS, Geethalakshmi S, Augustine A. 2023. Study of pharmacological profile of chloroform leaf extract of *Ludwigia perennis* - A wetland plant. *European J Med Plants* 34(8):41-53. DOI: 10.9734/ejmp/2023/v34i81154
- Torres-Hernández L, Sosa-del Castillo M, Pérez-Hernández Y, Rodríguez-Izquierdo L, Cortés-Martínez Y, Liriano-González R. 2022. Effect of polyethylene glycol-6000 on germination and early growth of *Phaseolus vulgaris* L. cv. 'Delicias'. *Cult Trop* 43(2):e06. DOI: 10.1234/ct.v43i2.1657
- Uddin MN, Afrin R, Uddin MJ, Uddin MJ, Alam AHMK, Rahman AA, Sadik G. 2015 *Vanda roxburghii* chloroform extract as a potential source of polyphenols with antioxidant and cholinesterase inhibitory activities: Identification of a strong phenolic antioxidant. *BMC Complement Altern Med* 15:195. DOI: 10.1186/s12906-015-0728-y
- Vinoth S, Sundari, Gurusaravanan P, Sivakumar S, Siva G, Kumar GP, ..., Jayabalan N. 2017. Evaluation of seagrass liquid extract on salt stress alleviation in tomato plants. *Asian J Plant Sci* 16:172-83. DOI: 10.3923/ajps.2017.172.183

- Wang L, Jayawardena TU, Yang HW, Lee HG, Kang MC, Sanjeeva KKA, Oh, JY, Jeon Y-J. 2020. Isolation, characterization, and antioxidant activity evaluation of a fucoidan from an enzymatic digest of the edible seaweed, *Hizikia fusiforme*. *Antioxidants* 9(5):363. DOI: 10.3390/antiox9050363
- Wong WL, Timms RE, Goh EM. 1988. Colorimetric determination of total tocopherols in palm oil, olein, and stearin. *J Am Oil Chem Soc* 65:258-61. DOI:10.1007/BF02636412
- Xu L, Geelen D. 2018. Developing biostimulants from agro-food and industrial by-products. *Front Plant Sci* 9:1567. DOI:10.3389/fpls.2018.01567
- Yakhin OI, Lubyantsov AA, Yakhin IA, Brown PH. 2017. Biostimulants in plant science: A global perspective. *Front Plant Sci* 7:2049. DOI:10.3389/fpls.2016.02049
- Yalçın S, Okudan ES, Karakaş Ö, Önem AN, Başkan KS. 2019. Identification and quantification of some phytohormones in seaweeds using UPLC-MS/MS. *J Liq Chromatogr Relat Technol* 42: 475-84. DOI:10.1080/10826076.2019.1625374
- Zhang L, Sun S, Liang Y, Li B, Ma S, Wang Z, Ma B, Li M. 2021. Nitrogen levels regulate sugar metabolism and transport in the shoot tips of crabapple plants. *Front Plant Sci* 12: 626149. DOI: 10.3389/fpls.2021.626149