

T.R.
GEBZE TECHNICAL UNIVERSITY
GRADUATE SCHOOL

***Cystoseira barbata* EXTRACTS AS BIOSIMULANTS IN**
SOILLESS CULTURE

HANDE MUTLU DURAK

A THESIS OF DOCTORATE
DEPARTMENT OF BIOTECHNOLOGY

ADVISOR: ASSIST. PROF. DR. BAHAR YILDIZ KUTMAN

JULY 2025

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GEBZE TEKNİK ÜNİVERSİTESİ
LİSANSÜSTÜ EĞİTİM ENSTİTÜSÜ

TOPRAKSIZ SİSTEMLERDE BİYOSTİMÜLANTLAR
OLARAK *Cystoseira barbata* ÖZÜTLERİ

HANDE MUTLU DURAK

DOKTORA TEZİ
BİYOTEKNOLOJİ ANABİLİM DALI

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To my beloved family

ABSTRACT

Soilless culture systems offer a promising alternative to traditional soil-based agriculture by overcoming many of its associated challenges. Integrating plant biostimulants that enhance plant growth and resilience into soilless farming systems represents a practical, safe, and sustainable strategy for modern agriculture. In particular, the growth-promoting properties of seaweed extracts make them valuable inputs for environmentally friendly production in soilless farming systems.

This thesis includes various characterization analyses to understand the mechanisms of action of different seaweed extracts (water, alkali and acid) obtained from *Cystoseira barbata*, a brown seaweed, which was collected from the Marmara Sea. The biostimulant activities of these extracts were evaluated in soilless cultivation systems using different crop plants, focusing on their effects on early growth and seedling development of wheat in perlite, wheat growth under salinity stress in hydroponics, yield and nutritional quality in hydroponically grown lettuce, and the efficacy of foliar applications in pepper cultivated in substrate-based system.

According to the perlite experiments, the application of *C. barbata* extracts to the germination medium had more positive effects on early plant growth compared to seed application. These findings suggest that extracts with varying compositions can be effective for supporting seedling performance and early development.

In hydroponic wheat experiments, the potential of using *C. barbata* extracts as biostimulants on wheat plants grown under two levels of salinity stress was evaluated. The extracts enhanced plant biomass and tolerance under both levels of salinity stress, indicating their potential to mitigate abiotic stress through stress adaptation mechanisms.

Application of *C. barbata* extracts led to considerable improvements in hydroponically grown lettuce under greenhouse conditions, having positive effects on growth parameters such as yield, root morphology, mineral levels and physiological quality factors, including phenolic and antioxidant capacity, carotenoid levels, glutathione, and vitamin C concentrations under greenhouse conditions. It was concluded that integrating the extracts into the nutrient solution could act as an effective biostimulant for leafy vegetables, enhancing plant performance and nutraceutical properties.

The results of the pepper experiment revealed that foliar application of *C. barbata* extracts, particularly the water and acid extracts, enhanced the yield and nutritional quality including phenolic content, antioxidant activity, vitamin C, glutathione, and soluble carbohydrate levels in plants grown in cocopeat-based medium. The experimental extracts also outperformed the commercial algae biostimulant product.

In conclusion, the findings of this study indicate that extracts obtained from the same algae source can significantly affect plant performance to different extents depending on their bioactive composition, which is influenced by the extraction method. These promising findings highlight the potential of *C. barbata* extracts as a sustainable and locally sourced biostimulant that can enhance plant performance, yield, quality and stress tolerance in soilless agricultural systems.

Keywords: *Cystoseira barbata* (*Gongoloria barbata*), Plant Biostimulants, Seaweed Extracts, Soilless Culture, Sustainable Agriculture

ÖZET

Topraksız tarım sistemleri, geleneksel toprak temelli tarımın getirdiği birçok zorluğun üstesinden gelerek, ona umut vadeden bir alternatif sunmaktadır. Bitki büyümesini ve dayanıklılığını artıran bitki biyostimülanlarını topraksız tarım sistemlerine entegre etmek, modern tarım için pratik, güvenli ve sürdürülebilir bir stratejiyi temsil eder. Özellikle deniz yosunu özütlerinin büyümeyi teşvik edici özellikleri, onları topraksız tarım sistemlerinde çevre dostu üretim için değerli girdiler haline getirir.

Bu tez çalışması, Marmara Denizi'nden toplanan kahverengi bir alg türü *Cystoseira barbata*'dan elde edilen farklı deniz yosunu özütlerinin (su, alkali ve asit) etki mekanizmalarının anlaşılmasına yönelik çeşitli karakterizasyon analizlerini içermektedir. Bu özütlerin biyostimülan aktiviteleri, farklı bitkiler kullanılarak topraksız yetiştirme sistemlerinde değerlendirilmiş, perlitte buğdayın erken büyümesi ve fide gelişimi, hidroponikte tuzluluk stresi altında buğday büyümesi, hidroponik olarak yetiştirilen marulda verim ve besin kalitesi ve substrat bazlı sistemde yetiştirilen biberde yaprak uygulamalarının etkinliğine odaklanılmıştır.

Perlit denemelerine göre, *C. barbata* özütlerinin çimlenme ortamına uygulanması, tohum uygulamasına kıyasla erken bitki büyümesi üzerinde daha olumlu etkilere sahipti. Bu bulgular, farklı bileşimlere sahip özütlerin fide performansını ve erken gelişimi desteklemede etkili olabileceğini göstermektedir.

Hidroponik buğday deneylerinde, *C. barbata* özütlerinin iki tuzluluk stresi seviyesi altında yetiştirilen buğday bitkilerinde biyostimülan olarak kullanılma potansiyeli değerlendirilmiştir. Özütler, her iki tuzluluk stresi seviyesinde de bitki biyokütlesini ve dayanıklılığını artırmış, bu da onların stresin adaptasyon mekanizmaları yoluyla abiyotik stresin hafifletilmesinde potansiyel taşıdığını göstermiştir.

C. barbata özütlerinin serada hidroponik olarak yetiştirilen marul bitkilerine uygulanması, verim, kök morfolojisi ve fenolik ve antioksidan kapasite, karotenoid düzeyleri, glutatyon ve C vitamini konsantrasyonları gibi fizyolojik kalite faktörleri üzerinde olumlu etkiler göstererek önemli gelişmelere yol açmıştır. Özütlerin besin çözeltisine entegre edilmesinin, yapraklı sebzeler için etkili bir biyostimülan görevi görebileceği, bitki performansını ve nutrasötik özelliklerini artırabileceği sonucuna varılmıştır.

Biber deneyinin sonuçları, özellikle su ve asit özütleri olmak üzere *C. barbata* özütlerinin yaprak uygulamasının, kokopit bazlı ortamda yetiştirilen bitkilerde verim ve fenolik içerik, antioksidan aktivite, C vitamini, glutatyon ve çözünür karbonhidrat seviyeleri gibi besin kalitesini artırdığını ortaya koymuştur. Ayrıca, deneysel özütlerin, ticari bir yosun bazlı biyostimülan ürününe kıyasla daha üstün performans gösterdiği belirlenmiştir.

Sonuç olarak, bu çalışmanın bulguları, aynı yosun kaynağından elde edilen özütlerin, ekstraksiyon yöntemine bağlı olarak değişen biyolojik olarak etkili bileşimleri sayesinde bitki performansını farklı derecelerde etkileyebileceğini göstermektedir. Bu umut verici bulgular, *Cystoseira barbata* özütlerinin sürdürülebilir ve yerel kaynaklı bir biyostimülan olarak, topraksız tarım sistemlerinde bitki performansını, verimi, ürün kalitesini ve stres toleransını artırma potansiyeline sahip olduğunu ortaya koymaktadır.

Anahtar Kelimeler: Bitki Biyostimülanları, *Cystoseira barbata* (*Gongoloria barbata*), Sürdürülebilir Tarım, Topraksız Tarım, Yosun Özütleri

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LIST OF SYMBOLS AND ABBREVIATIONS AND ACRONYMS

$(\text{CO}_3)^{-2}$	: Carbonate
(HCO_3^-)	: Bicarbonate
$(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$	: Ammonium heptamolybdate tetrahydrate
$(\text{SO}_4^-)_2$	: Sulphate
$^1\text{O}_2$	: Singlet oxygen
Abs	: Absorbance
ABTS	: 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonate)
Act	: Activity
ANOVA	: Analysis of variance
APX	: Ascorbate peroxidase
ASC	: Ascorbate
B	: Boron
BHT	: Butylated hydroxytoluene
<i>C.barbata</i>	: <i>Cystoseria barbata</i>
Ca	: Calcium
$\text{Ca}(\text{NO}_3)_2$	: Calcium nitrate
CAT	: Catalase
chl	: Chlorophyll
Cl^-	: Chloride
cm	: Centimeter
CO_2	: Carbon dioxide
Conc.	: Concentration
Cu	: Copper
$\text{CuSO}_4 \cdot 5(\text{H}_2\text{O})$	: Copper sulphate pentahydrate
cv.	: Cultivar
DAS	: Days After Sowing
DPPH	: 2,2-diphenyl-1-picrylhydrazyl
dH_2O	: Distilled water
dS	: Deci Siemens
Dehydroascorbic acid	: DHA
DW	: Dry weight
Dithiothreitol	: DTT
EC	: Electrical Conductivity
EC_e	: Saturated Paste Extract
ESP	: Exchangeable Sodium Percentage
FAO	: Food and Agriculture Organization
Fe	: Iron

Ferric ion	: Fe^{+2}
Ferrous ion	: Fe^{+3}
FeEDTA	: Iron ethylenediaminetetraacetate
FRAP	: Ferric-reducing antioxidant power
FW	: Fresh weight
g	: Gram
GAE	: Gallic Acid Equivalent
GB	: Glycine Betaine
GR	: Glutathione reductase
GSH	: Reduced glutathione
GSSG	: Oxidized Glutathione
h	: Hour
H_2O	: Water
H_2O_2	: Hydrogen peroxide
HCl	: Hydrochloric acid
HNO_3	: Nitric acid
HSD	: Tukey's honestly significant difference
IAA	: Indole-3-Acetic Acid
ICP-OES	: Inductively coupled plasma optical emission spectrometry
K	: Potassium
K_2SO_4	: Potassium sulphate
KCl	: Potassium chloride
kg	: Kilogram
KH_2PO_4	: Potassium dihydrogen phosphate
KI-I ₂	: Potassium iodide - Iodine
K-P	: Potassium phosphate buffer
KOH	: Potassium hydroxide
l	: Liter
LSD	: Fisher's least significant difference
m	: Meter
MDA	: Malondialdehyde
mg	: Miligram
Mg	: Magnesium
MgSO_4	: Magnesium sulphate
min	: Minute
ml	: Milliliter
mM	: Millimolar
Mn	: Manganese
$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	: Manganese sulphate monohydrate

N	: Nitrogen
<i>n.s.</i>	: not significant
Na	: Sodium
NaCl	: Sodium chloride
Na ₂ CO ₃	: Sodium carbonate
NaOH	: Sodium hydroxide
NADPH	: Nicotinamide adenine dinucleotide phosphate
NBT	: Nitro blue tetrazolium chloride
NEM	: N-ethylmaleimide
NH ₄ ⁺	: Ammonium
NO ₃ ⁻	: Nitrate
O ₂ ⁻	: Superoxide radical
OH ⁻	: Hydroxyl radical
P	: Phosphorus
Prt	: Protein
pH	: Power of hydrogen
ppm	: parts per million
REL	: Relative electrolyte leakage
ROS	: Reactive Oxygen Species
SOD	: Superoxide dismutase
Sp	: Specific
SWE	: Seaweed Extracts
TBA	: Thiobarbituric acid
TCA	: Trichloroacetic acid
TE	: Trolox Equivalent
Zn	: Zinc
ZnSO ₄ .7H ₂ O	: Zinc sulphate heptahydrate

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1. INTRODUCTION

1.1. Global Problems

The agricultural sector encounters many challenges, including climate change and the reduction of arable land with urbanization, industrialization, and desertification, all of adversely affect global food security and crop quality [Duhamel and Vandenkoornhuyse, 2013; Valoppi et al., 2021]. In addition, the excessive and uncontrolled use of agrochemicals such as fertilizers and pesticides to increase agricultural productivity causes, rather than benefits, environmental problems such as salinization and soil degradation [Lin et al., 2019]. On the other hand, only 10–20% of excessive fertilizer is taken through plants from the soil, and even minimal concentrations of pesticides impact target organisms [Mandal et al., 2023]. Moreover, chemical fertilizers present possible pollution problems in agriculture due to their contained pollutants, and their indiscriminate application frequently leads to the degradation of natural resources, and the release of harmful substances that directly impact human health into the soil, air, and water, ultimately leading to the contamination of natural resources [Rashmi et al., 2020].

It is estimated that the global population, currently over 8 billion, will reach around 10 billion by 2050 [Web1], increasing the demand for food by 25 to 70 percent over what is presently produced [Hunter et al., 2017]. For this reason, it will become even more important in the near future to achieve maximum product yield from a unit area without compromising product quality [Tan et al., 2006; Roupheal et al., 2018]. Considering all this, the development of sustainable and eco-friendly agricultural production systems to feed a growing population is a major concern that is closely linked to the United Nations' Sustainable Development Goals, including zero hunger and poverty [Francesca et al., 2020]. Moreover, the European Commission intends to substitute 30% of chemical fertilizers with bio-based alternatives by 2050 [Li et al., 2022a].

In intensive cropping systems, where more eco-friendly approaches are required to protect the environment and natural resources, the usage of biostimulants is gaining interest as a sustainable strategy to increase the efficiency of cropping systems while minimizing unnecessary chemical usage [Panfili et al., 2019].

Thus, the widespread application of biostimulants in agriculture as sustainable, environmentally friendly, and effective solutions may assist in feeding the increasing population, mitigating the effects of climate change, and decreasing reliance on chemical fertilizers, which are often excessively utilized in crop production systems [Malik et al., 2020; Hunter et al., 2017].

1.2. Plant Biostimulants

Plant biostimulants, often known as agricultural biostimulants, represent a promising and developing category of agricultural management products [Goñi et al., 2018]. They are compounds and/or microorganisms utilized in small amounts to plants that stimulate plant metabolism regardless of their nutrient content, and are distinct from fertilizers, pesticides, or soil improvers [Campobenedetto et al., 2021; Yakhin et al., 2017].

Biostimulants can be classified as seaweed extracts (SWEs), humic and fulvic acids, inorganic compounds, chitosan and other biopolymers, protein hydrolysates and other N-containing substances, botanicals, beneficial fungi and bacteria [du Jardin, 2015]. In agricultural production, plant biostimulants have become important products, typically formulated with one or more active compounds [Subramaniyan et al., 2023]. Additionally, these substances can be applied in small amounts, depending on their composition and intended use, by seed treatment, foliar spraying, or incorporating them into soil or soilless growing media such as perlite, cocopeat, vermiculite, or other soil alternatives [Calvo et al., 2014]. Biostimulants not only affect plants' primary metabolism through boosting the activity of photosynthesis and derived substances but can also induce secondary metabolism through triggering particular biosynthetic processes [Bulgari et al., 2019]. They may improve growth and development, improved quality traits, increased seed germination, and nutrient uptake and use efficiency, improved root system related parameters, and enhanced crop performance [Bulgari et al., 2015; Alvarez et al., 2024]. Biostimulants are also crucial in stressful

situations because they may boost the antioxidant system, stimulate the production of plant defense molecules, and thus make plants more resistant to stress factors [Nephali et al., 2020; Franzoni et al., 2022].

The identification and study of an individual compound within a biostimulant may yield unreliable and inaccurate outcomes, as the biostimulant effects on plants frequently arise from the interplay and synergistic effects of multiple bioactive substances [Bulgari et al., 2015; Rouphael and Colla, 2018]. The beneficial effects, however, differ across plant species, developmental stages, application methods, and times, and the exact mechanisms of action remain unclear [Colla et al., 2015].

Despite the beneficial effects of biostimulants on diverse abiotic stresses, recent European legislation on fertilizers emphasizes the necessity of scientific evidence validating the usefulness of a product classified as a biostimulant, along with a comprehensive elucidation of its mode of action mechanism [Regulation EU, 2019].

1.2.1. Seaweed Extracts

Macroalgae or seaweeds, are autotrophic, multicellular, and photosynthetic organisms which have the ability to colonize intricate, harsh, and challenging marine habitats worldwide [Bhattacharya et al., 2015]. Seaweed encounters persistent and occasionally severe fluctuations in light quantity, salinity, climate, and nutrient availability; consequently, they have developed the capacity to synthesize a wide range of secondary metabolites in their thallus, which is essential for swiftly managing and dealing with abiotic stressors [de Morais et al., 2015; Vaghela et al., 2023].

Seaweed extracts are one of the most promising and fastest-growing classes of biostimulants [Carmody et al., 2020] and constitute about one-third of the total worldwide biostimulant market [Eef et al., 2018]. Approximately 7.5 to 8.0 million kg of wet seaweed is harvested globally each year [Mughunth et al., 2024]. Seaweed biomass and its extracts have long been regarded as significant resources utilized for many purposes, such as agriculture, food, cosmetics, and pharmaceuticals [Carillo et al., 2020a]. Historically, the first known agricultural usage of algal biomass originated in ancient Rome as a soil amendment [Nedumaran et al., 2015; El Boukhari et al., 2020]. Furthermore, the first usage of SWE in liquid form in agricultural applications dates to the early 1950s [Craigie, 2011]. Macroalgae, as one of the most important

sustainable resources, plays a critical part in coastal marine ecosystems and consists of almost 10,000 species [Battacharyya et al., 2015]. They are primarily categorized into three classes according to their pigmentation: rhodophyta (red), chlorophyta (green), and phaeophyta (brown), [Khan et al., 2009].

Although certain seaweeds are prevalent across several geographic regions, others are endemic to certain locales [Ali et al., 2021]. Brown seaweeds with *Ascophyllum*, *Fucus*, and *Laminaria* are the dominant groups [du Jardin, 2015] and due to their abundance, fast-growth capability and distribution, they are the primary raw materials for the industrial production of extracts for agricultural practices [Carrasco-Gil et al. 2018; Rouphael and Colla, 2020]. The predominant marketable seaweed-based biostimulants are liquid extracts that differ in flavor, density, colour ranging from colorless to a very dark brownish-black, and content [Craigie, 2011]. In contrast to terrestrial plants, seaweed can be cultivated in ocean farms or open pond systems without requiring fresh water or arable land [Pereira and Cotas, 2024].

Alongside functional variety, characterized by distinct adaptation features to multiple habitats and pressures, the seaweed biochemical profile exhibits significant diversity owing to extensive taxonomic dispersion and evolutionary variation. It possesses a complex chemical composition comprising polysaccharides (alginate, fucoidan, laminarin, mannitol), unsaturated fatty acids, essential amino acid and bioactive peptides, vitamins (A, B12, C, D, E, K), and both macro- and micronutrients [Godlewska et al., 2016]. Seaweed extracts are also rich in antioxidant compounds like polyphenols and phlorotannins (eckol, phenolic acids, flavonoids, and lignans), and pigments (chlorophylls and carotenoids) [Michalak and Chojnacka, 2014]. Moreover, these extracts are abundant in plant growth regulators, like auxins, cytokinins, gibberellic acids, salicylic acid, kinetin and abscisic acid [Godlewska et al., 2017]. Moreover, it is important to emphasize that seaweed can synthesize unique substances not generated in terrestrial plants [Michalak and Chojnacka, 2014].

Macroalgae can produce several bioactive polysaccharides absent in terrestrial plants with their kind, quantity, and chemical composition varying according to the type of seaweed and environmental factors [Jiao et al., 2011; Vera et al., 2011]. Seaweed often has up to 76% polysaccharides by dry weight and acts as a promoter, demonstrating diverse biological actions when utilized on plants [Jayaraman et al., 2015].

Alginate, a polysaccharide present in the cell wall of seaweed in the form of alginic acid, makes up nearly half of the dry biomass of the algae [Holdt and Kraan, 2011]. These compounds are known to have numerous beneficial effects, like improving soil structure and water holding capacity and raising soil fertility through microbial activity stimulation [Mamede et al., 2023]. Alginate oligo-derivatives, which are made from degraded alginate, can boost root system and crop production while also reducing the adverse impacts of abiotic stressors, including salinity stress [Liu et al., 2013; Mukherjee and Patel, 2020].

Approximately 10% of the dry weight in algae consists of fucoidan, a sulfated polysaccharide with anti-inflammatory, antiviral, antitumor, and antioxidative properties; however, the chemical ingredients of this molecule can differ depending on the seaweed source and the timing of harvest [Synytsya et al., 2010; Song et al., 2012; Usov et al., 2022].

Laminarin comprises roughly 10% of the dry weight of seaweed, similar to fucoidan, with this percentage potentially increasing to 32% seasonally [Holdt and Kraan, 2011]. Due to its antioxidant activities, laminarin acts as a stimulant in various defense mechanisms, especially against pathogens, thanks to the activation of defense-related enzymes, Ca^{2+} influx, accumulation of stimulant compounds such as salicylic acid, and synthesis of phytoalexins [Aziz et al. 2003; Garde-Cerdán et al., 2017].

Mannitol, a sugar alcohol (polyol) produced during photosynthesis, is found naturally in several species, excluding archaea and mammals [Godswill, 2017]. It can constitute roughly 20% to 30% of the dry matter in seaweed [Adams et al., 2011; Wei et al., 2013]. Macroalgae, characterized by their rapid growth rates and significant ability for mannitol biosynthesis, constitute a viable and sustainable source for the huge-scale industrial production of mannitol, utilized in the food, health and pharmaceutical sectors. It functions as a vital compatible solute and antioxidant, contributing to cellular protection by maintaining turgor pressure, stabilizing lipid bilayers and membrane proteins, and mitigating oxidative stress [Hosseini et al., 2024].

Seaweed extracts exhibit bioactive properties when applied in low concentration to the plant or growth medium [Khan et al., 2009]. Besides enhancing growth and development, seaweed-based biostimulants are recognized for their beneficial effects on germination [Hernández-Herrera et al., 2016], nutrient uptake [Halpern et al.,

2015], root morphology [Rayorath et al., 2008], flowering [Ali et al., 2019], and fruit quality metrics [Kapur et al., 2018]. Moreover, algae-based biostimulants not only promote plant development but also mitigate several abiotic challenges, including drought [Xu et al., 2015; Elansary et al., 2016], salinity [Hernández-Herrera et al., 2022], cold [Nair et al., 2012; Bradáčová et al., 2016] and heat [Zhang and Ervin, 2008], by targeting several plant pathways.

The growth-promoting benefits of SWEs are recognized across various species [Battacharyya et al., 2015]; nevertheless, the heterogeneous and intricate composition of these substances complicates the identification of specific components responsible for their efficacy, hence obstructing a comprehensive understanding of the underlying mechanisms [Van Oosten et al., 2017]. While most of their chemical constituents and mechanisms of action remain inadequately understood, it is proposed that they function synergistically to elicit a favorable response within the plant mechanism, with each substance potentially acting either alone or collaboratively on diverse metabolic networks [Malaguti et al., 2002; Grundy et al., 2015]. Moreover, elucidating the biomolecular mechanisms of action of these varied compounds or their compositions on plants is a complex process that requires an integrated strategy that encompasses several disciplines, including genomes, proteomics, transcriptomics, and plant physiology [Carillo et al., 2020a].

Seaweed-based biostimulants have significant potential to be a viable tool in sustainable agriculture due to their rich bioactive components, high environmental compatibility, and being biodegradable and safe, in contrast to chemical fertilizers [Layek et al., 2018]. Moreover, the utilize of whole seaweed biomass as a biostimulant in agriculture is a more feasible and economical method than isolating a single molecule [Carillo et al., 2020a]. For this reason, the increasing interest in the development of novel products derived from SWEs has contributed to the development and advancement of innovative extraction methodologies [Kadam et al., 2013]. The increasing demand for a bioeconomy that uses resources more efficiently and sustainably requires the use of renewable biological resources instead of synthetic inputs in agricultural practices, which is possible by further integrating agriculture with blue biotechnology [Vieira et al., 2020].

On the other hand, there may be some difficulties in the use of SWEs in agriculture. Harvesting seaweed from marine ecosystems such as seas or oceans requires attention

to efficient harvesting techniques, extraction processes, rapid processing and storage methods to preserve the chemical composition and quality of the raw materials [Zodape, 2001]. Moreover, the variability stemming from differences in species, habitats, and environmental and climatic circumstances might complicate the maintenance of consistent quality and the optimization and standardization of the resulting product, and the many activities and uses of SWEs also complicate their commercial classification and labeling [du Jardin et al., 2020; Ali et al. 2021]. The prevalence of counterfeit products on the market, which include little seaweed-derived ingredients, mandates the implementation of rigorous quality standards to precisely determine, validate, and label the authenticity of their content [Leandro et al., 2020].

1.2.1.1. Seaweed Extraction Methods

Choosing and optimizing an efficient extraction technique is a vital beginning step to getting seaweed-based biostimulants, as variations in the content of bioactive compounds can be noted throughout the extraction of seaweed [Cikoš et al., 2018]. Seaweed extracts can be obtained by both physical and chemical processes [Godlewska et al., 2017]. The main objective of extraction methods is to maximize the production of target compounds, protect by-products, reduce energy consumption, explore recycling strategies to diminish waste creation, optimize the process, and enhance scalability [Michalak and Chojnacka, 2014].

Biologically active substances can recover from algal biomass primarily through traditional solvent extraction and hydrolysis methods, including hydrothermal, acid, or alkaline hydrolysis [Meillisa et al., 2013]. Firstly, the extraction of phenolics and phytohormones can be conducted using water extraction, a method that is straightforward, efficient, cost-effective, and environmentally sustainable without the need for organic solvents [Godlewska et al., 2016; Khalil et al., 2017]. Acid hydrolysis is an extraction technique employed to isolate polysaccharides mainly alginate and fucoidan, by removing fucose-containing sulfated polysaccharides from seaweed through treatment with strong acids like sulfuric or hydrochloric acid [Sharma et al., 2014]. The alkaline hydrolysis procedure, commonly employed in industry, offers significant extractability and medium breakdown of polysaccharides to oligomers, which are among the most bioactive constituents of SWE [Jayaraj et al., 2018; Shukla et al., 2019].

Moreover, various novel extraction strategies have been employed to maximize the isolation of different types of biologically active compounds from seaweed in a without solvent condition [Kisvarga et al., 2022], such as ultrasonic-assisted extraction (UAE) [Kadam et al., 2015], microwave-assisted extraction (MAE) [Xiao et al., 2013; Michalak et al., 2015], enzyme-assisted extraction (EAE) [Wijesinghe and Jeon, 2012], supercritical fluid extraction (SFE) [Conde et al., 2015] and pressure fluid extraction (PFE) [Perez-Vazquez et al., 2023].

Ultrasonic-assisted extraction utilizes sound waves with low frequency and high power produced in an ultrasonic bath or by an ultrasonic probe to create pressure fluctuations, which provide mechanical energy that breaks the cell, thereby diminishing particle size and enhancing the interaction between the solution and the obtained substances [Ying et al., 2011; Hahn et al., 2012; Mena-García et al., 2019]. This method has demonstrated efficacy in increasing the extraction of polysaccharides, pigments, and phenolic substances from different algae species [Florez-Fernandez et al., 2017; Carreira-Casais et al., 2021]. Finally, UAE can be utilized in conjunction with MAE and SFE, either alone or collectively, to significantly enhance extraction efficiency and diminish expenses [Kadam et al., 2013].

In MAE, the microwave system stimulates vibrations in water inside the cell, resulting in an elevation of intracellular fluid temperature, and a rise in temperature leads to water evaporation and the application of pressure on the cell walls, ultimately causing the cell wall to rupture and release intracellular contents into the surrounding environment [Hahn et al., 2012]. This technology offers reduced time and solvent usage, a more cost-effective alternative to SFE, and is mainly used to extract polysaccharides (fucoidan), pigments, and phenols from algal biomass [Kadam et al., 2013; Dulanlebit and Hernani, 2023].

The extraction of bioactive compounds from seaweeds via EAE may lead to degradation selectivity, high concentration of bioactive, and exhibit improved biological activity, therefore serving as a valuable option to overcome physical and chemical extraction limitations [Wijesinghe and Jeon, 2012]. The utilization of diverse enzymes such as protease, alginate lyase, α -amylase, agarase, cellulase, and pepsin to extract bioactive chemicals from seaweed is constrained in the market mostly due to the high prices associated with these enzymes [Sander and Murthy, 2009].

In SFE methods, CO₂ generally serves as a solvent, recognized for its cost-effectiveness, safety, nonflammability, and non-corrosiveness, and it can be readily and eliminated from the SWE [Pan et al., 2012; Crampon et al., 2011]. Moreover, CO₂ has a relatively low critical temperature and pressure, making it an outstanding solvent for the extraction of heat-sensitive bioactive chemicals from seaweeds, like carotenoids and pigments [Fiori et al., 2014]. This approach offers numerous advantages, including a rapid extraction ratio, a significant amount of productivity, efficiency, and safety, appropriate for industrial application [Crampon et al., 2011; Ibañez et al., 2011]; nevertheless, it also presents disadvantages such as substantial initial investment, the requirement for polar substances, and labor-intensive procedures.

Pressure fluid extraction is among the most promising methods, providing numerous advantages over conventional extraction techniques, including enhanced selectivity, significantly reduced processing time, and diminished usage of organic solvents [Ibañez et al., 2011; Jaime et al., 2005]. The PLE method, commonly employed for extracting antioxidants from SWE, utilizes water as a solvent while simultaneously applying elevated temperature and pressure to obtain the desired substances [Michalak and Chojnacka, 2014; Jacobsen et al., 2019], with the extraction temperature significantly influencing the method's efficiency and selectivity [Zakaria and Kamal, 2016].

Extraction efficiency may differ based on the seaweed species, solvent type and ratio, particle size, temperature, duration, pH, environmental factors, and other variables [Park et al., 2008]. Consequently, it is critical to determine the optimum and suitable reaction process to achieve maximum yield that is quantitative, non-destructive, time-saving, and efficient by adjusting all these influencing parameters [Wijesinghe and Jeon, 2012].

1.2.1.2. *Cystoseira barbata*

Cystoseira barbata, (syn. *Gongolaria barbata*), a species of brown algae, is classified within the order Fucales and belongs to the Sargassaceae family, and is located in the Adriatic, Black Sea, Mediterranean, Marmara, and Aegean Seas [Savonitto et al., 2021]. *C. barbata*, is regarded as generally resilient to eutrophication and is a long-

lived species typically found in shallow, protected, and well-illuminated marine coral environments, rock pools, and coastal marshes [Blanfuné et al., 2025]. Distinguished by a thickly branching tallus, these plants provide refuge, sustenance, and reproductive habitats for various organisms, thereby supporting ecosystem biodiversity [Thibaut et al., 2016; Chiarore et al., 2019]. Moreover, the prevalence of these species in their habitat signifies superior water quality [Manev and Petkova, 2021].

Although the biochemical content of *Cystoseira* varies for various reasons, it is generally rich in many vital bioactive compounds, including polysaccharides (fucoidan, alginate and laminarin), terpenoids, fatty acids and phlorotannins, and is known to have antioxidant properties [Ak and Türker 2018; Dobrinčić et al., 2021; Öztaşkent and Ak 2021; Orlando-Bonaca et al., 2021]. Moreover, *C. barbata* is classified under the Fucales order, recognized for its abundance of polyphenolic chemicals, including phenolics and flavonoids, which significantly enhance the antioxidant activity of macroalgae [Duan et al. 2006; Ak et al., 2022]. Various *Cystoseira* species have been shown to possess antiviral, antibacterial, anti-inflammatory, and antifungal properties [Orlando-Bonaca et al., 2021]. Furthermore, this edible alga is conventionally used as a functional food in the Black Sea regions [Pereira, 2017].

The proliferation, distribution, and viability of various algal species fluctuate due to natural environmental factors including water temperature, light, salinity, climate change, nutrients and anthropogenic stresses like habitat loss, pollution [Harley et al., 2012; Marletta and Lombardo, 2020]. The establishment of seaweed farms presents a viable answer to these issues [Zhang et al., 2022; Tullberg et al., 2022].

While the biostimulant potential of numerous *Cystoseira* species has been explored, *C. barbata* remains unexamined in this regard [Krid et al., 2024]. In the literature, a study was conducted on the effects of extracts obtained from *C. barbata* on the germination of various plants without mentioning their biostimulant effects [Demir et al., 2006]. On the other hand, the study published as part of this thesis describes for the first time the biochemical composition of *C. barbata* extracts and reveals their potential biostimulant effects on germination and growth of early wheat plants through substrate and seed applications [Mutlu-Durak et al., 2024].

1.3. Soilless Culture System

The soilless agriculture system, which is the fastest-growing sector of agriculture and has been adopted as an important technological component in modern greenhouse cultivation, plays a role as the driving force of agricultural production in overcoming various difficulties faced by soil-based agriculture [Hussain et al. 2014].

Although soilless culture systems are perceived as contemporary methods, the Hanging Gardens of Babylon and the wall paintings in the Deir el-Bahri temple showing Egyptians growing plants in soilless containers show that soilless agriculture has a history spanning approximately 4,000 years, dating back from ancient civilizations to the present day [Lakhiar et al., 2018; Waiba et al., 2019].

Higher yields can be obtained even in areas with adverse growing conditions thanks to eco-friendly soilless culture systems in which plants are grown by using many growing media other than soil, such as inorganic, organic materials, and synthetic substrates [Gruda, 2009; Hussain et al., 2014]. Beyond higher yields, soilless culture provides optimal growing conditions that consistently support reliable, healthy, and high-quality plant production compared to traditional open-field agriculture [Fussy and Papenbrock, 2022]. Additionally, these systems reduce the risk of soil-borne pathogens and eliminate the need for weeds control [Rajendran et al., 2024]. The widespread use of pesticides in soil-based agricultural systems, which contributes to environmental pollution, is avoided in these systems, leading to savings in time, labor, and overall costs [Savvas et al. 2013]. By applying nutrients and water to plants more equally, the efficiency of water and fertilizer use increases significantly compared to soil-based agriculture, thus helping to prevent waste by using fewer resources at the same time and making more production of the desired quality possible [Putra and Yuliando, 2015; Savvas, 2002].

Despite the significant benefits, soilless agriculture also has certain drawbacks [Fussy and Papenbrock, 2022; Resh, 2012]. The initial capital and energy expense is large, and large-scale commercial applications require technical expertise [Sonneveld, 2000; Resh, 2012]. Cultivating perennial plants is unfeasible in soilless agriculture, but often, high value-added annual crops are produced [AlShrouf, 2017]. Careful management of plant health is critical, as waterborne diseases can readily transmit between plants [Ikeda et al., 2002]. It is crucial to regulate pH, EC, and appropriate

nutrient concentration of the medium; failure to do so may restrict production performance and result in yield losses [Van et al., 2002].

Soilless culture systems are categorized into two types: substrate-culture, which utilizes a soilless medium for root growth, and hydroponic or water culture, where roots are mainly immersed in a nutrient solution lacking a substrate or soil in the rooting medium [Putra and Yuliando, 2015].

1.3.1. Substrate Culture

Substrate culture or solid media refers to a medium distinct from soil, using individually or in combinations of substrates that comprise either organic materials (peat, coco coir, bark, wood fiber, rice husks, biochar) or inorganic substances (sand, perlite, tuff, rockwool, vermiculite, zeolite and pumice) [Joshi et al. 2022; Mir et al., 2022]. This medium can exist in various forms to facilitate plant growth and development and must possess attributes such as superior water/nutrient retention capacity, high durability, biocompatibility, low bulk density, ample aeration space and devoid of pathogenic organism [Deepagoda et al., 2013; Hussain et al., 2014]. Furthermore, it allows more accurate and uncomplicated control over the supply and content of mineral nutrients and other components, allowing for more consistent crop performance and efficient resource management [Lee et al., 2022]. The selection of substrate material may differ based on the plant species, and the properties of the growing medium must fulfill all the plant's requirements [Fussy and Papenbrock, 2022]. The primary crops cultivated in substrate culture medium under greenhouse circumstances include tomato [Ghehsareh et al., 2011], pepper [Aslani et al., 2016] and strawberry [Tehranifar et al., 2006].

Coir fiber obtained from coconut mesocarp is a plant-based organic waste product, which is abundant in many Asian and Far Eastern countries, especially India and Sri Lanka, is exported worldwide from these countries [Abad et al., 2005; Raviv, 2013]. This fiber is extensively utilized, serving as a component of the growing medium for many crops in horticultural cultivation [Barrett et al., 2016]. Cocopeat culture possesses significant air and water holding capabilities, allowing roots to penetrate and develop inside the substrate readily [Kalaivani et al., 2019]. Coconut is entirely organic and may be readily recycled as a soil amendment and it does not contribute to

environmental pollution and is suitable for usage across numerous seasons due to its stability [Resh, 2022; Barker, 2021]. Conversely, when cocopeat is utilized in closed systems, microorganisms may proliferate in the substrate if the recirculated leachate is not properly treated. Coconut fibers insufficiently washed by the producer may retain high NaCl levels, requiring additional washing by the grower [Poulter, 2014]. It may possess potassium; therefore, the nutrient solution must be modified correspondingly, and the boards should be rinsed with a low-K nutrient solution [Ross et al., 2012].

Commercial production of perlite is based on a glassy volcanic rock that is pulverized, sieved and then heated to temperatures between 760 and 1100°C, and 70% of global perlite reserves are located on the Aegean coast of Türkiye [Doğan and Alkan, 2004]. An environmentally friendly and inert material with an extraordinary water retention capacity, capable of holding 3-4 times its weight in water, perlite is devoid of mineral nutrients and is often used as a sole substrate in soilless applications or potting soil mixes to reduce compaction. On the other hand, it has disadvantages such as high cost, high energy requirements during production, and aluminum toxicity at low pH levels [Raviv et al., 2019].

1.3.2. Hydroponic Culture

Hydroponic, one of the soilless cultures, is a plant-growing method in which the roots meet water and nutrients directly through aeration without using soil in the rooting environment [Fussy and Papenbrock, 2022]. The term hydroponics was originally described by Gericke in 1937, and the introduction of Hoagland's nutrient formula dramatically increased commercial crop yields in both solution culture and substrate culture systems [Gericke, 1937; Hoagland and Arnon, 1938; 1950]. Hydroponic systems can be more efficient and productive and at the same time more affordable in soilless production, compared to growing plants with soil and these systems are ideal for growing a wide variety of crops, including tomato, lettuce, and other leafy crops [Asao, 2012; Barrett et al., 2016; Sing et al., 2017]. Netherlands, a global pioneer in hydroponically cultivated vegetables and cut flowers, generates half of the total value of all fruits and vegetables produced in the nation through these systems [Fussy and Papenbrock, 2022].

The temperature, optimal concentration of the nutrient solution, pH, and EC values must be carefully regulated according to the specific requirements of the target plant to achieve maximum system efficiency and higher plant yield [Trejo-Tellez et al., 2012]. Electrical conductivity generally ranges from 0 to 5 dS/m in hydroponic cultivation, with recommendations for producers to maintain EC below 3 dS/m to promote optimal plant growth [Raviv et al., 2019]. In hydroponic cultivation, solution pH levels should generally be maintained between 5 and 7, with an optimal irrigation solution pH range of 5.5 to 6.5 [Savvas and Gruda, 2018].

Deep water culture, one of the oldest hydroponic systems, is a system in which plant production takes place in a relatively large container filled with nutrient solutions, with roots spreading freely in this liquid, supported by an air pump that oxygenates the water [Nursyahid et al., 2021]. One of the most widely used hydroponic systems, the Nutrient Film Technique, consists of long white channels with openings at the top for the placement of plants and involves growing plants by covering the roots with nutrient solutions without the use of substrate [Aires, 2018]. A thin layer of nutrients is applied to the roots by constantly or several times an hour pushing the nutrient solution through the root zone through foggers in an aeroponic system [Garzón et al., 2023].

As a cost-effective and easy-to-use practice to enhance crop production and decrease the need for chemical inputs, the addition of biostimulants from SWEs as growth-promoting products through nutrient solutions in soilless growing systems can be an important approach for sustainable agricultural production [Moncada et al., 2021; Miceli et al., 2021]. Although the integration of seaweed-based biostimulants into hydroponic systems in plant production is a promising, environmentally friendly production strategy, studies and their modes of action are still limited in the literature [Wang et al., 2022].

1.4. Salinity Stress

Salinity, a major abiotic stress factor affecting agriculture globally, causes significant losses in yield and quality, therefore poses a serious threat to world food security [Parihar et al., 2015]. Globally, salinity stress impacts about ~1 billion hectares of land across more than 100 nations, and is projected to affect 50% of arable land by 2050, resulting in considerable yield reductions in numerous crops [Atta et al., 2023].

Primary salinity results from natural processes like rock breakdown, which releases soluble salts like NaCl, CaCl₂, MgCl₂, SO₄⁻², and CO₃⁻² into the water. These salts then accumulate in the soil due to wind, precipitation, global warming, insufficient rainfall, and increased evapotranspiration [Zhu, 2001; Rouphael et al., 2018a]. Secondary salinity results from human activities like intensive agriculture, improper irrigation techniques, land clearing, the utilization of irrigation water with elevated salt concentrations, and inappropriate fertilizer [Rouphael et al., 2018a; Giordano et al., 2021].

Although salinity stress occurs initially triggers osmotic stress over the short term (ranging from minutes to days), prolonged exposure leads to ionic toxicity and ultimately results in oxidative stress in plants [Carillo et al., 2011]. Firstly, osmotic stress is intensified by elevated salt concentrations in the growth medium, which restricts water and nutrient absorption in plants by roots [Li et al., 2022b]. The diminished ability for water absorption induces osmotic disturbances that adversely impact stomatal aperture, transpiration, leaf expansion, root tip creation, new shoot development, and young leaf growth in plants, thus lowering nutrient usage efficiency and photosynthetic production [Annunziata et al., 2017; Negrao, et al., 2017]. Long-term exposure to salinity stress (ranging from days to weeks) induces ionic toxicity, leading to the accumulation of Na⁺ and Cl⁻ ions in plant cells and tissues, hence affecting many important plant processes, which disrupt metabolic balance and diminish plant growth and yield [Ibrahimova et al., 2021]. Ionic toxicity resulting from elevated ion concentrations that impair ionic homeostasis in plant cells manifests more gradually than osmotic stress and predominantly impacts the senescence of older leaves [Wungrampha et al., 2018].

Elevated Na⁺ concentrations in the environment diminish the availability of other ions, including K⁺, Ca²⁺, and Mg²⁺, because of competitive interactions, resulting in nutritional shortages and imbalances that adversely impact plant growth and development, eventually resulting in decreased yields [Atta et al., 2023; Pandit et al., 2024].

Under non-stress circumstances, reactive oxygen species (ROS), including singlet oxygen (¹O₂), hydrogen peroxide (H₂O₂), superoxide radical (O₂⁻), hydroxyl radical (OH⁻) mainly generated in mitochondria, chloroplast and peroxisome at low concentrations in plants function as signaling molecules in numerous critical processes

[**Hasanuzzaman et al., 2019**]. However, during stress conditions, as the closing of stomata leads to an accumulation of energy that is redirected to O₂, elevated ROS levels induce oxidative stress, resulting in detrimental effects on the plant, including DNA mutations, disruption of lipid metabolism and chlorophyll pigments, and protein degradation [**Naveed et al., 2020**].

The response of the plant to salinity stress may differ based on the amount, intensity and duration of the stress, and whether the onset is sudden or gradual, as well as the genotype and developmental stage of the plant [**Liang et al., 2018; dos Santos et al., 2022**].

1.4.1. Mechanism of Salinity Tolerance in Plants

Throughout the evolutionary process, plants have developed numerous adaptation mechanisms to endure saline conditions. These include selective ion absorption, accumulation, and excretion [**Shabala and Munns, 2012**]; control of hormonal equilibrium; alterations in evapotranspiration by decreasing of leaf size and adjustments in photosynthetic pigments [**Yasmeen et al., 2013**]; activation of antioxidant defence pathways; upregulation of stress-responsive genes; as well as expression of defensive proteins to mitigate salinity stress [**Ma et al., 2022**].

Under saline conditions, plants limit Na⁺ entry or accumulate this ion in their old tissues, preparing the ground for an environment where it can be eliminated later [**dos Santos et al., 2022**]. Ionic homeostasis, which regulates ion flux to establish a low Na⁺ concentration and a high K⁺ concentration in the cytosol, is crucial for the functionality of various cytosolic enzymes and for maintaining cell volume and membrane potential, as elevated Na⁺ levels impede K⁺ accumulation and stomatal regulation [**Hasegawa et al., 2000; Farooq et al., 2015**]. Na⁺/H⁺ antiporters, which are placed in the plasma membrane and tonoplast, are essential for preserving intracellular ion homeostasis by transporting Na⁺ from the cytoplasm into vacuoles or the extracellular environment [**Pehlivan et al., 2016; Cui et al., 2020**].

Osmotic adjustment is an adaptation mechanism that generally involves the accumulation of compatible inorganic ions as well as organic solutes including N containing osmolytes (polyamines, proline, glycine betaine), sugar alcohols (sorbitol, mannitol, pinitol), quaternary ammonium substances and sugars (sucrose and raffinose) synthesized and/or stored in the cytosol without causing any toxicity

problems to enable them to withstand ionic, oxidative and osmotic stress under salinity stress [Parihar et al., 2015; Kumar et al., 2021]. In addition, these compounds not only play an important role in osmotic adjustment but also demonstrate antioxidant properties by scavenging ROS, preserving membrane stability and the structure of proteins [Rahneshan et al., 2018].

When stressed environments scavenge high levels of ROS formation, plants can initiate protective defense mechanisms that encompass various enzymatic antioxidants, including catalase (CAT), glutathione reductase (GR), superoxide dismutase (SOD), ascorbate peroxidase (APX), peroxidase (POX) as well as non-enzymatic antioxidants such as ascorbate, glutathione (GSH), carotenoids, phenolic, flavonoids, and other secondary metabolites [Bacha et al., 2017; Giordano et al., 2021].

1.4.2. Salinity Stress in Soilless Systems

Hydroponic culture systems provide a regulated setting for investigating plant response to salinity stress. These systems offer accurate regulation of salt levels in the nutrient solution and enhance the monitoring of root as well as shoot growth patterns [Tester and Davenport, 2003]. Researchers can apply different degrees of salinity stress on wheat genotypes through a hydroponic system, thereby reducing confounding variables linked to soil-based systems, such as soil property heterogeneity and microbial interactions, thereby facilitating the systematic assessment of salinity tolerance traits among many genotypes and offer significant insights to the fundamental physiological and molecular processes associated with stress responses [Fellahi et al., 2024]. Hydroponic culture is considered more appropriate than soil screens for large-scale screening of many seedlings [Mujeeb-Kazi et al., 2019; Kulkarni et al., 2021].

Salinity stress, resulting from elevated salt concentrations in soil or water [Rouphael et al., 2017], is typically linked to soil-based agriculture; however, it also serves as a limiting factor for soilless agricultural systems due to NaCl accumulation from contaminated fertilizers and inadequate water quality [Katsoulas and Voogt, 2014;]. Soil or water with an EC exceeding 4 dS m^{-1} , corresponding to 40 mM NaCl, is classified as saline [Ibrahimova et al., 2021]. The elevated levels of NaCl in the

rooting medium may lead to leaf turgor loss due to osmotic effects, Cl and Na⁺ toxicity in various plant tissues, and potential nutritional imbalance [Alfosea-Simón et al., 2020].

To obtain optimum yields from plants grown in soilless farming systems, it is vital to use high quality water with low salt concentration in the formulation of a balanced nutrient solution. In contrast, increasing difficulties in supplying high quality water will lead to the use of saline water in soilless farming systems, and a nutrient solution with an EC exceeding the tolerance threshold of many vegetable crops will reduce yield and quality in plant production [Mariani and Ferrante, 2017; Vetrano et al., 2020].

Numerous studies indicated that the vegetative growth performance and yield of wheat cultivated hydroponically under saline stress diminished [Ashraf and Ashraf, 2016]. Moreover, Mutlu-Durak et al. (2023) reported that salinity stress resulted in diminished growth and development in hydroponically grown maize. Another study showed that elevated EC of the nutrient solution in a soilless system adversely affected tomato yield and overall growth [Krauss et al., 2006; Alfosea-Simón et al., 2020]. Ünlükara et al. (2008) reported similar findings, indicating a decrease in lettuce production following irrigation with water exhibiting a high EC level of 6.0 mS/cm.

In today's world, where the global climate crisis is becoming increasingly severe, as high-quality water resources become increasingly scarce and access to high-quality water for agricultural applications is becoming increasingly difficult [Assouline et al., 2015]. For this reason, plants grown in soilless systems may experience salt stress, and unless innovative strategies are developed to increase plant salt tolerance, problems associated with salinity stress in agricultural production will be inevitable [Niu et al., 2018; Kaloterakis et al., 2021]. Therefore, advancing knowledge and developing sustainable approaches for the effective use of saline water in horticulture, as well as mitigating salt stress in saline environments, are becoming priorities for vegetable growers and researchers [Hegazi et al., 2015].

Although the limited applications of seaweed-derived biostimulants in hydroponic systems [du Jardin, 2015; Sambo et al., 2019], recent studies indicate that SWEs can enhance plant yields by favorably influencing physiological processes and mitigating the adverse effects of salt stress [Deolu-Ajayi et al., 2022; Mughunth et al., 2024].

Consequently, the incorporation of algae-based biostimulants to enhance plant stress tolerance represents a sustainable and innovative approach in agricultural practices [Carillo et al., 2020a; Hernández-Herrera et al., 2022].

Specifically, SWEs can bolster plant defense mechanisms by activating certain metabolic pathways and/or stimulating formation of secondary metabolites, including non-enzymatic antioxidants, that enhance the antioxidant response and improve photosynthesis, growth, production, and quality [Rai et al., 2021; Dell'Aversana et al., 2021; Khan et al., 2022]. Overall, the augmenting and priming effects of SWEs on plant defense against abiotic stress can be ascribed to the biochemical composition and their ability to trigger a cascade of physiological and molecular responses within the plant [Yakhin et al., 2017]. Compatible solutes, which bolster plant resistance to various abiotic stresses, accumulate in plant cells in response to osmotic stress and can mitigate the detrimental effects of salt stress by maintaining cell volume, while also enhancing stress tolerance through the regulation of signaling pathways linked to the stress response [Ghosh et al., 2021]. SWE-based biostimulants have elevated levels of many osmolytes that may be partially taken by the plant and/or stimulate a mild and temporary osmotic stress [Di Stasio et al., 2018]. Ongoing osmotic priming during the growing period enhances the plant's ability to withstand subsequent stress, initiating a series of morphological and physiological adaptation mechanisms that improve the plant's response to unforeseen and seasonal stressors [Fleming et al., 2019]. SWEs encompass several advantageous compounds devoid of specialized osmotic action that may facilitate plant development and yield. They also interact with stress-protective compounds, including secondary metabolites such as phenolic compounds with non-enzymatic antioxidant properties, and exhibit hormone-like activity [Sharma et al., 2016; Muthu-Pandian et al., 2019]. Moreover, SWEs also comprise numerous biologically active components, such as complex polysaccharides and phycocolloids (fucoidan, alginate), which have demonstrated efficacy in inducing salinity tolerance in plants [Drira et al., 2021; Zou et al., 2019].

1.5. Crop Quality

The quality attributes developed throughout the plant's growth cycle, from initial development to the harvest stage, encompass a range of factors, including agricultural

traits (fruit size, yield potential, and resistance to pathogens), sensory characteristics (color, aroma, flavor, texture firmness, and morphological features), and nutritional and biochemical composition (phenolic compounds and antioxidant capacity) [Di Vittori et al., 2018]. These multifaceted attributes are not only shaped by genetic and environmental influences but also by agricultural practices and are critical in determining consumer safety, acceptance, and marketability [Rouphael et al., 2018b; Kutman, 2023].

Nitrogen (N) is crucial for crop quality, as its type, delivery technique, and dosage greatly affect plant growth and in green leafy crops, increased nitrogen levels promote chlorophyll production, leading to a more vibrant color that is more attractive to consumers [Barickman and Kopsell, 2016]. Larger fruit size is generally seen as an indicator of superior quality, but attaining the genetically predetermined size requires adequate nitrogen availability; yet excessive nitrogen can adversely impact on overall size and, subsequently, market quality [Schellenberg et al., 2009; Carranca et al., 2018]. Calcium (Ca) is an essential macroelement that influences the quality of fruits and vegetables; its lack frequently results in physiological diseases that undermine visual and structural integrity, overall quality [de Freitas and Mitcham, 2012]. Potassium (K), another important macronutrient, when low, can worsen physiological problems such as inadequate coloring, consequently reducing visual quality [Morgan et al., 2005]. Conversely, sufficient K application has been demonstrated to enhance flavor characteristics in diverse fruits, such as melons, apples, and cherries, by elevating total soluble solids (TSS), augmenting sugar content (sweetness), and regulating acidity [Alva et al., 2006; Lester et al., 2006; Solhjoo et al., 2017; Yener and Altuntas, 2020]. Sulfur (S), especially as organosulfur metabolites, significantly enhances the flavor, aroma and nutraceutical attributes of horticulture crops, highlighting its relevance for sensory as well as nutritional quality [Huchette et al., 2007]. Boron (B) shortage among micronutrients is linked to visual defects that diminish overall product quality [Rerkasem et al., 2020].

Crops not only supply vital nutrients but also encompass several kinds of antioxidants and health-enhancing compounds [Kutman, 2023]. The nutritional status of plants can substantially affect the levels of both helpful metabolites and antinutritional substances [Omirou et al., 2009]. Consequently, plant nutrition is interconnected with human

nutrition through the nutritional value of crops [Bouis and Welch, 2010; Bouis and Saltzman, 2017].

Phytochemicals, polyphenolic compounds in plants, are known for their physiological effects, including stress tolerance, color, odor, aroma, and flavor enhancement, as well as their capacity to improve quality parameters [Picchi et al. 2012]. The content and levels of phytochemicals in plants, which are considerable quality indicators due to their positive effects on human health including preventing many diseases as well as anti-cancer, antioxidant and anti-aging activity, can be affected by environmental factors and agricultural practices [Lako et al. 2007; Fratianni et al., 2020].

When growing a particular crop variety under a particular set of circumstances, the amounts of mineral elements needed to optimize yield are seldom the same as the amount needed to optimize quality [Kutman, 2023]. A frequently noted correlation between yield and quality attributes is elucidated by the "dilution effect," whereby heightened yields result in a diminished concentration of minerals or other vital substances as they are dispersed throughout a greater biomass [Marles, 2017; Wang et al., 2018; Kutman et al., 2023]. In a different situation, the peak achievable yield is reached at a nutrient input level that fails to improve quality; beyond this threshold, increased mineral supply may enhance certain quality traits without further augmenting production [Kutman, 2023]. Identifying the ideal conditions that concurrently enhance all aspects of crop quality is exceedingly difficult, as agronomic methods that improve one quality attribute may be inappropriate for enhancing others. At this moment, the use of plant biostimulants in agriculture provides a sustainable strategy for achieving high yield and improved product quality, two key characteristics for assuring economic production while also protecting the environment and human health.

In addition to enhancing harvestable crop yield, SWEs have been documented to augment the nutrient quality of various plants, including apple [Soppelsa et al., 2018], broccoli [Lola-Luz et al., 2014], cucumber [Valencia et al., 2018], lettuce [Yusuf et al., 2019], orange [Fornes et al., 2002], pepper [Paradićović et al., 2011], spinach [Fan et al., 2011; Xu and Lescovar, 2015], strawberry [Kapur et al., 2018], tomato [Ali et al., 2016].

The flavor and taste of fruit are primarily influenced by the levels of TSS, total acids, and their respective ratios [Krüger et al., 2012], thus increasing TSS in fruits by applications of algae-based biostimulants contributes to overall fruit quality [Kapur et al., 2018]. The modulation of photosynthesis and secondary metabolism by biostimulants, including SWEs, can lead to improvements in fruit and vegetable quality [Colla et al., 2017] and at the same time, the increase in secondary metabolites can contribute to human health by reducing many diseases caused by oxidative stress [Sangha et al., 2014].

Following the treatment of SWE, the fruits exhibit increased concentrations of beneficial and flavor-enhancing components, including anthocyanins, soluble carbohydrates including fructose and sucrose, polyphenols, and ascorbic acid [Valencia et al., 2018; Frioni et al., 2018; Kergosien et al., 2023]. Seaweed extracts from *A. nodosum* were shown to increase the anthocyanins and total phenolic contents in grapevines and berries, while in spinach, they increased the levels of phenolics, flavonoids, and antioxidants, and in strawberry plants, they improved the edible quality of the fruit by increasing TSS, sucrose, and fructose [Fan et al., 2011; Frioni et al., 2018; Kapur et al., 2018]. Another study has shown that the application of brown SWE on cucumbers significantly enhanced fruit quality by elevating total phenol content, antioxidant capacity, and vitamin C levels, alongside improving yield [Valencia et al., 2018]. Moreover, the shelf-life appears to be prolonged by the treatment of spinach [Fan et al., 2014], tomato [Widyastuti et al., 2019], grapes [Norrie and Keathley, 2006] with brown macroalgae extracts.

1.6. Wheat

Wheat, the third most produced crop globally after rice and corn, was first cultivated during the Neolithic Revolution about ten thousand years ago [Khalid et al., 2023]. Wheat is a staple crop for human nutrition and global food security, comprising thousands of varieties, including bread or common wheat (*Triticum aestivum*) and durum or pasta wheat (*Triticum durum*) [Çakmak and Kutman, 2018]. According to 2023 global wheat production data, China, India, and Russia are the three greatest wheat producers globally, collectively accounting for nearly 41% of total wheat production, while Türkiye ranks 9th [Web2].

Triticum durum, a wheat species of considerable economic significance worldwide and in Türkiye, is characterized by its elevated yellow pigment content and hard, vitreous endosperm, primarily employed in the manufacture of food products including pasta, spaghetti, bulgur, and couscous [Kiliç, 2010; Tedone et al., 2017; Özülkü, 2022]. Sarıçanak-98, a Turkish durum wheat cultivar, has become popular in southeastern Türkiye due to its high production possibility, resilience to widespread wheat diseases in the area, and tolerance to suboptimal soil conditions, especially those related to microelement shortages [Yildirim et al., 2013].

Wheat is not only a significant source of carbohydrates but also offers considerable quantities of important or beneficial health components, namely protein (albumin, globulin, glutenin), vitamins including B and E, lipids, minerals, dietary fiber, and phytochemicals that could aid in maintaining a balanced diet [Shewry and Hey, 2015; De Santis et al., 2021].

Global climate change with increasing drought may create salt stress in previously unaffected arable lands and worsen the effects of existing salt stress, thus negatively affecting wheat crop productivity [Miransari and Smith, 2019]. Consequently, considering the place of wheat in global food security, it will become even more important as a promising and sustainable approach to utilize the power of plant biostimulant including SWEs, which mitigate the negative impacts of stress for sustainable wheat production in saline regions, which will cause even more devastating results in the near future [Sellami et al., 2025].

Numerous studies have indicated that extracts from various macroalgae species function as biostimulants, offering multiple advantages in promoting early-stage or vegetative growth in wheat, including enhanced root elongation, increased chlorophyll content, improved osmoprotection mechanisms, higher relative water content, reduced oxidative stress, greater stress tolerance, and improved mineral nutrient uptake [Latique et al., 2017; Ali et al., 2021; Mutlu-Durak et al., 2024].

1.7. Lettuce

Lettuce (*Lactuca sativa* L.), belonging to Asteraceae family, is one of the most popular leafy vegetables worldwide, widely cultivated and consumed primarily in the raw form [Kim et al., 2016; Ottaiano et al., 2021]. Lettuce, with its short growth cycle and ease

of cultivation, is an ideal candidate for year-round hydroponic production and remains a leading choice among leafy vegetables in soilless farming systems [Dasgan et al., 2023]. According to 2023 data, China produces more than 50% of the world's lettuce, while Türkiye, where most of the production is carried out in the Aegean, Mediterranean and Marmara regions, ranks sixth globally with a lettuce production volume of 577,773 tons [Web3].

Lettuce plays a critical role in the Mediterranean diet and as a source of plant nutrients with high levels of health-enhancing attributes, including antioxidants molecules including carotenoids, phenolic compounds like polyphenols, and flavonoids, and is also rich in vitamins (A, C, K and E), fiber, carotenoids, low calorie, low levels of fat, some macro and microelements (Fe, Ca) and also several other minerals [Mou, 2012; Ahmed et al., 2021]. The amount and quality of these features may fluctuate based on many pre-harvest conditions, including agronomic strategies [Sofa et al., 2016].

Lollo Rosso, a rapidly proliferating lettuce variety characterized by its vivid red pigmentation [Cammarisano et al., 2020], is abundant in phytonutrients including phenolic acids, flavonoids, and anthocyanins and excessive consumption of these chemicals has been linked to a diminished occurrence of chronic and degenerative disorders [Tsormpatsidis et al., 2008; Sublett et al., 2018].

The integration of open field and greenhouse environments, alongside contemporary production technologies like vertical farming and the potential for year-round cultivation with a brief growth cycle, is significantly enhancing the economic significance of lettuce and providing advantages that promote its extensive cultivation [Kacar and Ulukapi, 2025]. This reliance on synthetic fertilizers for lettuce production raises substantial concerns regarding environmental impact and sustainability; therefore, the potential application of biostimulants as an alternative or supplementary input is crucial for a sustainable approach [Hassan et al. 2021; Hong et al. 2022].

Key parameters considered in the quality assessment of lettuce include soluble carbohydrates, proteins, certain phenolic compounds (flavonoids) and photosynthetic pigments (chlorophyll and anthocyanins) that contribute to taste and aroma [Wang et al., 2022]. These compounds are not only involved in the metabolic processes in the plant but are also important for human health due to their antioxidant properties [Lee

et al., 2017]. The effects of plant biostimulants on lettuce are not limited to increasing yield [Di Mola et al., 2019], they also augment nutritional quality by enhancing antioxidant levels [Chrysargyris et al., 2018; Di Mola et al., 2019; Ottaiano et al., 2021] and inducing the uptake of macro- and micronutrients [Noroozlo et al., 2019; Yaseen and Takacs-Hajos, 2022]. There are many studies in the literature about the treatment with SWEs on hydroponically grown lettuce plants [Oñez et al., 2025; Miceli et al., 2021]. These applications have been reported to enhance plant growth and improve yield [Sandepogu et al., 2019; Di Mola et al., 2019] and many quality traits like chlorophyll and carotenoids content, antioxidant activity [Jung and Kim et al., 2020], stomatal conductance, potassium content [Chrysargyris et al., 2018] as well as postharvest quality parameters [Miceli et al., 2021].

1.8. Pepper

Pepper (*Capsicum* spp.) commercialized in many fruit colors and shapes varieties, is one of the most economically important crops worldwide, as it provides productivity, fruit quality, and favorable market prices for growers [Melo et al., 2020]. Pepper, whose origin dates back to 7500 BC, is based on the tropical regions of America, and it is thought to have spread to Europe and then Africa and Asia after Columbus discovered the American continent [Penella and Calatayud, 2018].

Pepper is an valuable agricultural product not only due to its economic value but also because of the nutritional benefits of its fruits in human diet, which serve as a rich source of mineral elements (Ca, Fe, K, Mg), natural pigments and antioxidant components including carotenoids, capsaicinoids, polyphenolic compounds (quercetin) and pro-vitamin A and E (tocopherols), vitamin C [Paradić et al., 2011; García-Jiménez et al., 2018; Hervert-Hernández et al., 2010; Barrajió-Catalán et al., 2020]. The concentration and profile of these compounds display significant diversity within genotypes and are influenced by various factors, including fruit developmental stage, cultivation methods, and post-harvest processing techniques [Wahyuni et al., 2013]. These molecules, beneficial for human health due to their anti-aging, antioxidant and anti-cancer properties, significantly contribute to the prevention of several diseases, including cardiovascular, immunological, and digestive

disorders, when consumed consistently and in sufficient amounts [Paradić et al., 2011; Mwebi et al., 2020].

The recommended daily intake (RDI) of vitamin C for woman is 90 mg and 75 for men [Monsen, 2000], a 100 g portion of pepper generally provides about 100% of the necessary daily intake of vitamin C and tocopherol, while supplying about 5–10% of the daily requirement for provitamin A [Rosa-Martínez et al., 2021; Wahyuni et al., 2013].

Capsicum annuum is the one the most extensively distributed and economically valuable species within the Capsicum family [Yildiz et al., 2020]. Pepper, brought to Istanbul in the 16th century throughout the Ottoman Empire [Yayman et al., 2024], displays considerable morphological variation, including various colour, forms, bitterness levels, and flavor profiles [Wang and Bosland, 2006]. Moreover, Türkiye ranks among the world's leading pepper-producing nations, contributing 2.6 million tons to the global pepper production of 36.1 million tons in 2020 [Karataş et al., 2024]. Unlike pepper varieties grown all over the world, such as bell pepper, long green pepper is widely grown, mostly native to Türkiye, and can be consumed fresh or used in a variety of ways, including dishes, fried foods, pickles, ready meals, frozen products, canned products and medicine [Başaran and Engindeniz, 2015]. This crop exhibits significant adaptability and may be cultivated in various production systems, encompassing both open-field and greenhouse conditions [Chatzisyneon et al., 2017; Melo et al., 2020].

Numerous studies have shown that foliar application of SWE to pepper increased growth parameters, fruit number and yield [Ali et al., 2022], nutritional value, mineral content and quality [Mohamed et al., 2021], antioxidant activity [Ashour et al., 2021], as well as an extended shelf life [Khan et al., 2018].

2. PURPOSE OF THE STUDY

While it is known that SWEs derived from brown algae are widely used as biostimulants in agricultural practices, studies specifically focusing on *C. barbata* remain extremely limited. To date, no studies have explored the production of plant biostimulants from *C. barbata* using different extraction techniques or evaluating their effects in soilless agricultural systems.

This thesis aimed to investigate the biostimulant potential of *C. barbata*, a locally available brown seaweed, by comparing the biochemical properties and plant effects of its water, acid, and alkali extracts obtained through different extraction methods. To achieve this, the extracts were characterized in terms of mineral content, total phenolics, amino acids, antioxidant capacity, and auxin-like activity.

The effects of these extracts on growth, development, yield, and quality were evaluated under different soilless cultivation systems (perlite, deep-water culture, and substrate culture) using multiple application methods.

Two perlite-based experiments were conducted by applying *C. barbata* extracts directly to seeds or to the growth medium to evaluate their performance on wheat (*Triticum durum* cv. Sariçanak-98) germination and seedling development, identifying the most effective extract type and optimum application levels (low, medium, high).

Hydroponic experiments were conducted to evaluate the potential of *C. barbata* extracts to alleviate the stress-induced effect on wheat exposed to salinity stress in a model system and to investigate the underlying tolerance mechanisms through physiological analyses.

The performance of the extracts was also evaluated in lettuce (*Lactuca sativa* L. cv. Lollo Rosso) and pepper (*Capsicum annuum* cv. Fiyaka F1) under soilless conditions. In lettuce, various concentrations of the extracts were added to the nutrient solution to assess their effects on yield, and quality. In pepper, foliar applications of the extracts were compared with a commercial seaweed-based product in terms of yield and fruit quality.

3. MATERIAL AND METHOD

3.1. Collection and Preservation of *C. barbata*

Cystoseira barbata was gathered in February 2020 from the shallow areas (maximum depth of 1 m) along the Tuzla/Istanbul coast (40° 49' 30" N, 29° 16' 44" E) in the Marmara Sea (**Figure 3.1A-B**). The samples of algae were obtained by separating the seaweed without compromising their attachment points where they attach themselves to the stone (**Figure 3.1C**).

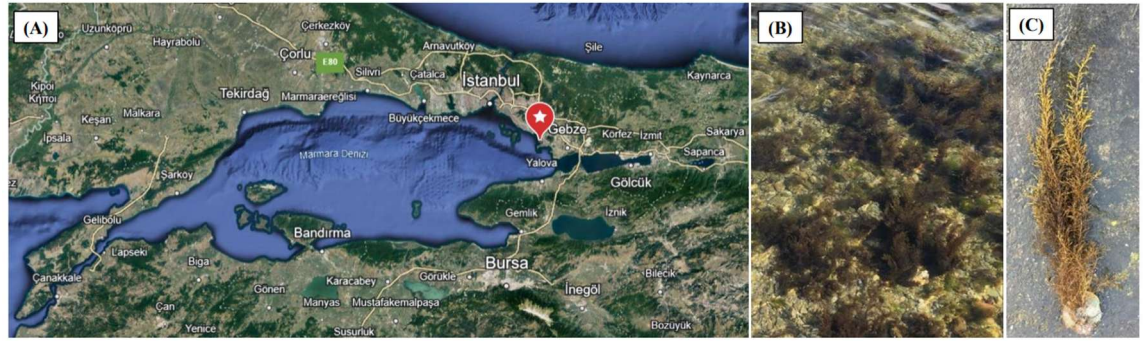


Figure 3.1: *Cystoseira barbata* collected from the Tuzla / Istanbul coast of the Sea of Marmara (40° 49' 30" N, 29° 16' 44" E) (A), algae biomass found in the area (B), and detailed picture of the seaweed growing on the stone (C) [Mutlu-Durak et al., 2024]

The harvested samples were then transported to the laboratory on the same day, where they were rinsed three times with deionized water to ensure the removal of extraneous substances and excess seawater. Some of these seaweeds were dried in an oven at 60°C for 3 days, ground into fine powder with a laboratory blender, and stored in ziplock bags. The remaining part of the collected and washed seaweed was stored at -80°C as a frozen sample after draining the excess water.

3.2. Preparation of *C.barbata* Extracts

The extraction of *C. barbata* was conducted by using three different methods: water, acid, and alkali extractions as described below

The obtaining of water extracts from dried seaweed was executed utilizing the methodology outlined by **Sharma et al., [2014]**. One hundred grams of dried and pulverized seaweeds were mixed with deionized water to reach a total volume of one liter and maintained at 70°C for 3 hours with constant stirring by a magnetic stirrer. Following filtration with cheesecloth and centrifugation at 8000 g for 15 minutes, the supernatant was obtained.

Acid extraction was carried out according to the method described by **Ertani et al., [2018]** and **Shukla et al., [2019]**. 500 g of frozen seaweed samples was ground with the help of a laboratory blender with deionized water into a total volume of 1 L. The pH of this mixture was reduced to 3.0 ± 0.1 by using H_2SO_4 . The mixture was incubated in a hot water bath at 45°C for 30 minutes and then centrifuged at 8000 g for 15 minutes. After filtration using a cheesecloth, the pH of the supernatant was neutralized to $pH 7.0 \pm 0.1$ using KOH.

Alkali extraction is a prevalent approach for producing biostimulants from brown seaweeds [**Shukla et al., 2019**], functioning as both an extraction and hydrolysis process. This extraction was conducted following the methodologies outlined by **Craigie [2011]** and **Shukla et al., [2019]**. The frozen seaweed samples were processed according to the acid extraction methodology up to the centrifugation step, as previously outlined. In contrast to the acid extraction procedure, following the incubation period at 45 °C for 30 minutes, KOH was introduced to the extract, and its pH was set to 12.0 ± 0.1 . The extracts were placed in a water bath at 80°C for 3 hours, followed by the centrifugation step was repeated. Following filtration using cheesecloth, the liquid phase was obtained, and the pH was set to 7.0 ± 0.1 using H_2SO_4 . These extracts were preserved at -80°C until utilization.

3.3. Characterization of *C. barbata* Extracts

The concentrations of polysaccharides (alginic acid, fucoidan, and laminarin) and mannitol in SWEs were quantified through assays conducted by Boğaziçi University (**Table 3.1**). **Table 3.1** indicates that the largest concentrations of polysaccharides (alginic acid and fucoidan) were found in water (11.6 g l^{-1} and 45.9 g l^{-1}), alkali (5.0 g l^{-1} and 42.4 g l^{-1}), and acid (1.0 g l^{-1} and 36.0 g l^{-1}), respectively. Fucoidan constituted the predominant polysaccharide in all extracts. In contrast to other polysaccharides,

the minimal quantity of laminarin was seen in the water extraction method. The concentration of mannitol, a sugar alcohol, in *C. barbata* extracts ranged from 1.1 to 3.0 g l⁻¹ of seaweed extract.

Table 3.1: Effect of different types of seaweed extractions (Water extracts, Alkali extracts, Acid extracts) on the contents of polysaccharides (alginic acid, fucoidan and laminarin) and mannitol (g l⁻¹) of SWEs.

Extraction	Alginic Acid	Fucoidan	Laminarin	Mannitol
	(g l ⁻¹)			
Water	11.6 ± 0.2	45.9 ± 0.1	4.2 ± 0.1	3.0 ± 0.3
Alkali	5.0 ± 0.1	42.4 ± 0.2	6.5 ± 0.0	1.1 ± 0.1
Acid	1.0 ± 0.0	36.0 ± 0.0	5.9 ± 0.0	2.9 ± 0.4

Mineral analysis, total phenolic substance, total free amino acid, and auxin-like activity were determined in these extracts.

3.3.1. Mineral Analysis

The mineral analysis was conducted following the methodology modified by **Ceylan et al., [2016]**. Approximately 0.5 ml of each extract was allocated to digestive tubes. The samples were subjected to acid digestion with 2.0 mL of 30% hydrogen peroxide (H₂O₂) and 5.0 mL of 65% nitric acid (HNO₃). The wet digestion of SWEs was conducted using a microwave device (Mars Express; CEM Corp., Matthews, NC, USA). After the samples were completely cooled, the total volume was adjusted to 20 mL by the addition of double-deionized water, followed by filtration using filter papers (Macherey-Nagel, Ø125 mm, blue band). The quantities of macronutrients (Ca, K, Mg, S, P) and micronutrients (B, Cu, Fe, Mn, Zn) in extracts were quantified using inductively coupled plasma optical emission spectrometry (ICP-OES) (Agilent 5800, USA).

3.3.2. Total Phenolic Substance Analysis

The total amount of phenolic substances of the SWEs was measured using the Folin-Ciocalteu method [**Singleton and Rossi, 1965**]. In the reaction, 400 µl of 0.5 N Folin reagent and 400 µl of 10% Na₂CO₃ solution were mixed with 20 µl of the sample, and

the total volume was calibrated to 1.5 ml with distilled water. Gallic acid solutions (0-1.6 mg/ml) were prepared as standards and underwent the same procedure as the samples. After a 30-minute incubation, the absorbance (Abs) values of the standards and samples at 760 nm were measured using a spectrophotometer (Peak Instruments C-7100S), and the content of phenolic compounds in the samples was ascertained by the established standard curve. Results were quantified in grams of Gallic Acid Equivalent (GAE) per liter (g GAE l⁻¹) of fresh extracts.

3.3.3. Total Free Amino Acid Analysis

The total amount of free amino acids in SWEs was measured by the method outlined by **Sadavisam and Manickam [2005]**. 0.2 M citrate buffer was made utilizing citric acid and pH was adjusted to 5 with 10 M NaOH. 0.5% (w/v) ninhydrin was solubilized in ethanol. A solution of ninhydrin was mixed with citrate buffer at a ratio of 1:1 and 0.05% (w/v) SnCl₂.2H₂O was dissolved in this mixture. This ninhydrin reagent was freshly prepared for analysis. Glutamic acid was solubilized in K-P buffer and used as the amino acid standard. 1 ml of ninhydrin reagent was combined with 0.1 ml of sample or standard and the mixture was incubated in a 90 °C water bath for 10 minutes. Absorbance values were measured at 570 nm using a UV/Vis spectrophotometer. Results were quantified in g of Glutamic acid Equivalent per liter (g l⁻¹) of fresh extracts.

3.3.4. Auxin-like Activity Analysis

To determine the hormonal effects of SWEs, **Colla et al., [2014]** used the "corn coleoptile elongation rate test" method. Corn seeds were germinated in darkness for 7 days under controlled climate chamber settings. Following this, the apical sections of the coleoptiles, measuring 3-4 mm in length from an approximate total length of 3 cm, were excised, and a 2 cm segment was extracted from the remaining portion. Ten coleoptile segments were put in 10 cm diameter petri dishes with 20 ml of liquid in each. The required solutions for this purpose are: indole-3-acetic acid (IAA) at a concentration of 1.75 mg/L, filtered distilled water, or SWE including Water Extract (0.04%), Alkali Extract (0.1%), and Acid Extract (0.1%). Four independent replications were conducted for each application, and the experiment lasted 48 hours.

After this duration, coleoptile lengths were quantified utilizing the Image J software, and the auxin-like impacts of SWEs were assessed by contrasting them with IAA.

3.3.5. Determination of Antioxidant Capacity

3.3.5.1. ABTS assay

Seaweed extracts were tested for their ability to scavenge free radicals using the 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonate; ABTS) method, as detailed by **Re et al., [1999]**. An aqueous solution containing 7 mM ABTS and 2.45 mM potassium persulfate ($K_2S_2O_8$) was combined in equal volumes to create the $ABTS^{•+}$ stock solution. Before being used, the combination was stabilized for up to 16 hours in a dark environment. Following incubation, ethanol was added to the dark blue-green $ABTS^{•+}$ stock solution to achieve the ideal Abs value (~ 0.70) at 734 nm. After combining 10 μ l of SWE or standard with 1 ml of diluted ABTS solution and letting it sit in the dark for 10 minutes, the final Abs were measured at 734 nm to assess the $ABTS^{•+}$ scavenging activity. The following formula was used to determine the ABTS radical's scavenging activity: Scavenging activity (%) = $((A_0 - A_1)/A_0) * 100$, where A_0 represents the control Abs and A_1 represents the extract solution Abs. Trolox [($\pm$)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid] solutions (0–1.5 mM) were utilized as standards. The data are given as mmol Trolox equivalent (TE) per liter (mmol TE l^{-1}).

3.3.5.2. DPPH assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) method was conducted using the methodologies established by **Miller and Rice-Evans [1997]** and **Sanchez and Calixto [1998]**. 100 μ l of the SWEs or standard was combined with 3.9 ml of 100 μ M DPPH in methanol, and the reaction mixture was left to sit at room temperature in the dark for 120 minutes. A spectrophotometer was used to detect Abs at 515 nm following incubation. A calibration curve in the range of 0–1.5 mM Trolox solutions was used to compute the values. The scavenging activity formula was used to determine the samples' percentage of DPPH decolorization. The findings were expressed as mmol TE l^{-1} .

3.3.5.3. FRAP assay

A revised version of the Ferric-reducing antioxidant power (FRAP) method, as described by **Benzie and Strain [1996]**, was employed. The FRAP reagent was prepared daily by combining 300 mM acetate buffer (pH 3.6), 10 mM tripyridyl triazine (TPTZ) in 40 mM HCl, and 20 mM ferric chloride ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) in a 10:1:1 (v/v/v) ratio. Incubate 30 μL of sample solution or Trolox standards, 90 μL of deionized water, and 900 μL of freshly made FRAP reagent and mixture in a water bath at 37°C for 30 minutes. A spectrophotometer was then used to detect the change in Abs caused by the antioxidants in the SWEs reducing the ferric-TPTZ) complex at 593 nm. The FRAP assay also employed Trolox as the standard solution to create a standard curve (0-1.0 mM), with the results represented in mmol TE l^{-1} .

3.4. Determination of the Effects of *C. barbata* Extracts on Germination and Early Growth Periods in Wheat

3.4.1. Plant Material and Growth Conditions

This thesis study employed durum wheat seeds (*Triticum durum* cv. Sarıçanak-98), which were developed and registered in 1998 by the GAP International Agricultural Research and Training Center.

The two perlite experiments were performed in a growth chamber under regulated environmental circumstances with light/dark intervals of 16/8 hours, temperatures of $25/20^\circ\text{C}$, and relative humidity levels of 60/70.

All thesis study, with the exception of the germination experiment, were conducted in a Venlo-type, automatic-controlled glass greenhouse equipped with central heating and an evaporative cooling system at the Biotechnology Institute of Gebze Technical University in Kocaeli, Türkiye. The temperature in this research greenhouse with geographical coordinates of $40^\circ 48' 24''$ N and $29^\circ 21' 46''$ E is kept at around $25 \pm 5^\circ\text{C}$ during the day and $20 \pm 5^\circ\text{C}$ at night. There is supplementary lighting and also a thermal curtain system that can be used to extend the daytime and increase the light intensity in the winter months.

Two perlite experiments were conducted to investigate the impact of *C. barbata* extracts on wheat seed germination and early plant growth. The first of these was about

adding the extracts to perlite, the growth medium, while the other experiment was to directly impregnate the seeds with SWEs.

3.4.2. Application of SWEs to Perlite Medium (Substrate Experiment)

The objective of this perlite experiment was to evaluate the efficacy of SWEs derived from *C. barbata* as a biostimulant and to assess the impact of these extracts, when administered directly to a perlite medium, on wheat germination and short-term growth. The water holding capacity of this quantity of perlite was ascertained to be 150 mL. In this context, 40 g of dry perlite was created in 300 ml plastic containers by including water with varying concentrations of SWE, in which wheat seeds were germinated. It will be consistently irrigated with deionized water during the germination phase, following its water requirements. This experiment examined three levels of seaweed (low, medium, and high) from each of the three extraction methods water extracts (2, 4 and 6 mL were diluted in 150 mL deionized water) and alkali and acid extract (0.5, 1.0 and 1.5 mL per 150 mL deionized water) resulting in a total of ten application groups, each with five duplicates, alongside a control group (water). The germination and development of wheat seeds were monitored for 6 days.

At the end of the experiment, the germination percentage (%) was determined as follows:

$$\text{Germination Percentage (\%)} = \left(\frac{\text{number of germinated seeds}}{\text{number of planted seeds}} \right) * 100.$$

Furthermore, the fresh weights (FW) of both the shoots and roots of all wheat plants were measured. Ten plants were randomly selected from each of the five replicate boxes for the analysis of shoot and root esterase activity. Shoots and roots from an additional set of 10 plants from each box were utilized for shoot height measurements and comprehensive root imaging investigations. The remaining plants were dried and utilized for mineral analysis.

3.4.3. Seed Soaking with SWEs (Seed Treatment)

This investigation utilized SWEs as seed treatment agents, and their impact on seedling performance and early seedling viability was observed. Experiments were performed

in a perlite media under controlled growth chamber conditions. To achieve this, 40 wheat seeds were immersed in the same solutions as in the first experiment with SWE for 16 hours at ambient temperature. Upon completion of this procedure, seeds were sown in 300 ml plastic containers filled with perlite saturated with water and observed for 6 days. Following the water requirements, they were irrigated with deionized water during the germination phase. This experiment included three levels of seaweed (low, medium, and high) selected from each of the three extraction methods (water, alkali, and acid), along with a control group treated only with water, resulting in a total of ten application groups and five repetitions for each group.

At the end of the 6th day, similar to the first experiment germination %, fresh weights of shoots and roots, esterase activities in shoots and roots, shoot length, and comprehensive root imaging studies were also conducted.

3.5. Investigation of The Effects of *C. barbata* Extract Applications on Salinity Stress Resistance and Related Physiological Parameters in Wheat

For use in hydroponic culture experiments, wheat seeds were germinated for approximately one week in perlite growth medium impregnated with a solution containing 2 mM Ca in the form of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$. They were watered with deionized water throughout the germination period according to their water needs. At the end of a week, the plants were transferred to pots with lids made of lightproof black plastic containing 4.5 l of nutrient solution. A total of 15 one-week-old wheat seedlings were placed in 5 holes opened at equal distances in the pot lids, with 3 plants in each hole, using sponge cut to surround the root collar.

When plants were transferred to the hydroponic system, they were first grown in half-strength nutrient solution conditions to prevent roots from experiencing transplant shock and then transferred to the full-strength nutrient solution medium 2 days later. Nutrient solutions were prepared using only analytical grade chemicals and high-quality deionized water and were renewed once a week. The ventilation of the pots was provided continuously with the aquarium pump. The prepared pots were placed randomly in the greenhouse using the randomized plots trial pattern.

The contents of the hydroponic nutrient solution used in the wheat experiment were as follows: the full-strength nutrient solution contained 4 mM Ca (NO₃)₂·4H₂O, 0.2 mM KH₂PO₄, 1.2 mM K₂SO₄, 0.1 mM KCl, 0.75 mM MgSO₄·7H₂O, 75 µM Fe (in the form of FeEDTA), 5 µM H₃BO₃, 2 µM MnSO₄·H₂O, 3 µM ZnSO₄·7H₂O, 0.6 µM CuSO₄·5H₂O, 0.50 µM (NH₄)₆Mo₇O₂₄·4H₂O.

3.5.1. Determination of The Effective *C.barbata* Extracts Application Levels on Plant Growth in a Hydroponic Environment

This study aimed to determine of types and application doses of *C. barbata* extracts that positively influence the growth of wheat plants grown under greenhouse circumstances added to the nutrient solution in a hydroponic medium on the growth and mineral nutrition of wheat.

The experiment consisted of 40 pots, including three SWE application levels from each of the three extraction methods, along with a control group receiving no SWE treatment. Each application group included 4 pot replications in the experimental design. Seaweed extracts were incorporated into the hydroponic system concurrently with the solution change when the plants were 14 and 21 days old. The experiment concluded after the plants reached 28 days old.

The concentrations of SWE to be utilized were established as follows:

- Water extraction: 10, 20, and 40 ml of extracts were incorporated into 4.5 l of nutrient solution.
- Alkali extraction: 2.5, 5, and 10 ml of extracts were incorporated into 4.5 l of nutrient solution.
- Acid extraction: 2.5, 5, and 10 ml of extracts were incorporated into 4.5 l of nutritional solution.

At the end of experiment, shoots and roots were harvested separately. The DW of shoots and roots were measured. The roots were meticulously rinsed with adequate deionized water before harvest, dried at 70°C for three days, weighed, and pulverized into a fine powder; these samples were utilized for mineral analysis in the tissues.

3.5.2. Determination of Effective Salt Stress Application Levels on Plant Growth in a Hydroponic Environment

The second experiment was carried out to determine the effective salt stress application levels on plant growth in a hydroponic environment. Within the scope of this study, wheat plants grown in hydroponic and greenhouse conditions were exposed to 5 different salt levels (30 mM, 60 mM, 90 mM, 120 mM, 150 mM NaCl) apart from the control application. In the experiment, there were 4 pots in parallel in each application group, and there was a total of 24 pots. Salt applications were started 1 week after the plants were given a full-strength nutrient solution under hydroponic conditions; the specified salt doses were applied to the solution together with the nutrients at each solution change. The experiment was 2 weeks after the salt application started.

During harvest, the shoot parts and roots of all plants were harvested separately and dried at 70°C for 3 days, then weighed to determine their dry weight. Salt doses that reduce the weight of plant shoot parts by 15-25% and 20-30% were determined and were used as salt levels.

3.5.3. Effects of Selected *C. barbata* Extract Applications on Salinity Stress Resistance and Physiological Parameters

In this experiment, in which the potential of *C. barbata* extracts on the tolerance of wheat to salinity stress was investigated, plants were grown as described in the first hydroponic experiment. One week after the plants were grown in a full-strength nutrient solution, salt stress, and SWEs were added to the nutrient solution of the plants simultaneously with the solution changes during the solution change. This experiment consisted of 4 SWEs (1 dose chosen based on the first hydroponic experiment, alongside a control application without SWEs), 2 salt doses (salt levels determined from the second hydroponic results, in addition to a control), and 4 pots parallel for each application group, resulting in a total of 48 pots. The experiment was terminated 2 weeks after the SWEs, and salt application was started as described in the second hydroponic experiment.

All shoot parts and roots in the pot were harvested separately. The roots were gently washed with plenty of deionized water during harvest. The 6 out of 15 plants from each pot were dried at 70°C for 3 days, weighed and ground into fine powder, and these samples were used to determine the minerals in the tissues. During this analysis,

in addition to the mentioned minerals, the amount of Na in the tissues was also measured with the method explained under the title of mineral analysis. These samples were also used for the measurement of glycine betaine, the details of which are given below. As soon as the second oldest leaf of 1 plant in each pot was cut, it was subjected to the REL test, the method of which is explained below.

All leaves from the remaining eight plants in each pot were collected for analyses requiring fresh samples. The leaves were cut into fragments with a maximum width of 5 mm, and the pooled material was divided into three subsamples. Each fragment was weighed to determine its fresh weight for subsequent analyses. The samples were then individually packaged and immediately flash-frozen in liquid nitrogen. In these shoot samples, protein concentration, activities of antioxidative enzymes (SOD, CAT, APX, GR), free proline, and malondialdehyde (MDA) levels were determined as described below.

3.6. Determination of The Effects of *C. barbata* Extracts on Yield and Quality Parameters of Lettuce (*Lactuca sativa* L. cv Lollo Rosso) in the Soilless Culture System

3.6.1. Plant Materials and Growth Conditions

In this study, lettuce (*Lactuca sativa* L. cv. Lollo Rosso), is red lettuce variety from the Genta Prestige brand, was used. It features red, frilled, and curly leaf morphology, exhibiting a compact structure, appropriate for growth in open fields or greenhouses. The development period typically spans 45 to 60 days, contingent upon conditions, and it thrives in mild to fairly warm climate.

This study aimed to identify the types and application dosages of SWEs which positively affect the growth of lettuce plants cultivated under greenhouse conditions and to elucidate the consequences of these applications. Greenhouse conditions were as described above for the wheat hydroponic experiment.

3.6.2. Experimental Designs and Seaweed Extract Treatments

The contents of the hydroponic nutrient solution used in the lettuce experiment were as follows: the hydroponic nutrient solution contained 4.65 mM Ca (NO₃)₂·4H₂O, 0.35

mM KH_2PO_4 , 1.35 mM KNO_3 , 0.65 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.15 mM KCl , 50 μM Fe (in the form of FeEDTA), 20 μM H_3BO_3 , 5 μM $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 5 μM $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 1 μM $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and 0.50 μM $(\text{Na}_2) \text{MoO}_4 \cdot 2\text{H}_2\text{O}$.

In this experiment, lettuce seeds were germinated in vermiculite, moistened with a solution containing 2 mM Ca in the form of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ for 10 days under greenhouse conditions. They were watered with deionized water throughout the germination period according to their water needs. After 10 days, the lettuce seedlings considered appropriate for transplantation were relocated to pots with a lid made of lightproof black plastic containing 4.5 l of nutrient solution.

After 10 days, the lettuce seedlings deemed appropriate for transplantation were moved to pots covered with a lid made of lightproof black plastic containing 4.5 l of nutrient solution, with 5 holes opened at equal distances in the pot lids, with 1 plant in each hole, using sponge cut to surround the root collar.

The experiment was carried out similarly to the first hydroponic wheat experiment. This experiment had 40 pots, incorporating three seaweed extract levels selected from each of the three extraction processes, a control group without SWE, and four repetitions per application group. All pots had 4 lettuce plants, thinned to 3 lettuce plants when the plants were approximately 18 days after transferring (DAT).

Seaweed extracts were applied to the nutrient solution at the same time by changing the solution 5 times at one-week intervals. In the lettuce hydroponic experiment, the application rates of SWEs obtained from *C. barbata* were the same as the application doses used in the first wheat hydroponic experiment, as described above (3.4.3.1).

Detailed root imaging analysis of one lettuce root from each pot was performed using the WinRhizo root imaging device in exactly half of the experiment (18 DAT). The experiment was terminated when the plants were about 5 weeks old.

At the end of this experiment, the shoot and root part of lettuce plant in each pot were separate independently. Lettuce roots were meticulously washed with abundant deionized water and dried at 70°C for three days and weighed. Lettuce shoots also dried, weighed and grinded into a fine powder; these samples were utilized to determine the minerals in the tissues. Dry matter ratio was calculated by dividing the DW of shoot parts by the FW of shoot parts, and root/shoot parts ratio was calculated by dividing the DW of roots by the DW of shoot parts.

All leaves from these plants were cut into fragments with a maximum width of 5 mm. The pooled samples were then divided into three portions; each fragment was individually weighed to record FW for subsequent analyses. Afterwards, the samples were separately packaged and immediately flash-frozen in liquid nitrogen, then stored at -80°C . The fresh samples were used for phenolic content, antioxidant activity (ABTS, DPPH, FRAP), vitamin C, glutathione, and carotenoids analyses as described below.

3.7. Comparison of Foliar Applications of *C. barbata* Extracts and a Commercial Seaweed-Based Product on Yield and Fruit Quality of Pepper

3.7.1. Plant Materials and Growth Conditions

In this study, the pepper seeds (*Capsicum annuum* cv. Fiyaka F1) were used as plant material in the substrate culture experiment. Fiyaka F1 is an early kind of pepper characterized by its sweetness. It is a hybrid pepper variety ideal for cultivation in both greenhouse and open field cultivation. This plant is appropriate for industrial pickling and fresh market consumption, exhibiting a yield that persists throughout the season. Adapts exceptionally well to spring and summer crops.

3.7.2. Experimental Designs and Seaweed Extract Treatments

In this experiment, the effects of three different SWEs (water, alkali, and acid) obtained from *C. barbata* and Acadian as commercial seaweed-based biostimulant products were investigated on pepper (*Capsicum annuum* cv. Fiyaka) plants grown in cocopeat medium as substrate culture as foliar application under greenhouse conditions. Greenhouse conditions were as described above for the wheat hydroponic experiment.

The Acadian algae-based biostimulant, derived from *Ascophyllum nodosum* through proprietary biochemical extraction procedures to retain bioactive components, is marketed in liquid form by Acadian Plant Health (Canada).

In this study, pepper seeds were germinated in vermiculite moistened with a solution containing 2 mM Ca in the form of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ for 45 d under greenhouse

conditions. During this period, they were watered at regular intervals according to their water needs and watered once a week with nutrient solution. After 45 days, the pepper seedlings considered appropriate for transplantation were relocated to pots with a total volume of roughly 9.5 l, packed with well-moistened cocopeat.

The pots received 10 mM $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 0.5 mM KH_2PO_4 , 3 mM KNO_3 , 1.5 mM $\text{MgSO}_4 \cdot \text{H}_2\text{O}$, 0.3 mM KCl , 50 μM Fe (in the form of FeEDTA), 10 μM H_3BO_3 , 5 μM $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 5 μM $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 1 μM $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.50 μM $(\text{Na}_2)\text{MoO}_4 \cdot 2\text{H}_2\text{O}$. Fertilisation was conducted twice weekly, and approximately one-quarter of the nutrient solution was drained from the bottom of the pots to avoid excessive mineral buildup in the growth medium.

There were 4 replications from each treatment group in the experiment consisting of 20 pots. After the plants were transferred to the pots, the experiment lasted approximately 80 days and during this period, 5 foliar applications were applied to the plants at approximately 2-week intervals and a total of 6 harvests were carried out. The pepper plants were watered with a nutrient solution twice a week. In all harvests, care was taken to collect peppers that reached a certain size, thus ensuring a homogeneous harvest.

Foliar applications were prepared as follows: 10 ml per l for the commercial product, as indicated on the label; 20 ml per l for the water extract; and 10 ml per l for the alkali and acid extracts. Water was sprayed on pepper plants in the control group. This application was applied under greenhouse conditions and in a windless environment, except at times when the sun was not directly on the plants, such as at noon. To increase the effectiveness of applications on leaves, 2% Tween was prepared as a stock solution and Tween was added from the stock solution to each SWE application solution at 2/10 000.

The fruit number, weight and yield of the total peppers collected during the 80-day growing period were taken. The collected peppers were washed and removed of pedicle and seeds and then cut into small pieces (~2 g) and then they were packaged separately and immediately shocked in liquid nitrogen to store at -80°C . Some of these samples were dried in a 65°C for 3 days and ground, and the samples turned into fine powder were used in mineral analysis and total soluble sugar analysis in fruits. The fresh samples were used for phenolic content, antioxidant activity (ABTS, DPPH,

FRAP), vitamin C, glutathione, and chlorophyll pigment (chl_a, chl_b and caretonoids) analyses as described below.

3.8. Plant Analysis

3.8.1. Mineral Analysis of Plant Samples

Approximately 0.2 grams of each dried and finely powdered wheat, lettuce as well as pepper fruits were measured and put into digestion tubes. Microwave digestion was conducted as outlined in section 3.3.1, and mineral analysis was executed by ICP-OES.

3.8.2. Esterase Activity Analysis

The esterase activity in the obtained shoot parts and roots was carried out with the method developed by **Junge and Klees [1984]** and modified by **Ertani et al., [2019]**. According to this method, 1 g of fresh shoot parts or root sample was pulverized with liquid nitrogen in a non-ball form, and then 10 ml of 0.1 M potassium phosphate (K-P) buffer (pH 7.0) was added and homogenized. The sample was centrifuged at 15000 g for 15 minutes at 2°C and the supernatant was transferred to new eppendorfs and utilized as an enzyme source. For esterase measurement, 1 ml of α -naphthyl acetate solution was added to the plant extracts and followed at 600 nm for 20 minutes at 37°C, and the results were reported as % esterase activity by comparing them with the value measured in the control (=100%).

3.8.3. Detailed Root Imaging Analysis (WinRHIZO)

The roots of ten wheat seedlings from each repetition box in perlite experiments and one root of lettuce were analyzed using the WinRhizo STD4800 Pro (Regent Instruments) advanced root imaging and analysis system. This apparatus and software were utilized to ascertain the alterations in wheat root morphology induced by SWEs derived from various extraction methods. The root positioning device facilitated the placement of the roots on the scanner, allowing for the measurement of root length, surface area, volume, and average diameter parameters. The alterations in the root tips and the branching configurations of the roots were elucidated.

3.8.4. Total Phenolic Content Analysis

The Folin-Ciocalteu method was employed to quantify the total phenolic content in the plant samples [Singleton et al., 1999]. Plant extracts were initially generated utilizing 70% methanol at a ratio of 1:25 (w/v) for lettuce and 1:40 for pepper fruit [Kalużewicz et al., 2017]. The samples were extracted using a mixing mill with liquid nitrogen, thereafter, centrifuged at 4 °C for 15 minutes at 8000 g, and aliquoted for total phenolic and total antioxidant analysis. This approach relies on a redox reaction wherein phenolics are oxidized and the Folin reagent is reduced in an alkaline environment, leading to the development of a blue hue. 400 µl of Folin reagent (0.5 N) and 400 µl of sodium carbonate (Na_2CO_3) (10%) solution was mixed with 20 µl of the sample or standard. The total volume of the mixture was adjusted to 1.5 ml with distilled water (dH_2O) to facilitate the reaction. Standards of gallic acid at various concentrations ($0.125\text{--}1\text{ mg ml}^{-1}$) were prepared. Following a 30-minute incubation in darkness, the Abs of the standards and samples was assessed at 760 nm and quantified utilizing the standard curve. The values were expressed as mg GAE per 100 g of FW.

3.8.5. Glycine Betaine Analysis

Glycine betaine analysis was performed according to the method developed by Grieve and Grattan (1983). 20 ml of pure water was added to 0.5 g of dry powder sample and shaken at room temperature for 24 hours and then filtered. 1 ml of 2 N sulfuric acid (H_2SO_4) was added to 1 ml of filtered sample and 0.5 ml of this mixture was cooled in an ice bath for 1 hour and then 0.2 ml of pre-cooled KI- I_2 reagent was added. The KI- I_2 reagent was prepared by dissolving 15.7 g of iodine (I_2) and 20 g of potassium iodide (KI) in 100 ml of water. The mixture was kept at 0-4°C for 16 hours and then centrifuged at 4000 g at 0°C for 15 minutes. After carefully removing the liquid phase from the medium, the period-iodide crystals remaining at the bottom were dissolved by vortexing in 9 ml of 1,2-dichloroethane. Glycine betaine solutions of different concentrations were prepared and used as a standard in 1 N H_2SO_4 . The Abs of the standards and samples at 360nm were measured with a spectrophotometer. The glycine betaine concentrations in the samples were calculated using the drawn standard curve.

3.8.6. Relative Electrolyte Leakage (REL) Analysis

The fresh leaf samples taken for relative electrolyte analysis were washed with deionized water and placed in capped tubes containing 50 ml of deionized water so that the entire leaf remained in the water. After the prepared tubes were kept at 25°C for 6 hours, the conductivity of the water was measured with a conductivity meter (EC meter), and the EC₁ value was determined for each of the samples. After the values read were recorded, the samples were boiled in a water bath for 30 minutes and the EC₂ values were determined.

Finally, the REL value is calculated as follows:

$$REL = (EC_1/EC_2) * 100\%. \quad (3.1)$$

3.8.7. Extraction for Protein and Antioxidative Enzyme Assays

A K-P buffer with a pH of 7.6 was prepared using 0.1 M KH₂PO₄ and 0.1 M K₂HPO₄ to be used in the extraction of frozen wheat shoot samples. 0.1 mM Na₃-EDTA (Titriplex III) was added to this extraction buffer. 0.5 g of frozen fresh green parts or root samples were ground into fine powder in an iron ball mill with the help of liquid nitrogen, and then 5 ml of buffer was added to homogenize the samples. The homogenate was centrifuged at 4°C for 15 minutes at 4600 g, and the supernatant was transferred to new tubes. The centrifugation process was repeated at 4°C and 15000 g, and 250 µl aliquots were taken from the liquid phase and stored at -80°C. These samples were used in protein and antioxidative enzyme analyses.

3.8.8. Bradford Protein Analysis

Protein concentration was determined using the linearized Bradford technique as outlined by **Zor and Selinger [1996]**. According to this method, a solution was prepared by adding 50 ml of pure ethanol, 100 ml of 85% ortho-phosphoric acid, and 450 ml of pure water to 100 mg of Coomassie Brilliant blue. 100 ml of glycerin and enough pure water to complete the final volume of 1 l were added to this solution, which was filtered with coarse filter paper. This solution, called Bradford reagent, was kept in the refrigerator at 4°C for at least 24 hours before use.

Protein standards at various concentrations (0, 100, 200, 400, 800 mg/ml) to be used for drawing the standard curve were prepared by dissolving Albumin (fraction V) in K-P buffer. The 5 ml of Bradford reagent was applied to 50-150 µl of extracts or standards and after waiting for 5 minutes, Abs values were measured at both 450 nm and 590 nm using a spectrophotometer. After calculating the Abs (590) / Abs (450) ratio for each measurement, the standard curve was drawn and the protein concentration in the wheat samples was determined.

3.8.9. Antioxidative Enzyme Activities

To determine the impacts of SWEs on antioxidative enzyme activity under salinity stress, the extraction of wheat samples obtained from the third hydroponic wheat experiment was carried out as described in 3.6.8. Total activities of antioxidative enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) in wheat samples were measured spectrophotometrically, and specific enzyme activities were calculated by dividing by protein concentration as described below.

3.8.9.1. Superoxide dismutase (SOD) Analysis

The activity of the SOD enzyme was assessed spectrophotometrically utilizing the methodology established by **Giannopolitis and Ries [1977]** and partially refined by **Mengütay et al., [2013]**. For this, 100 µl of plant extract was mixed with 500 µl of 50 mM Na₂CO₃, 500 µl of 12 mM L-methionine and 500 µl of 75 µM NBT, and the total volume was completed to 4.5 ml with K-P buffer containing 0.1 mM Titriplex III. 500 µl of 2 µM riboflavin was added to these prepared mixtures at the last moment to start the reaction and kept under strong light for 8 minutes. Then, the inhibition of photochemical reduction of p-nitro blue tetrazolium chloride (NBT) by SOD was monitored at 560 nm wavelength. One unit of SOD activity is defined as the activity that reduces NBT activity by 50%.

3.8.9.2. Catalase (CAT) Analysis

For the measurement of catalase activity, K-P buffer (pH 7.6) containing 100 mM H₂O₂ and 0.1 mM Titriplex III was prepared. K-P buffer was added to 100 µl H₂O₂

solution and 50 µl plant extract to complete the volume of the mixture to 1 ml. Catalase activity was calculated by following the decrease in H₂O₂ Abs spectrophotometrically for 2 minutes at 240 nm wavelength [Kutman et al., 2013].

3.8.9.3. Ascorbate peroxidase (APX) Analysis

The activity of ascorbate peroxidase enzyme was determined by the method developed by Nakano and Asada [1981]. According to this method, 100 µl of 12 mM H₂O₂, 100 µl of 0.25 mM ascorbic acid (C₆H₈O₆), and 50 µl of plant extract were added to K-P buffer containing 0.1 mM Na₃-EDTA (Titriplex III) to complete the total volume to 1 ml. The average depletion rate of L-ascorbic acid was calculated by measuring Abs values at 290 nm spectrophotometrically and determining APX activity.

3.8.9.4. Glutathione reductase (GR) Analysis

The activity of the glutathione reductase enzyme was measured using the method developed by Foyer and Halliwell [1976] and partially modified by Mengutay et al., [2013]. To determine the activity of the GR enzyme, 100 µl of 0.5 mM oxidized glutathione (GSSG), 100 µl of 0.12 mM nicotinamide adenine dinucleotide phosphate (NADPH), 200 µl of plant extract and K-P buffer containing 0.1 mM Titriplex III were added to each sample to complete the mixture to 1 ml. Glutathione reductase enzyme activity was determined by following the Abs at a wavelength of 340 nm and the oxidation of NADPH was determined spectrophotometrically, and Abs values were monitored for 2 minutes to find the average NADPH-Na₄ depletion rate.

3.8.10. Malondialdehyde (MDA) Analysis

Malondialdehyde (MDA) analysis was performed by the spectrophotometric method developed by Heath and Packer [1968] and improved by Hodges et al., [1999]. For use in extraction, approximately 0.5 g of frozen leaf sample was homogenized in an iron ball mill with liquid nitrogen and homogenized with 25 ml of 80% ethanol. The homogenized mixture was centrifuged at 3000 g for 10 minutes and the liquid phase was transferred to a clean tube for use. Firstly, two separate solutions were prepared as follows:

-TBA: 20% trichloroacetic acid (TCA) + 0.01% butylated hydroxy toluene (BHT)

+TBA: 20% TCA + 0.01% BHT + 0.65% thiobarbituric acid (TBA)

For each sample, 1 ml of -TBA or 1 ml of +TBA solution was added to 1 ml of plant extract taken in a tube and incubated in a water bath at 95°C for 25 minutes and then immediately cooled in an ice bath. The liquid phases of the mixtures, which were then centrifuged at 3000 g for 10 minutes, were transferred to clean tubes and their Abs at 440, 532 and 600 nm were measured with a spectrophotometer. The MDA concentration in the samples was calculated using the following formulas:

$$A = [(Abs_{532}+TBA) - (Abs_{600}+TBA) - (Abs_{532}-TBA-Abs_{600}-TBA)]$$

$$B = [(Abs_{440}+TBA - Abs_{600}+TBA) 0.0571]$$

$$MDA (nmol \cdot ml^{-1}) = [(A-B) / 157\ 000] 10^6 \quad (3.2)$$

3.8.11. Free Proline Analysis

In the analysis of free proline of wheat samples obtained from the third hydroponic wheat experiment, **Bates et al., [1973]** was utilized. To extract proline, approximately 0.5 g of frozen sample was homogenized in an iron ball mill using liquid nitrogen and then 10 ml of a 3% water-based sulfosalicylic acid solution was added and homogenized. The homogenate was filtered with Whatman filter paper and used. 2 ml of each sample was mixed in a tube with 2 ml of glacial acetic acid and 2 ml of acid-ninhydrin solution (1.25 g of ninhydrin is dissolved in 30 ml of glacial acetic acid and 20 ml of 6 M phosphoric acid by heating and can be stored at 4°C for 24 hours) and incubated at 100°C for 1 hour and then cooled in an ice bath. After the mixture cooled, 4 ml of toluene was added, and this new mixture was vortexed. The toluene phase containing the chromophore was transferred to a new container and used for free proline analysis. To use as a standard, proline solutions prepared at different concentrations in 3% water-based sulfosalicylic acid solution were used. The Abs of the standards and samples at 520 nm were determined by spectrophotometer and the amount of proline in the samples was determined by drawing the standard curve.

3.8.12. Total Antioxidant Capacity of Plant Samples

Antioxidant activity of plants was performed, as mentioned above in 3.2.6. The plant's (lettuce and pepper) antioxidant activities were assessed using the same methanolic extraction as the total phenolic content assay [Mannino et al., 2020]. The plant extracts were made with 70% metaphosphoric acid, with lettuce at a 1:5 (w/v) ratio and pepper at a 1:10 (w/v) ratio. The results were given in terms of μmol Trolox equivalent (TE) per 100 g FW ($\mu\text{mol TE } 100 \text{ g}^{-1} \text{ FW}$).

3.8.13. Ascorbic acid Analysis

Total ascorbic acid (AsA) and reduced AsA were measured in plant species (lettuce and pepper) [Kampfenkel et al., 1995; Ilahy et al., 2011]. Plant extracts were initially prepared using 5% metaphosphoric acid at a 1:5 (w/v) ratio for lettuce and 1:10 (w/v) ratio for pepper. The samples were extracted using a mixing mill with liquid nitrogen, thereafter, centrifuged at 4 °C for 15 minutes at 8000 g, and aliquoted for glutathione quantification. The analysis entails the reduction of ferric iron (Fe^{+3}) to ferrous iron (Fe^{+2}) by ASA, succeeded by the spectrophotometric identification of Fe^{+2} complexes with 2,2-dipyridyl. The preincubation of the sample with dithiothreitol (DTT) reduces the oxidized form of dehydroascorbic acid (DHA) to AsA. Excess DTT is subsequently eliminated using N-ethylmaleimide (NEM), and the total AsA is quantified. The Abs of the samples or standards was measured at 525 nm by UV-Vis spectrophotometry. The findings were presented as milligrams of total AsA and milligrams of reduced AsA per 100 g of FW.

3.8.14. Glutathione Analysis

Aliquots of the 5% metaphosphoric acid extract from the lettuce employed for ascorbic acid (AsA) analysis were utilized to assess GSH [Kampfenkel et al., 1995]. A K-P buffer (150 mM) with EDTA (5 mM) at pH 7.4 was included into the extracts [Zhou et al., 2022]. The resultant mixture was subjected to centrifugation at 4 °C at 8000 g for 15 minutes. Subsequently, DTNB was introduced into the reaction vessel at a concentration of 6 mM. The Abs was measured at 412 nm following a 20-minute

incubation period. Results were quantified in mg GSH per 100 g of FW, employing a standard curve derived from different amounts of GSH.

3.8.15. Chlorophyll and Total Carotenoid Analysis

The lettuce and pepper samples were extracted using 80% acetone at 1:100 (w/v) ratio and 1:20 (w/v) ratio using a homogenizer, respectively [Wellburn, 1994]. The homogenate was then centrifuged at 4°C at 8000 g for 15 minutes. The measurement has been facilitated by diluting the plant sample (100 µl) with 80% acetone (1900 µl). The Abs of the samples was measured at 470 nm, 646.8 nm, and 663.2 nm. The following equations were utilized for calculations:

The calculation formula is below:

$$C_a = 12.25\text{Abs}_{663.2} - 2.79\text{Abs}_{646.8}$$

$$C_b = 21.5\text{Abs}_{646.8} - 5.1\text{Abs}_{663.2}$$

$$C_{x+c} = (1000\text{Abs}_{470} - 1.82C_a - 85.02C_b) / 198 \quad (3.3)$$

3.8.16. Total Soluble Carbohydrate Analysis

The method employed to quantify soluble carbohydrates by spectrophotometer was derived from the methodology outlined by Yemm and Wills [1954] and Mengutay et al., [2013], with some alterations. The anthrone reagent interacts with the polysaccharides in the solution, resulting in a green-blue color. Dried and ground pepper samples were initially extracted at a ratio of 1:100 (w/v) using 80% ethanol. Subsequently, 0.6 g of anthrone was combined with 0.4 L of 20% ethanol and sulfuric acid (98%) in a 1:3 ratio. Plant samples (0.25 ml) and anthrone reagent (4 ml) were subsequently combined and incubated for 20 minutes at 90°C in a water bath. Following refrigeration on ice, the Abs were measured at 612 nm. The standard curve was generated utilizing various amounts of D-glucose. The concentrations of soluble carbohydrates were quantified as mg g⁻¹ DW.

3.8.17. Statistical Analysis

For statistical analysis, JMP software (18.0.0) was employed. The importance of the treatment effects and their interactions on the observed attributes for each experiment was assessed using one-way analysis of variance (ANOVA). Where ANOVA indicated a significant effect, a post-hoc test at 5% significance was employed to ascertain significant differences between means. The Fisher protected least significant difference (LSD) test ($p < 0.05$) was employed in the experiments of this thesis study. The datasets obtained from the biochemical characterization of SWEs contained three replicates for each group. The only exception was auxin-like activity analysis, where 4 replicates were used for each treatment group. For the germination experiments, a completely randomized design was used with 5 replicate.

The first hydroponic wheat experiment showed means and standard deviations of 5 replicates; second and third were 4. In the 3rd wheat experiment, the interaction between salinity and SWE treatments was analyzed, employing the LSD test for instances where ANOVA indicated a statistically significant difference as at two-factor variance assessment. Lack of lettering indicates absence of significant effect according to ANOVA.

In the lettuce and pepper experiment, the values represent the means and standard deviations of 4 repeats.

4. RESULTS

4.1. Biochemical Characterization of *C. barbata* extracts

The concentrations of macro and micronutrients contained within SWEs are outlined in **Table 4.1**. According to ANOVA, the effect of seaweed extraction type on macronutrients as well as micronutrients (except for Zn) of SWEs was significant (**Table 4.2**). The Ca concentration was the highest in the acid extract, showing a statistically significant difference, whereas it was similar in water and alkali extracts (**Table 4.1A**). All SWEs contained K and S concentrations exceeding 1 g per l, with the alkali extract exhibiting the highest levels of both elements. The results showed that the water and acid extracts had similar K⁺ and Mg²⁺ concentrations. Furthermore, the alkali and acid extracts exhibited similar levels of P. The alkali extract contains approximately double the amount of Fe compared to the acid extract (**Table 4.1B**). Moreover, B and Mn were the most abundant micronutrients after Fe in the SWEs. Finally, the measured concentrations of Cu and Zn were < 1 mg l⁻¹.

Table 4.1: Essential (A) macro- (Ca, K, Mg, P and S) and (B) micro- (B, Cu, Fe, Mn and Zn) element concentrations of the SWEs

(A) Macronutrient Concentrations (g l⁻¹)						
SWE	Ca	K	Mg	P	S	
Water	0.32 ± 0.01 b	5.42 ± 0.30 b	0.32 ± 0.01 a	0.11 ± 0.01 a	1.31 ± 0.07 c	
Alkali	0.31 ± 0.01 b	9.37 ± 0.31 a	0.14 ± 0.00 b	0.07 ± 0.00 b	5.05 ± 0.16 a	
Acid	0.89 ± 0.01 a	5.64 ± 0.06 b	0.31 ± 0.00 a	0.07 ± 0.00 b	3.02 ± 0.01 b	
(B) Micronutrient Concentrations (mg l⁻¹)						
SWE	B	Cu	Fe	Mn	Zn	
Water	5.75 ± 0.29 a	0.23 ± 0.15 b	11.08 ± 0.23 b	2.11 ± 0.11 b	0.23 ± 0.17	
Alkali	5.05 ± 0.30 b	0.43 ± 0.02 a	16.53 ± 1.47 a	1.83 ± 0.08 c	0.41 ± 0.19	
Acid	3.35 ± 0.06 c	0.07 ± 0.04 b	7.52 ± 0.13 c	3.16 ± 0.02 a	0.29 ± 0.23	

Values are mean and standard deviations of three replicates. Different letters indicate significant differences between means according to Fisher's protected LSD test ($p < 0.05$). Lack of lettering indicates absence of significant effect according to ANOVA.

The significance of the effect of extraction type on the biochemical characterization of SWEs, as determined by one-way analysis of variance (ANOVA), is presented in **Table 4.2**.

Table 4.2: Significance of the biochemical characterization of SWEs according to one-way analysis of variance (ANOVA) ($\alpha = 0.05$)

Source of Variation	DF ¹	Ca	K	Mg	P	S
Extracts	2	***	***	***	***	***
Source of Variation	DF	B	Cu	Fe	Mn	Zn
Extracts	2	***	**	***	***	<i>n.s.</i> ²
Source of Variation	DF	Total Phenolic	Total Free Amino acid			
Extracts	2	***	***			
Source of Variation	DF	ABTS	DPPH	FRAP	Auxin-like activity	
Extracts	2	***	***	***	*	

DF¹, degree of freedom; *n.s.*²: not significant; * $0.01 < p < 0.05$; ** $0.001 < p < 0.01$; *** $p < 0.001$

ANOVA indicated a significant effect of extraction type on total phenolic, free amino acid in SWEs (**Table 4.2**). The biochemical characterization results of several SWEs, as presented below, demonstrate that the total phenolic content, total free amino acids of the extracts are influenced by the extraction techniques (**Table 4.3**). Despite the alkali extract exhibiting the highest overall phenolic content, it concurrently displayed the lowest concentration of free amino acids. Conversely, the water extract exhibited the highest levels of total free amino acids.

Table 4.3: Total phenolic and total free amino acids concentrations of SWEs

Extraction Types	Total Phenolic	Total Free Amino acid
	(g l ⁻¹)	
Water	0.71 ± 0.08 b	2.31 ± 0.10 a
Alkali	2.00 ± 0.03 a	1.64 ± 0.13 b
Acid	0.64 ± 0.01 b	2.17 ± 0.08 a

Values are means and standard deviations of three replicates. Different letters indicate significant differences between means according to the Fisher's protected LSD test ($p < 0.05$).

ANOVA indicated that the effects of extraction types on the antioxidant activities of SWEs were significant (**Table 4.2**). The antioxidant activities of SWEs have been reported by 3 different methods (**Table 4.4**). The alkali extract exhibited the strongest antioxidant activity, with 2–3 times greater efficacy than both the water and acid extracts, as corroborated by consistent results from all three analyses. On the other hand, the antioxidant activity observed in both water and acid extracts did not show substantial variation.

Table 4.4: The antioxidant activities (ABTS, DPPH, and FRAP) of SWEs

Extraction Types	ABTS	DPPH	FRAP
		(mmol TE l ⁻¹)	
Water	0.22 ± 0.00 c	6.37 ± 0.53 b	7.47 ± 0.12 b
Alkali	0.74 ± 0.00 a	16.71 ± 0.94 a	15.70 ± 0.19 a
Acid	0.24 ± 0.00 b	6.01 ± 0.06 b	5.05 ± 0.82 c

Values are means and standard deviations of three replicates. Different letters indicate significant differences between means according to the Fisher's protected LSD test ($p < 0.05$).

ANOVA demonstrated that the effect of extraction type had a significant effect on auxin-like activity of SWEs (**Table 4.2**). For the auxin like activity test, the elongation rate of maize coleoptiles after 2 days of incubation in darkness was assessed under several solutions: acid extract (0.1%), alkali extract (0.1%), water extract (0.04%), IAA (Indole-3-Acetic Acid) (1.75 mg L⁻¹) as reference, and a control (water) (**Figure 4.1**). The utilization of alkali extracts promoted the highest elongation rate, surpassing that of IAA application. In comparison to the control application, the coleoptile

elongation rate increased by 20%, 25%, and 30% with treatments of water, acid, and alkali extracts, respectively, whereas IAA resulted in an approximate 25% increase in length.

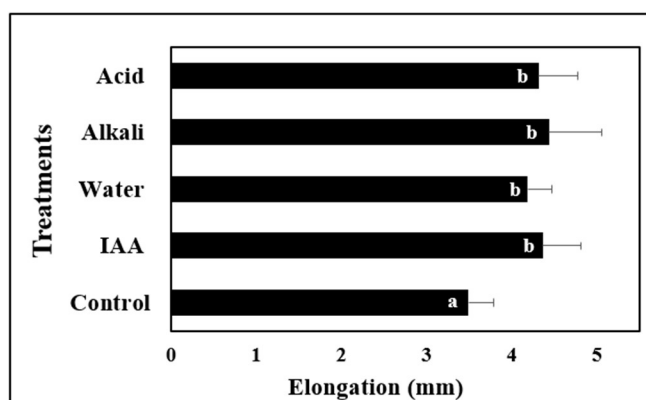


Figure 4.1: Elongation rate of maize coleoptiles utilizing alternative SWEs (water, alkali, and acid), IAA (1.75 mg l^{-1}), and control. Values are mean and standard deviations of four replicates. Different letters indicate significant differences between means according to Fisher's protected LSD test ($p < 0.05$).

4.2. Determination of the effects of *C. barbata* extracts on germination and early growth periods in wheat

Two experiments were conducted within the scope of perlite experiments: the first is the substrate experiment, in which SWEs were applied directly to the perlite, and the second is the seed soaking experiment, in which extracts were used as seed treatment. The effects of extraction type on early growth parameters in the substrate experiment determined by one-way analysis of variance (ANOVA) are shown in **Table 4.5A** and in **Table 4.5B** for the seed treatment.

Table 4.5: Significance of the effects of SWEs on reported traits on wheat (*Triticum durum* cv. Sariçanak-98) plants (6 days after sowing (DAS)) grown in perlite experiment (A) Substrate Treatment and (B) Seed Treatment according to one-way analysis of variance (ANOVA) ($\alpha = 0.05$)

A) Substrate Treatments						
Source of Variation	DF ¹	Germination Percentage	Shoot Height	Shoot FW	Shoot Esterase Activity	Root Esterase Activity
Seaweed Extracts	2	<i>n.s.</i> ²	***	***	<i>n.s.</i>	<i>n.s.</i>
Source of Variation	DF	Root FW	Root Length	Root SA	Root Volume	Avg. Diameter
Seaweed Extracts	2	*	*	**	**	*
Source of Variation	DF	Ca	K	Mg	P	S
Seaweed Extracts	2	***	***	**	***	***
Source of Variation	DF	Cu	Fe	Mn	Zn	
Seaweed Extracts	2	<i>n.s.</i>	*	**	***	
B) Seed Treatments						
Source of Variation	DF	Germination Percentage	Shoot Height	Shoot FW	Shoot Esterase Activity	Root Esterase Activity
Seaweed Extracts	2	<i>n.s.</i>	***	*	***	***
Source of Variation	DF	Root FW	Root Length	Root SA	Root Volume	Avg. Diameter
Seaweed Extracts	2	*	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>

DF¹, degree of freedom; *n.s.*²: not significant; * $0.01 < p < 0.05$; ** $0.001 < p < 0.01$; *** $p < 0.001$

4.2.1. Application of SWEs to Perlite Medium (Substrate Experiment)

The final germination rate was determined at the end of the experiment, which involved directly providing perlite to the SWEs (**Figure 4.2**). The applications were shown to have no statistically significant effect on the wheat germination rate, with similar outcomes noted in germination percentages (**Table 4.5A**). These applications demonstrated an average germination rate of over 90%. Upon comparing various SWEs and their concentrations with the control group, it was noted that there was no apparent beneficial or negative impact on the germination of wheat seeds.

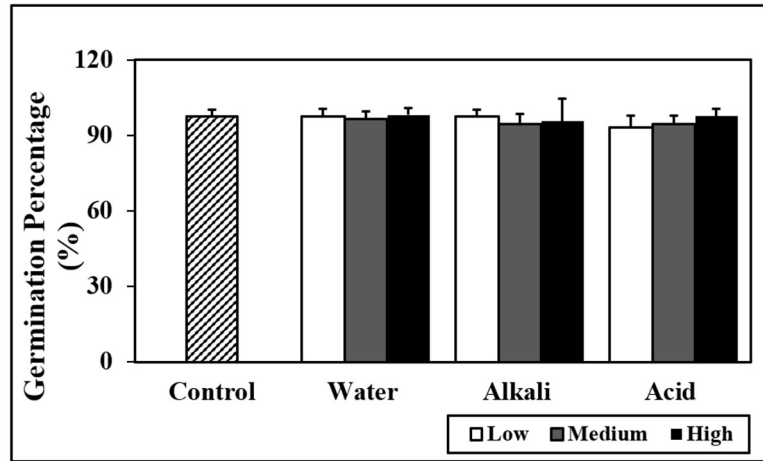


Figure 4.2: Effect of substrate treatments (Control (Water); SWEs: Water extracts (Low, Medium, High); Alkali extracts (Low, Medium, High); Acid extract (Low, Medium, High)) on germination percentage (%) of wheat (*Triticum durum* cv. Sariçanak-98) seedlings at 6 days after sowing (6 DAS)) grown in perlite.

In the substrate treatment experiment, all seedlings subjected to any of the substrates (water, alkali, and acid extracts) exhibited superior shoot and root growth, as well as enhanced seedling performance, in comparison to the control group (**Figure 4.3, Figure 4.4**). According to ANOVA, the seedling height as well as shoot biomass were statistically significantly affected by SWEs application (**Table 4.5A**). The findings of the measured shoot growth parameters from the substrate experiment investigating the impacts of SWEs are presented in **Figure 4.4**. Substrate treatment with SWEs resulted in a more vigorous and uniform germination stage. In the substrate experiment, control (non-treated) plants had the lowest shoot height and shoot biomass (**Figure 4.3, Figure 4.4**). The substrate application with all SWEs (except for low concentration of acid extracts) resulted in a statistically significant increase in shoot height (**Figure 4.4A**). The treatment with SWEs resulted in an approximate 20% increase in seedling height in the substrate experiment. The highest shoot biomass was measured in wheat seedlings grown in a growth medium treated with a high concentration of all SWEs (**Figure 4.3, Figure 4.4B**). Furthermore, shoot FW was increased by around 30% using these extracts. Visually, the substrate treatments were more apparent than the seed soaking treatments in the differences caused by the SWEs (**Figure 4.3, Figure 4.7**).

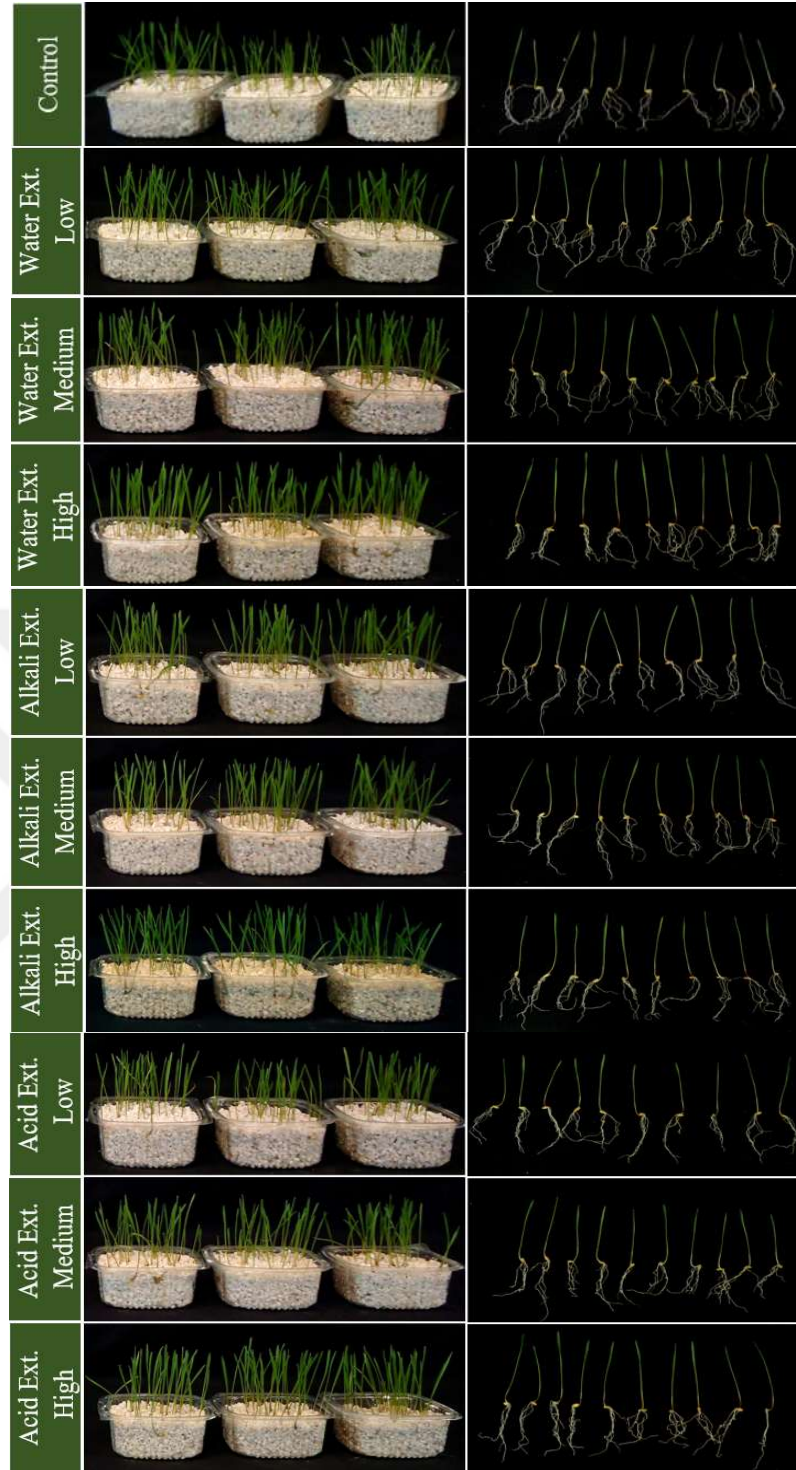


Figure 4.3: Effect of substrate treatments (Control (Water); SWEs: Water extracts (Low, Medium, High); Alkali extracts (Low, Medium, High); Acid extracts (Low, Medium, High)) on perlite-grown 6 day-old-wheat (*Triticum durum* cv. Sarıçanak-98) seedlings under growth chamber conditions.

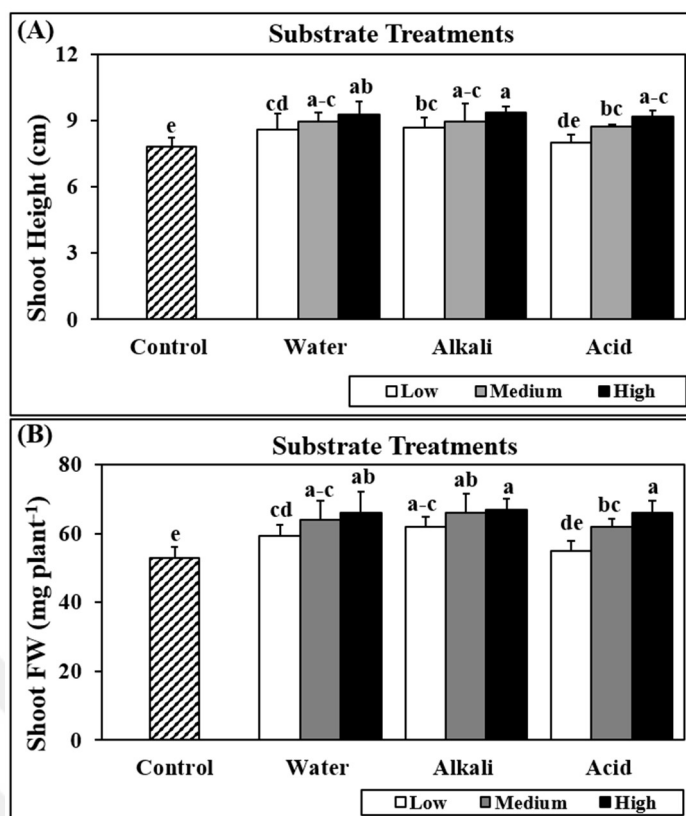


Figure 4.4: Effect of substrate treatments (Control (Water); SWEs: Water extracts (Low, Medium, High); Alkali extracts (Low, Medium, High); Acid extract (Low, Medium, High)) on (A) shoot height, (B) shoot fresh weight (FW) of wheat (*Triticum durum* cv. Sariçanak-98) seedlings at 6 days after sowing (6 DAS)) grown in perlite.

According to the ANOVA results, the application of substrates containing a variety of SWEs at varying concentrations did not have any significant and consistent impact on the outcomes of the shoot and root esterase activity (Table 4.5A, Figure 4.5).

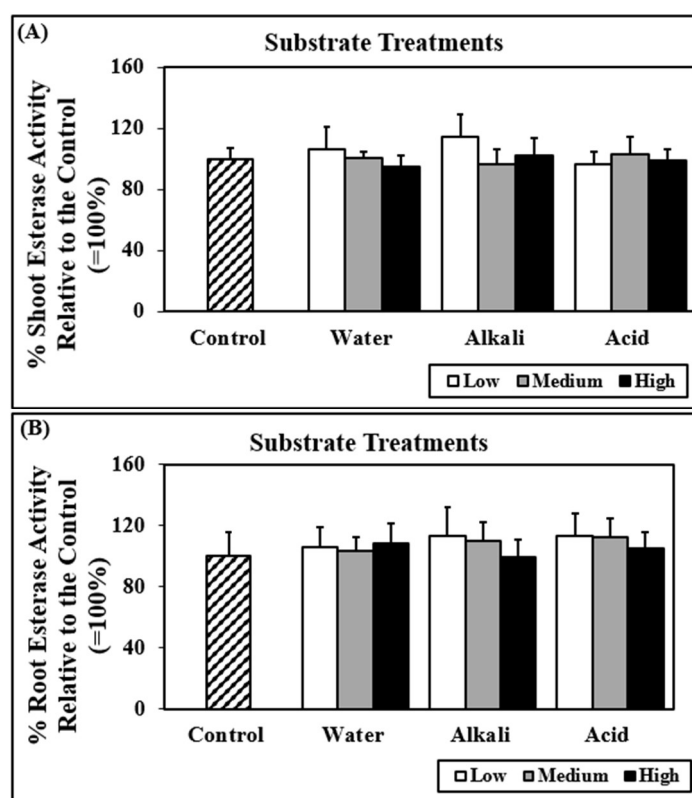


Figure 4.5: Effect of substrate treatments (Control (Water); SWEs: Water extracts (Low, Medium, High); Alkali extracts (Low, Medium, High); Acid extract (Low, Medium, High)) on (A) shoot and (B) root esterase activity of wheat (*Triticum durum* cv. Sarıçanak-98) seedlings at 6 days after sowing (6 DAS)) grown in perlite.

A study of mineral elements was conducted to verify the substantial enhancement noted in the growth characteristics of the substrate treatment groups (Table 4.6). ANOVA analyses indicated that the substrate treatment with SWEs had a significant impact on the shoot macroelement concentration of wheat (Table 4.5A). In the substrate experiment, the concentrations of K and S in the shoots were enhanced with the SWEs treatment, while the concentrations of Ca and P were reduced, and the Mg concentration remained the same. The treatments showed the most significant impact on K compared to all assessed macronutrients. The concentration of K was directly correlated with the increasing amounts of SWEs, with the greatest value observed in plants treated with water extracts. Thanks to this treatment, K concentration nearly tripled in the wheat shoots compared to the control. Conversely, the elevations in S concentrations were less significant, not surpassing 20% in all of the treatments. The reductions in shoot concentrations of both P and Ca following SWE treatments were

primarily dose-dependent, with the most significant and consistent declines recorded for Ca, which surpassed 50% at the highest treatment level of the water extract.

Table 4.6: Changes in shoot macronutrient (Ca, K, Mg, P, S) concentrations of wheat (*Triticum durum* cv. Sarıçanak-98) seedlings with different substrate treatments

Treatments	Ca (mg g ⁻¹)	K (mg g ⁻¹)	Mg (mg g ⁻¹)	P (mg g ⁻¹)	S (mg g ⁻¹)
Control	3.5 ± 0.2 a	12.6 ± 0.4 g	1.3 ± 0.0 ab	7.9 ± 0.1 a	2.7 ± 0.1 e
Water - L	2.8 ± 0.2 c	21.9 ± 0.6 d	1.4 ± 0.1 ab	7.4 ± 0.2 b	2.8 ± 0.1 de
Water - M	2.1 ± 0.1 e	29.7 ± 1.5 b	1.3 ± 0.0 ab	6.8 ± 0.3 c-e	2.8 ± 0.1 de
Water - H	1.6 ± 0.1 f	36.8 ± 2.4 a	1.4 ± 0.1 a	7.3 ± 0.5 b	3.1 ± 0.2 ab
Alkali - L	2.9 ± 0.3 bc	14.8 ± 0.7 f	1.3 ± 0.0 bc	7.0 ± 0.1 cd	2.9 ± 0.1 cd
Alkali - M	2.8 ± 0.4 c	18.2 ± 2.4 e	1.3 ± 0.1 ab	6.9 ± 0.1 c-e	3.0 ± 0.1 bc
Alkali - H	2.5 ± 0.2 d	26.8 ± 1.9 c	1.3 ± 0.0 bc	6.8 ± 0.2 de	3.2 ± 0.1 a
Acid - L	3.1 ± 0.2 b	14.3 ± 0.4 fg	1.3 ± 0.0 bc	7.1 ± 0.2 bc	2.9 ± 0.0 cd
Acid - M	3.0 ± 0.1 bc	15.2 ± 1.3 f	1.4 ± 0.1 ab	6.6 ± 0.5 e	2.9 ± 0.2 bc
Acid - H	2.4 ± 0.1 d	18.5 ± 0.5 e	1.2 ± 0.0 c	6.2 ± 0.1 f	2.7 ± 0.0 e

Values are mean and standard deviations of five replicates. Different letters indicate significant differences between means according to Fisher's protected LSD test ($p < 0.05$).

ANOVA indicated that shoot microelement concentrations, with the exception of Cu, were statistically significantly influenced by SWEs (**Table 4.5A**). In general, seaweed applications slightly increased or decreased micronutrient concentrations (**Table 4.7**). No notable alterations were detected in shoot Cu concentrations. There was not a consistent effect of SWEs as substrate treatments on shoot Fe concentration, which ranged from 52 to 61 mg kg⁻¹. On the other hand, medium or high levels of SWE application caused a reduction in Zn and Mn concentrations. The lowest Zn concentration was observed in the high level of the acid extract treatment.

Table 4.7: Changes in shoot micronutrient (Cu, Fe, Mn, Zn) shoot concentrations of wheat (*Triticum durum* cv. Sariçanak-98) seedlings with different substrate treatments

Treatments	Cu (mg kg ⁻¹)	Fe (mg kg ⁻¹)	Mn (mg kg ⁻¹)	Zn (mg kg ⁻¹)
Control	10.4 ± 1.4	56 ± 3 a-c	34 ± 3 a	38 ± 3 a
Water - L	10.4 ± 1.0	61 ± 3 a	32 ± 2 a	35 ± 2 a-c
Water - M	9.7 ± 1.1	58 ± 2 a-c	27 ± 1 c	31 ± 2 c-e
Water - H	9.7 ± 1.2	61 ± 6 a	27 ± 3 c	32 ± 2 c-e
Alkali - L	9.4 ± 0.4	55 ± 3 a-c	32 ± 2 a	37 ± 1 ab
Alkali - M	8.6 ± 1.7	54 ± 0 bc	28 ± 6 bc	30 ± 5 de
Alkali - H	8.6 ± 0.2	58 ± 8 ab	28 ± 2 bc	33 ± 4 cd
Acid - L	9.3 ± 0.4	53 ± 2 bc	31 ± 1 ab	33 ± 1 b-d
Acid - M	9.3 ± 0.8	61 ± 10 a	31 ± 2 ab	34 ± 5 a-d
Acid - H	8.9 ± 1.0	52 ± 2 c	29 ± 2 bc	29 ± 1 e

Values are mean and standard deviations of five replicates. Different letters indicate significant differences between means according to Fisher's protected LSD test ($p < 0.05$).

According to ANOVA, the factors associated with the root system were significantly influenced by SWEs (**Table 4.5A**). The positive effects of SWEs on root biomass and system-related parameters were also observed in the substrate experiment (**Table 4.8**). The SWEs treatment statistically significantly enhanced the root biomass, except for the low concentration of acid extract application. Moreover, the root biomass of wheat seedlings that were treated with high levels of acid extract was approximately 25% higher when compared with control plants. Moreover, the maximum root biomass, almost 25% greater than that of the control plants, was achieved with the treatment of the water extract at a medium level or with the use of acid or alkali extracts at high levels. In addition to the positive effects seen in root FW, when the parameters for root morphology were examined, the highest root length, root surface area, and root volume were observed in wheat seedlings subjected to a medium concentration of water extract. The notable enhancements in these metrics achieved with this application varied from 20% to 30% in comparison to the control. Finally, the average root diameter was increased in response to SWEs, especially acid extract at high levels.

Table 4.8: Effect of substrate treatments on root growth and morphology parameters (FW; Length; SA, Surface Area; Vol, Volume) of wheat (*Triticum durum* cv. Sarıçanak-98) seedlings

Treatments	Root FW (mg plant ⁻¹)	Root Length (cm plant ⁻¹)	Root SA (cm ² plant ⁻¹)	Root Vol. (mm ³ plant ⁻¹)	Avg. Diameter (mm)
Control	67 ± 3 c	23 ± 1 c	3.9 ± 0.2 c	53 ± 5 c	0.55 ± 0.03 c
Water- L	77 ± 9 ab	26 ± 2 ab	4.6 ± 0.3 ab	65 ± 4 ab	0.57 ± 0.02 a-c
Water-M	84 ± 9 ab	27 ± 2 a	4.9 ± 0.4 a	69 ± 5 a	0.57 ± 0.01 bc
Water-H	79 ± 6 ab	24 ± 2 bc	4.4 ± 0.3 b	62 ± 3 b	0.57 ± 0.02 ab
Alkali-L	78 ± 5 ab	25 ± 1 ab	4.5 ± 0.3 b	62 ± 5 b	0.56 ± 0.01 bc
Alkali-M	77 ± 8 ab	24 ± 3 bc	4.4 ± 0.5 b	62 ± 6 b	0.57 ± 0.01 a-c
Alkali-H	82 ± 9 ab	26 ± 2 ab	4.6 ± 0.3 ab	65 ± 4 ab	0.57 ± 0.01 bc
Acid-L	76 ± 8 bc	25 ± 0 bc	4.4 ± 0.3 b	62 ± 7 b	0.56 ± 0.03 bc
Acid-M	83 ± 5 ab	25 ± 1 bc	4.6 ± 0.1 ab	67 ± 4 ab	0.58 ± 0.03 ab
Acid-H	85 ± 6 a	24 ± 2 bc	4.4 ± 0.3 b	66 ± 5 ab	0.59 ± 0.02 a

Values are mean and standard deviations of five replicates. Different letters indicate significant differences between means according to Fisher's protected LSD test ($p < 0.05$). Lack of lettering indicates absence of significant effect according to ANOVA.

4.2.2. Seed Soaking with SWEs (Seed Treatment)

In the seed soaking experiment, firstly, the final germination percentage was calculated, and the results are shown in **Figure 4.6**. Like the germination rate outcomes in the substrate treatment experiment, various applications of SWE did not adversely affect the germination of wheat seedlings, nor were any additional benefits observed. The mean germination rate across all applications exceeds 90%. Algae extracts exhibited comparable effects on wheat seed germination; however, these effects were not statistically significant (**Table 4.5B**).

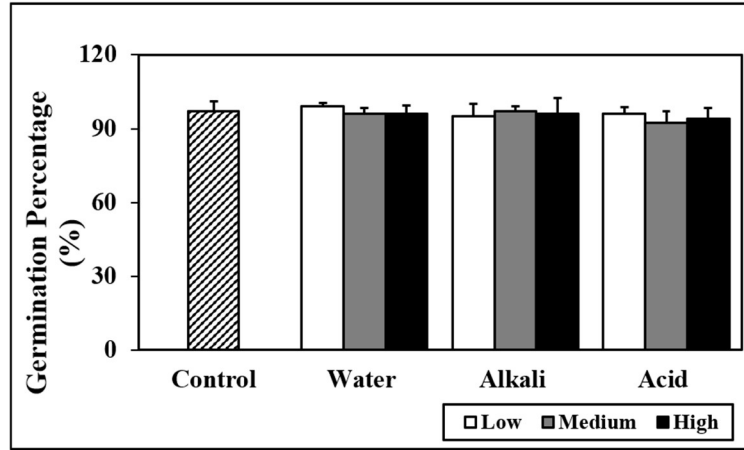


Figure 4.6: Effect of seed soaking (Control (Water); SWEs: Water extracts (Low, Medium, High); Alkali extracts (Low, Medium, High); Acid extract (Low, Medium, High)) on germination percentage (%) of wheat (*Triticum durum* cv. Sariçanak-98) seedlings at 6 days after sowing (6 DAS)) grown in perlite

The effects of soaking treatment of wheat seeds with SWEs on early seedling growth and development are shown in **Figure 4.7**. According to ANOVA, the shoot height as well as shoot biomass were statistically significantly affected by SWEs as seed treatment agents (**Table 4.5B**). The positive effects of seaweed applications on the early growth of wheat seedlings are observed. Conversely, the observed favorable effects on shoot biomass and height were less apparent and not consistently influenced by dose. All SWEs significantly improved seedling height in the seed soaking experiment (**Figure 4.7, Figure 4.8A**). The application of SWEs provided approximately %20 an increase in wheat seedling height. Moreover, the maximum seedling height was observed in medium rate of alkali extract-treated plant (**Figure 4.7, Figure 4.8B**). Seaweed applications as seed soaking agents caused only %10 an enhancement on shoot FW. However, the effects are not as obvious as with substrate applications. Overall, SWE-treated plants had higher shoot and root length, and development compared to the control.

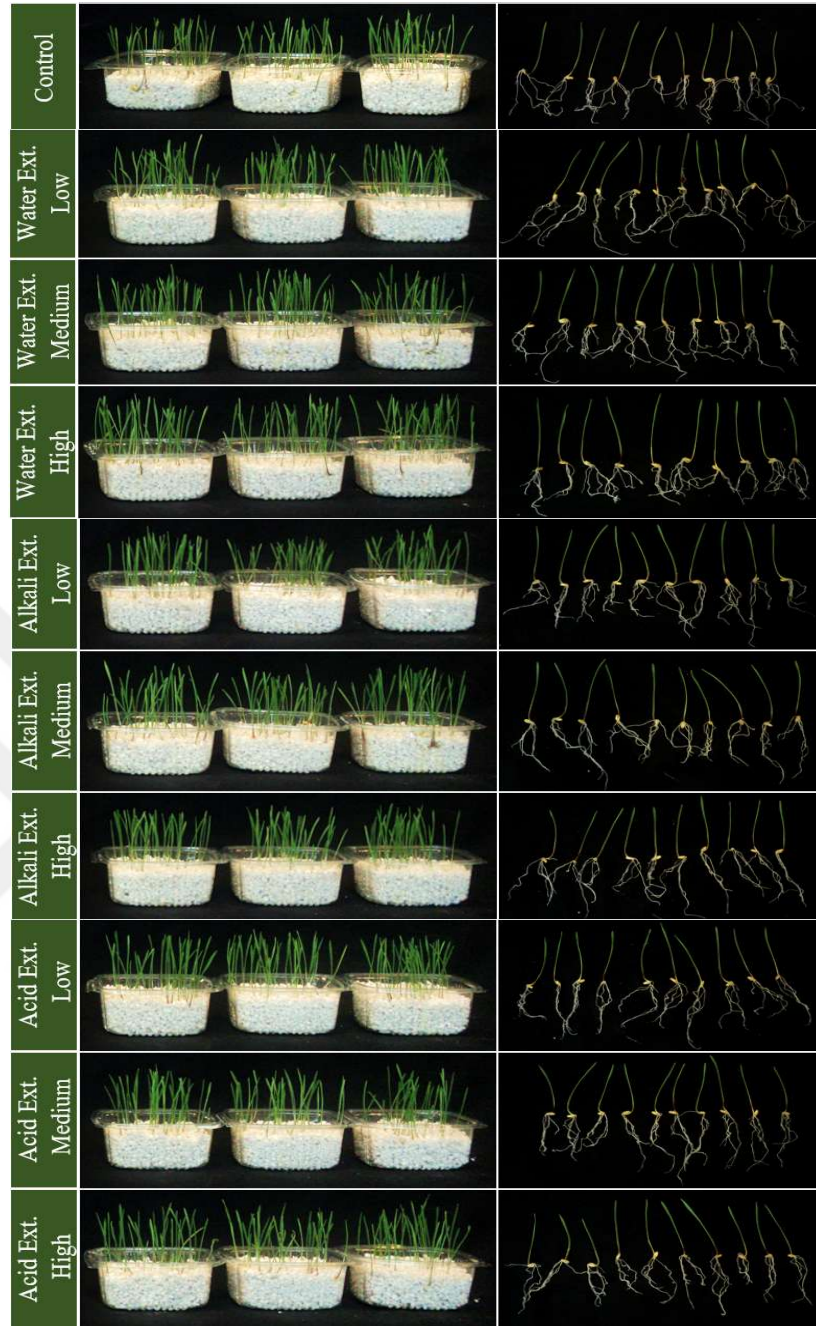


Figure 4.7: Effect of seed soaking (Control (Water); SWEs: Water extracts (Low, Medium, High); Alkali extracts (Low, Medium, High); Acid extracts (Low, Medium, High)) on perlite-grown 6 day-old-wheat (*Triticum durum* cv. Sarıçanak-98) seedlings under growth chamber conditions

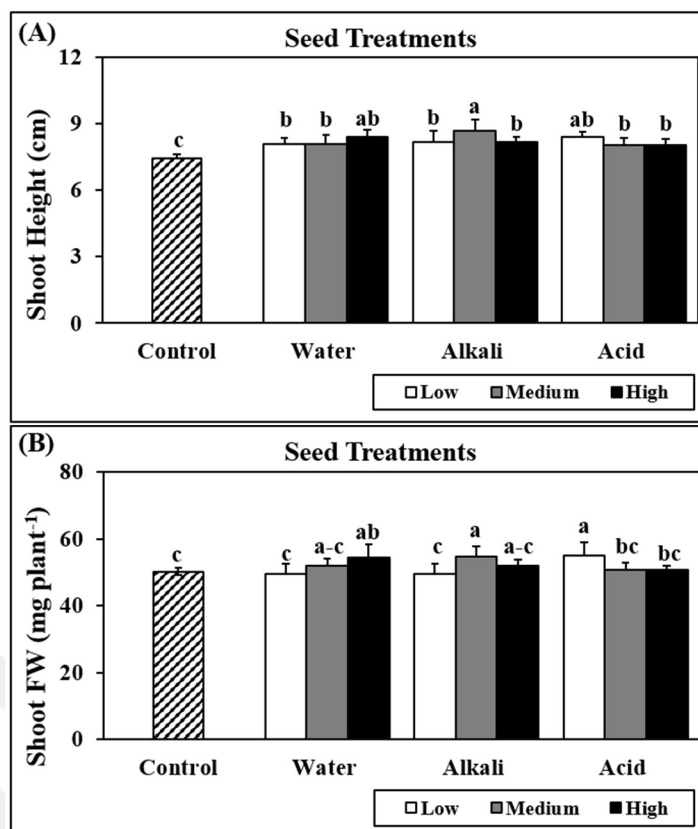


Figure 4.8: Effect of seed treatments (Control (Water); SWEs: Water extracts (Low, Medium, High); Alkali extracts (Low, Medium, High); Acid extract (Low, Medium, High)) on (A) shoot height, (B) shoot fresh weight (FW) of wheat (*Triticum durum* cv. Sariçanak-98) seedlings at 6 days after sowing (6 DAS)) grown in perlite

ANOVA indicated that soaking treatment with SWEs significantly influenced esterase activity in comparison to the control, however not in a dose-dependent manner (**Table 4.5B, Figure 4.9**). The high levels of the studied alkali and acid extracts correlated with the most notable effects on root esterase activity, which increased by as much as 40%.

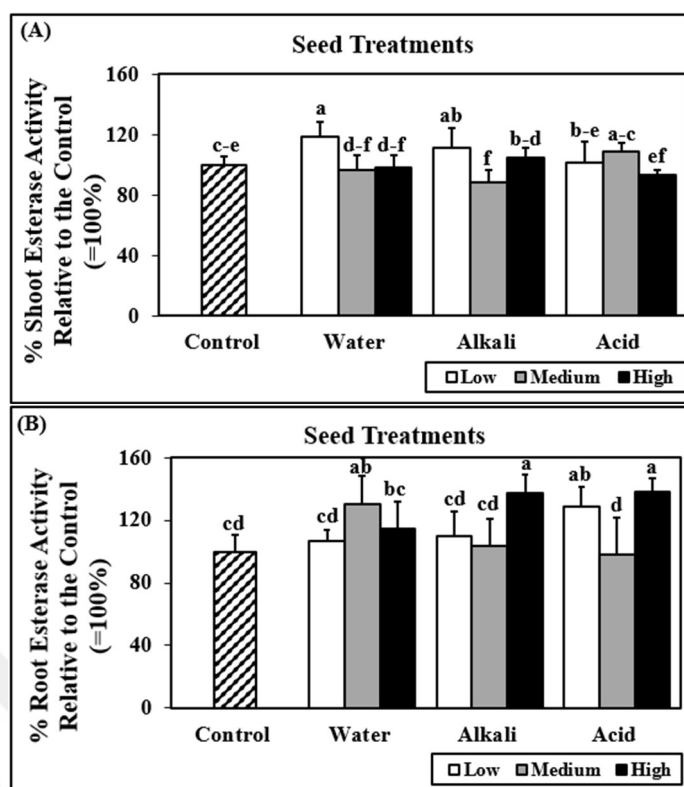


Figure 4.9: Effect of seed treatments (Control (Water); SWEs: Water extracts (Low, Medium, High); Alkali extracts (Low, Medium, High); Acid extract (Low, Medium, High)) on (A) shoot and (B) root esterase activity of wheat (*Triticum durum* cv. Sarıçanak-98) seedlings at 6 days after sowing (6 DAS)) grown in perlite

The positive effects observed on root biomass and morphological parameters in substrate experiments were not obtained in the seed soaking experiment (Table 4.8, Table 4.9). It was observed that seed soaking applications with SWE, irrespective of the application doses, caused insignificant changes in certain wheat root parameters (Table 4.5B, Table 4.9). Only a slight decrease in root biomass was observed with low dose alkali SWEs application.

Table 4.9: Effect of seed treatments on root growth and morphology parameters (FW; Length; SA, Surface Area; Vol, Volume) of wheat (*Triticum durum* cv. Sarıçanak-98) seedlings

Treatments	Root FW (mg plant ⁻¹)	Root Length (cm plant ⁻¹)	Root SA (cm ² plant ⁻¹)	Root Vol. (mm ³ plant ⁻¹)	Avg. Diameter (mm)
Control	68 ± 5 ab	21 ± 2	4.1 ± 0.4	62 ± 11	0.61 ± 0.06
Water- L	69 ± 3 ab	25 ± 1	4.6 ± 0.4	69 ± 11	0.59 ± 0.04
Water- M	70 ± 1 ab	22 ± 1	4.1 ± 0.2	61 ± 4	0.59 ± 0.01
Water- H	66 ± 4 bc	21 ± 2	4.0 ± 0.3	60 ± 3	0.61 ± 0.02
Alkali-L	62 ± 3 c	21 ± 2	4.1 ± 0.4	63 ± 5	0.61 ± 0.01
Alkali-M	68 ± 4 ab	22 ± 1	4.2 ± 0.1	66 ± 4	0.62 ± 0.03
Alkali-H	68 ± 2 ab	22 ± 2	4.1 ± 0.2	61 ± 4	0.59 ± 0.03
Acid-L	67 ± 6 ab	21 ± 3	3.9 ± 0.4	59 ± 5	0.60 ± 0.03
Acid-M	71 ± 3 a	22 ± 2	4.2 ± 0.4	62 ± 4	0.60 ± 0.01
Acid-H	71 ± 4 a	22 ± 3	4.2 ± 0.4	64 ± 4	0.61 ± 0.02

Values are mean and standard deviations of five replicates. Different letters indicate significant differences between means according to Fisher's protected LSD test ($p < 0.05$). Lack of lettering indicates absence of significant effect according to ANOVA.

4.3. Investigation of the Effects of *C. barbata* Extract Applications on Salinity Stress Resistance and Related Physiological Parameters in Wheat

4.3.1. Determination of the Effective *C.barbata* Extracts Application Levels on Plant Growth in a Hydroponic Environment

The different application levels of water, alkali, and acid extracts obtained from *C. barbata* were tested by giving them directly to the nutrient solution. These applications positively influenced the vegetative growth and development of approximately one-month-old wheat plants grown hydroponically (**Figure 4.10**). The application of SWEs in hydroponic medium caused statistically significant improvements in the shoot and root DW of wheat plants (**Table 4.10A**, **Figure 4.11**). The advantageous impact on growth and development is distinctly evident in both the shoot and root systems of wheat plants cultivated hydroponically with the administration of SWE, in contrast to those grown without it (**Figure 4.10**, **Figure 4.11**). Wheat plants subjected to medium rate of water extract exhibited an increase of around 35% in dry weight of

the shoot compared to control plants (**Figure 4.11A**). Conversely, although acid applications did not yield a statistically significant improvement relative to the control plant, it was noted that low-dose acid applications resulted in a 16% enhancement in shoot DW. As the result of shoot DW, statistically significant effects of SWE on root DW were determined (**Figure 4.11B**). In applications of water and alkali extracts at medium and high levels, notable increases in root DW were recorded compared to the control plants, with the medium rate of water extract application yielding the highest shoot and root biomass. Root DW rose by roughly 40% with a medium rate of water extract and 35% with high alkali extract treatments compared to control plants. As a result, it was observed that the biomass and vigor of wheat plants grown in the nutrient solution containing SWE increased, whereas the root and shoot growth of control plants was weak and, therefore, less developed compared to the others (**Figure 4.10, Figure 4.11**). Consequently, the most effective doses of SWEs (water (medium), alkali (low) and acid (low)) were selected for the third hydroponic experiment.



Figure 4.10: Effect of SWEs (Control (Water); Water extracts (Low, Medium, High); Alkali extracts (Low, Medium, High); Acid extracts (Low, Medium, High)) on hydroponically grown 28 day-old-wheat (*Triticum durum* cv. Sarıçanak-98) seedlings under greenhouse conditions.

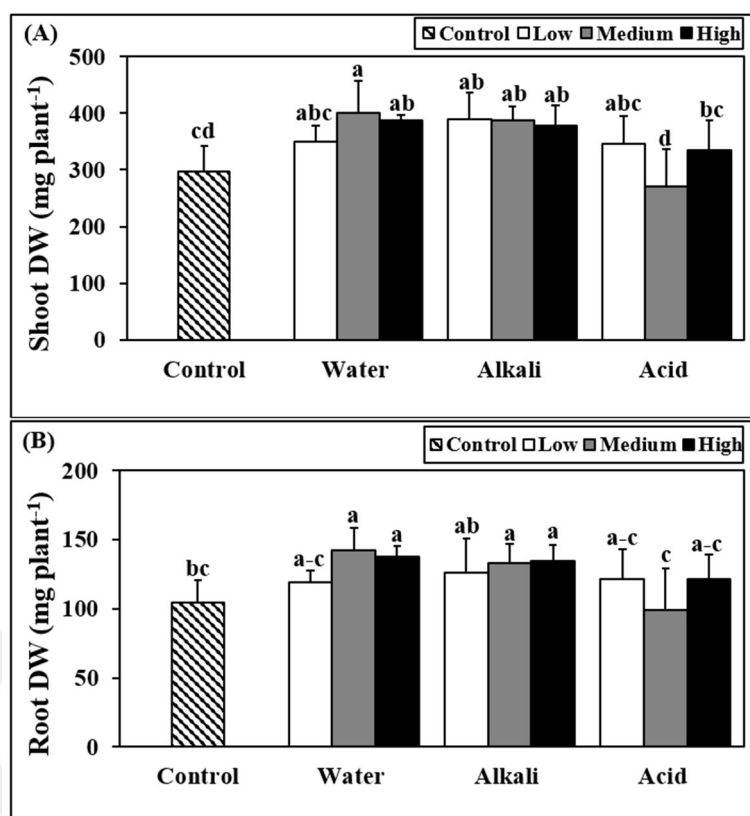


Figure 4.11: Effect of SWEs (Control (Water); Water extracts (Low, Medium, High); Alkali extracts (Low, Medium, High); Acid extracts (Low, Medium, High)) on (A) shoot DW and (B) root DW of wheat.

The effects of the extraction type on growth parameters and macro and micronutrient concentrations of SWEs in hydroponically grown wheat plants, as determined by one-way ANOVA, are shown in **Table 4.10A**. Additionally, the significance of extraction type on shoot and root dry matter in the second hydroponic experiment is detailed in **Table 4.10B**.

Table 4.10: Significance of the effects of SWE on reported traits on wheat (*Triticum durum* cv. Sariçanak-98) plants grown in (A) first hydroponic experiment (B) second hydroponic experiment according to analysis of variance (ANOVA) ($\alpha = 0.05$).

A) First Hydroponic Experiment						
Source of Variation	DF ¹	Shoot DW	Root DW			
Seaweed Extract	3	**	*			
Source of Variation	DF	P	K	Ca	Mg	S
Seaweed Extract	3	<i>n.s.</i>	***	***	***	<i>n.s.</i> ²
Source of Variation	DF	Fe	Zn	Mn	Cu	Mo
Seaweed Extract	3	**	***	*	***	<i>n.s.</i>
B) Second Hydroponic Experiment						
Source of Variation	DF	Shoot DW	Root DW			
Salinity Stress	1	***	***			

DF¹, degree of freedom; *n.s.*²: not significant; * $0.01 < p < 0.05$; ** $0.001 < p < 0.01$; *** $p < 0.001$

The effect of adding SWEs directly to the nutrient solution on macronutrient concentration in hydroponically grown wheat plants are shown in **Table 4.11**. The concentrations of P and S in the shoots of hydroponically grown wheat plants were not substantially influenced by *C. barbata* extracts compared to the control group; nevertheless, K, Ca, and Mg concentrations exhibited statistically significant changes (**Table 4.10A**, **Table 4. 11**). Application of high rate of water extracts increased K concentrations by approximately 10% relative to the control group. Conversely, all SWEs administered at high doses and water extracts given at moderate levels resulted in a substantial reduction in Ca, while moderate applications of acid extracts led to a considerable rise. Finally, all SWEs, apart from the medium rate of acid extracts, markedly diminished Mg concentration; yet the plants exhibited no indications of Ca or Mg deficiency.

Table 4.11: Changes in shoot macronutrient (P, K, Ca, Mg, S) concentrations of wheat (*Triticum durum* cv. Sariçanak-98) seedlings in response to SWEs.

Seaweed Extracts	P (mg g ⁻¹)	K (mg g ⁻¹)	Ca (mg g ⁻¹)	Mg (mg g ⁻¹)	S (mg g ⁻¹)
Control	7.5 ± 0.2	52 ± 1 bc	9.1 ± 0.3 b	2.6 ± 0.2 a	4.5 ± 0.2
Water-L	7.3 ± 0.2	53 ± 1 bc	8.9 ± 0.2 b-d	2.3 ± 0.0 b	4.5 ± 0.1
Water-M	7.0 ± 0.7	51 ± 2 b-d	8.4 ± 0.5 de	2.2 ± 0.1 bc	4.4 ± 0.1
Water-H	7.8 ± 0.1	57 ± 1 a	8.2 ± 0.1 e	2.2 ± 0.0 bc	4.6 ± 0.0
Alkali-L	7.3 ± 0.7	54 ± 4 ab	8.7 ± 0.6 b-e	2.3 ± 0.2 bc	4.7 ± 0.1
Alkali-M	6.7 ± 0.2	49 ± 1 d	8.8 ± 0.3 b-e	2.2 ± 0.0 bc	4.8 ± 0.0
Alkali-H	6.7 ± 0.8	51 ± 3 cd	8.3 ± 0.5 de	2.0 ± 0.2 c	4.9 ± 0.5
Acid-L	7.0 ± 0.7	50 ± 3 cd	9.1 ± 0.6 bc	2.3 ± 0.1 bc	4.6 ± 0.0
Acid-M	7.5 ± 0.7	51 ± 2 cd	10.4 ± 0.7 a	2.6 ± 0.3 a	4.6 ± 0.3
Acid-H	6.8 ± 0.5	50 ± 2 cd	8.5 ± 0.2 c-e	2.1 ± 0.0 bc	4.5 ± 0.0

Values are mean and standard deviations of five replicates. Different letters indicate significant differences between means according to Fisher's protected LSD test ($p < 0.05$). Lack of lettering indicates absence of significant effect according to ANOVA.

The effects of SWEs added to hydroponic medium on wheat shoot microelement (Fe, Zn, Mn, Cu and B) concentrations are shown in **Table 4.12**. The treatments of SWEs considerably influenced the concentrations of micronutrients in shoots except for Mo (**Table 4.10A, Table 4.12**). The application of water extracts and moderate doses of acid extracts from plants resulted in an approximate 10% enhancement in Fe concentration in the shoot tissues; however, this increase was not statistically significant. Conversely, although the quantities of Zn, Mn and Cu diminished with the treatments, no micronutrient deficiencies were detected in the plants.

Table 4.12: Changes in shoot micronutrient (Fe, Zn, Mn, Cu, Mo) concentrations of wheat (*Triticum durum* cv. Sariçanak-98) seedlings in response to SWEs.

Seaweed Extracts	Fe (mg kg ⁻¹)	Zn (mg kg ⁻¹)	Mn (mg kg ⁻¹)	Cu (mg kg ⁻¹)	Mo (mg kg ⁻¹)
Control	198 ± 12 ab	75 ± 7 a	138 ± 9 a	15 ± 1 a	6.8 ± 0.3
Water-L	187 ± 9 b	52 ± 2 b	126 ± 7 ab	14 ± 1 b-d	6.9 ± 0.4
Water-M	220 ± 14 a	49 ± 1 bc	120 ± 8 bc	14 ± 1 a-c	6.2 ± 0.3
Water-H	189 ± 12 b	47 ± 4 bc	126 ± 8 ab	14 ± 0 a-c	6.8 ± 0.5
Alkali-L	179 ± 23 bc	53 ± 8 b	127 ± 17 ab	15 ± 1 ab	6.6 ± 1.3
Alkali-M	184 ± 19 bc	53 ± 4 b	124 ± 5 ab	13 ± 0 cd	6.7 ± 0.3
Alkali-H	177 ± 15 bc	47 ± 4 bc	113 ± 20 bc	13 ± 1 d-f	6.4 ± 0.4
Acid-L	158 ± 20 c	50 ± 2 b	124 ± 12 ab	13 ± 0 de	6.4 ± 0.2
Acid-M	220 ± 34 a	44 ± 7 cd	105 ± 19 c	12 ± 2 ef	6.5 ± 0.1
Acid-H	185 ± 14 b	39 ± 2 d	117 ± 6 bc	11 ± 1 f	6.3 ± 0.6

Values are mean and standard deviations of five replicates. Different letters indicate significant differences between means according to Fisher's protected LSD test ($p < 0.05$). Lack of lettering indicates absence of significant effect according to ANOVA.

4.3.2. Determination of Effective Salt Stress Application Levels on Plant Growth in a Hydroponic Environment

In this experiment, the impact of salinity stress on hydroponically grown wheat plants was investigated by incorporating five distinct NaCl concentrations (0, 30, 90, 120, and 150 mM) directly into the nutrient solution (**Figure 4.12**). ANOVA results indicate that salinity treatments had a decreasing effect on both shoot and root biomass (**Table 4.10B**). In comparison to control plants that received no salt application, escalating salt concentration progressively decreased wheat growth and development. The control plant exhibited superior vegetative growth and enhanced development in the hydroponic system, characterized by an increased number of shoots and roots (**Figure 4.12, Figure 4.13**). Moreover, as the salt concentration increased, shoot biomass progressively decreased, with reductions of 10%, 20%, 30%, 35%, and 40% corresponding to the application of 30, 60, 90, 120, and 150 mM NaCl, respectively (**Figure 4.13A**). Increased salt levels negatively affected not only shoot biomass but also root biomass of wheat (**Figure 4.12, Figure 4.13B**). High salt concentrations of

120 and 150 mM NaCl statistically significantly reduced wheat root biomass compared to the control. As expected, plants had the highest shoot and root biomass under control conditions, while plants growing at 150 mM had the lowest shoot and root biomass. As a result, the 60 and 90 mM NaCl levels were selected for the third hydroponic experiment because this level caused the reduction of 20-30 % shoot and root biomass and so led to moderate stress on hydroponically grown wheat.



Figure 4.12: Effect of different salinity stress levels (Control (0 mM NaCl, 30 mM NaCl, 60 mM NaCl, 90 mM NaCl, 120 mM NaCl, 150 mM NaCl) on hydroponically grown 28 day-old-wheat (*Triticum durum* cv. Sarıçanak-98) seedlings under greenhouse conditions.

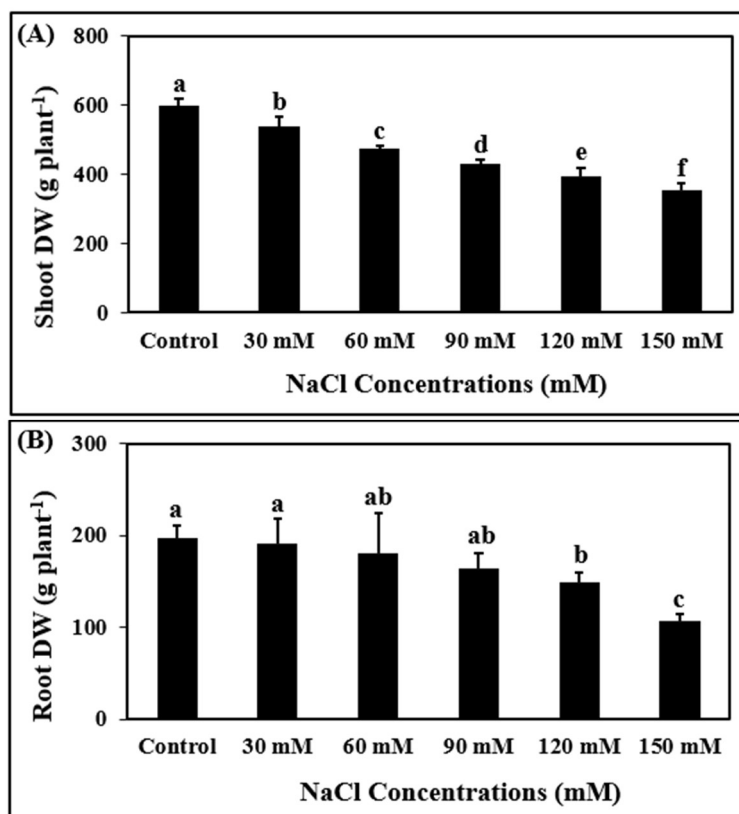


Figure 4.13: Effect of different salinity stress levels (Control (0 mM NaCl, 30 mM NaCl, 60 mM NaCl, 90 mM NaCl, 120 mM NaCl, 150 mM NaCl) on (A) shoot DW per plant and (A) root DW per plant of wheat.

4.3.3. Effects of Selected *C. barbata* Extract Applications on Salinity Stress Resistance and Physiological Parameters

The third hydroponic wheat experiment utilized the most effective doses of SWEs (water extract (medium), alkali (low) and acid extracts (low)) determined in the first hydroponic experiment (**Figure 4.11**) and the salinity stress levels (60 mM and 90 mM) chosen from the second hydroponic experiment (**Figure 4.13**). The impact of incorporating water, alkali, and acid extracts derived from *C. barbata* into hydroponic medium was evaluated in deep water culture under both control (0 mM) and saline conditions (60 mM, 90 mM) (**Figure 4.14**).

The impact of salt stress on plant growth was evaluated by quantifying the dry weight of the shoot and root of hydroponically cultivated wheat (**Figure 4.15**). According to a two-way analysis of variance, the interaction of salinity and biostimulant treatment was found to be significant on the shoot biomass (**Table 4.13, Figure 4.15**). On average, shoot dry weight decreased by approximately 20% and 25% under 60 mM

and 90 mM salinity stress treatments, respectively (**Figure 4.15A**). Under control conditions, water, and alkali extract treatments statistically significantly enhanced shoot DW. Regardless of stress, the most positive effects on shoot and root biomass were obtained with water, acid and alkali extract applications, respectively (**Figure 4.15**). All treatments of SWEs positively influenced shoot DW under 60 mM salt stress, with water extract treatment resulting in a statistically significant increase of 17% compared to the control. On the other hand, when compared to the control plants, acid extract applications enhanced the shoot DW by 23% under high salinity conditions.

The biostimulant and salinity applications as well as the interaction of salinity and biostimulants caused a significant effect on root DW (**Table 4.13, Figure 4.15B**). The root biomass of wheat plants was significantly impacted by salinity stress, resulting in a 15% reduction (**Figure 4.15B**). The application of water extract to the nutrient solution increased hydroponically grown wheat root biomass up to 36% under 60 mM salt stress. Moreover, the acid extract treatment significantly increased root biomass compared to the control, with up to 50% under high salinity conditions.

Overall, the addition of SWEs to the nutrient solution positively influenced vegetative growth performance relative to untreated plants under both non-stressed and stressed circumstances (**Figure 4.14, Figure 4.15**).



Figure 4.14: Effect of biostimulant applications (Control; Water Ext.; Alkali Ext.; Acid Ext.) and salinity stress levels (0 mM NaCl, 60 mM NaCl, 90 mM NaCl) on hydroponically grown 28 day-old-wheat (*Triticum durum* cv. Sarıçanak-98) seedlings under greenhouse conditions.

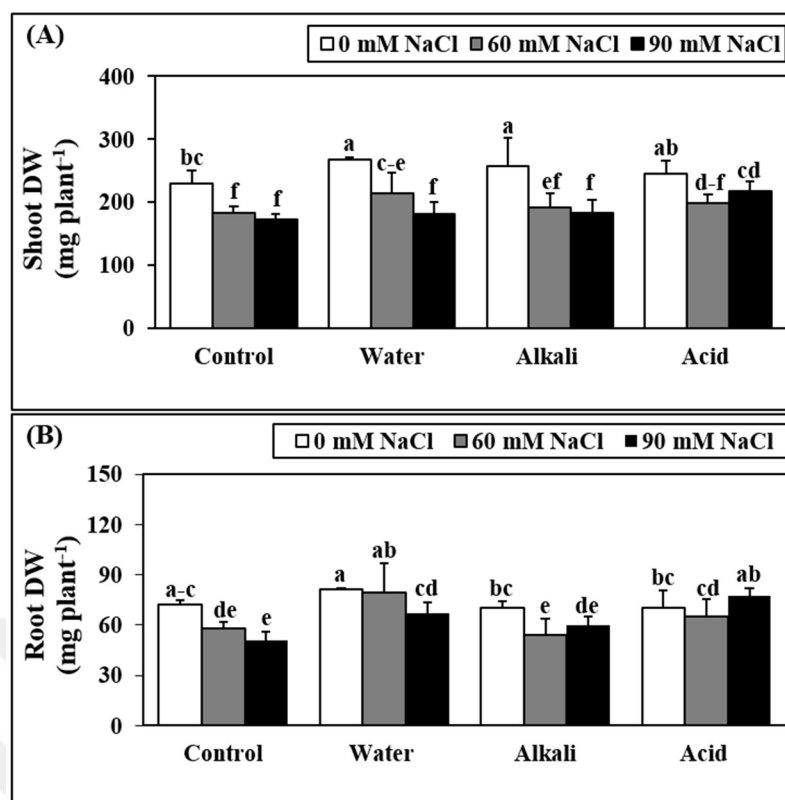


Figure 4.15: Effect of SWEs (Control; Water; Alkali; Acid) and salinity stress levels (0 mM NaCl, 60 mM NaCl, 90 mM NaCl) on (A) shoot DW and (B) Root DW of hydroponically grown wheat.

The single effect of SWE and salinity stress treatments and their combined interaction of SWE treatment and salinity stress treatments on the measured parameters in hydroponically grown wheat plants were shown by two-way ANOVA (**Table 4.13**).

Table 4.13: Significance of the effects of SWE on reported traits on wheat (*Triticum durum* cv. Sarıçanak-98) plants grown in third hydroponic experiment according to two-way analysis of variance (ANOVA) ($\alpha = 0.05$).

Source of Variation	DF	Shoot DW	Root DW	REL	Protein	APX Total
Seaweed Extract (A)	3	**	***	***	<i>n.s.</i>	***
Salinity Stress (B)	2	***	***	***	<i>n.s.</i>	***
A x B	3	*	**	*	<i>n.s.</i>	***
Source of Variation	DF	CAT Total	GR Total	SOD Total	APX Sp	CAT Sp
Seaweed Extract (A)	3	<i>n.s.</i>	<i>n.s.</i>	**	***	<i>n.s.</i>
Salinity Stress (B)	2	*	***	*	***	<i>n.s.</i>
A x B	3	<i>n.s.</i>	<i>n.s.</i>	*	***	<i>n.s.</i>
Source of Variation	DF	GR Sp	SOD Sp	MDA	Glycine Betaine	Proline
Seaweed Extract (A)	3	<i>n.s.</i>	<i>n.s.</i>	**	<i>n.s.</i>	***
Salinity Stress (B)	2	*	*	***	<i>n.s.</i>	***
A x B	3	<i>n.s.</i>	*	*	*	*
Source of Variation	DF	Ca	Mg	P	S	
Seaweed Extract (A)	3	**	*	***	***	
Salinity Stress (B)	2	***	***	***	<i>n.s.</i>	
A x B	3	**	*	<i>n.s.</i>	**	
Source of Variation	DF	Fe	Zn	Mn	Cu	Mo
Seaweed Extract (A)	3	*	***	<i>n.s.</i>	**	*
Salinity Stress (B)	2	***	<i>n.s.</i>	<i>n.s.</i>	***	***
A x B	3	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
Source of Variation	DF	Na	K	Na/K		
Seaweed Extract (A)	3	<i>n.s.</i>	***	<i>n.s.</i>		
Salinity Stress (B)	2	***	***	***		
A x B	3	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>		

DF¹, degree of freedom; *n.s.*²: not significant; * 0.01 < p < 0.05; ** 0.001 < p < 0.01; *** p < 0.001

A two-way analysis of variance indicated that biostimulant and salinity treatments and their interaction significantly influenced REL (Table 4.13, Figure 4.16). Overall, control plants exhibited the highest REL value, while the lowest REL values were recorded with water, acid, and alkali applications, respectively. Salinity treatment in the absence of SWE application caused significant changes in REL, however the REL values were statistically unchanged under non-saline conditions. Under 60 mM NaCl conditions, all SWE extracts significantly reduced REL values compared to control. On the other hand, only water-based SWE markedly reduced REL in comparison to the control under high salinity conditions.

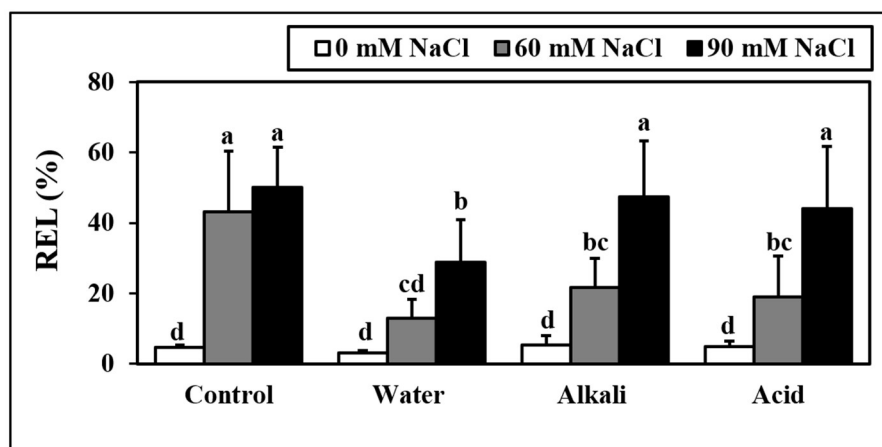


Figure 4.16: Effect of SWEs (Control; Water; Alkali; Acid) and salinity stress levels (0 mM NaCl, 60 mM NaCl, 90 mM NaCl) on REL values (EC_1/EC_2 (%)) of hydroponically grown wheat.

The effects of SWEs on the macronutrient concentrations of hydroponically grown wheat are shown in **Table 4.13**. The macronutrient concentration was affected by the interaction of biostimulant and salinity stress, with the exception of P, which remained unaffected (**Table 4.14**). Among the applications, the control plants had the lowest P, Mg and S concentrations. On the other hand, salinity stress caused Ca and Mg concentration to decrease by approximately 40% and 30%, respectively, while it did not affect S concentration significantly. In general, P concentration tended to increase with increasing salinity stress levels. Under high-stress conditions, the water extract resulted in a slight decrease in Ca concentration in wheat. Mg concentration did not change statistically with SWE treatments under stress conditions. Under stressful circumstances, S concentration in wheat plants treated with alkali and acid extracts exceeded those of control plants.

Table 4.14: Effect of SWEs (Control; Water; Alkali; Acid) and salinity stress levels (0 mM NaCl, 60 mM NaCl, 90 mM NaCl) on macronutrient concentrations (P, Ca, Mg and S) of hydroponically grown wheat (*Triticum durum* cv. Sariçanak-98).

Seaweed Extracts	NaCl (mM)	P (mg g ⁻¹)	Ca (mg g ⁻¹)	Mg (mg g ⁻¹)	S (mg g ⁻¹)
Control	0	11 ± 1	9.6 ± 0.2 c	2.4 ± 0.1 c	4.2 ± 0.1 e
	60	15 ± 1	6.3 ± 0.2 e	1.8 ± 0.1 de	4.7 ± 0.2 d
	90	15 ± 0	7.0 ± 0.8 d	1.8 ± 0.2 de	4.9 ± 0.3 a-d
Water	0	14 ± 1	10.4 ± 0.4 b	2.7 ± 0.2 ab	5.0 ± 0.1 a-c
	60	17 ± 1	6.2 ± 0.7 e	1.8 ± 0.1 de	4.8 ± 0.3 b-d
	90	18 ± 1	6.3 ± 0.2 e	1.7 ± 0.1 e	4.7 ± 0.4 cd
Alkali	0	12 ± 1	10.7 ± 0.2 b	2.5 ± 0.1 bc	4.8 ± 0.2 cd
	60	16 ± 2	6.7 ± 0.6 de	1.9 ± 0.2 d	5.0 ± 0.2 a-c
	90	16 ± 0	7.1 ± 0.3 d	1.9 ± 0.1 de	5.1 ± 0.1 a-c
Acid	0	13 ± 1	11.5 ± 0.5 a	2.7 ± 0.1 a	5.1 ± 0.1 a
	60	16 ± 1	6.6 ± 0.4 de	1.8 ± 0.1 de	4.9 ± 0.1 a-d
	90	16 ± 1	6.7 ± 0.0 de	1.8 ± 0.1 de	5.1 ± 0.1 ab

Values are mean and standard deviations of four replicates. Different letters indicate significant differences between means according to Fisher's protected LSD test ($p < 0.05$). Lack of lettering indicates absence of significant effect according to ANOVA.

The interaction of biostimulant and salinity stress treatments had no significant effect on the concentration of any micronutrients (Table 4.13). A slight increase in Cu, Mo and B concentration was observed with stress application (Table 4.15). Furthermore, low salinity stress resulted in an increase of approximately 23% in Fe concentration, while high salt stress led to a 50% increase. Seaweed treatments enhanced shoot Fe concentration, with alkali extracts specifically increasing Fe concentration by 28% under high salt level. Although salinity stress did not influence Zn concentration, shoot Zn levels in hydroponically grown wheat ranged from 33 to 43 mg kg⁻¹. Neither salt stress nor the application of SWEs significantly influenced the Mn concentration in wheat. The Cu concentration in hydroponically grown wheat, which increased with stress, tended to decrease with SWE treatments. Overall, the highest Mo levels were observed in acid extract-treated plants compared to other plants under saline conditions.

Table 4.15: Effect of SWEs (Control; Water; Alkali; Acid) and salinity stress levels (0 mM NaCl, 60 mM NaCl, 90 mM NaCl) on micronutrient concentrations (Fe, Zn, Mn, Cu, and Mo) of hydroponically grown wheat (*Triticum durum* cv. Sariçanak-98).

Seaweed Extracts	NaCl (mM)	Fe (mg kg ⁻¹)	Zn (mg kg ⁻¹)	Mn (mg kg ⁻¹)	Cu (mg kg ⁻¹)	Mo (mg kg ⁻¹)
Control	0	100 ± 12	37 ± 3	79 ± 6	15 ± 1	9 ± 1
	60	131 ± 15	43 ± 6	92 ± 14	19 ± 2	13 ± 2
	90	152 ± 18	43 ± 4	89 ± 14	19 ± 2	12 ± 1
Water	0	118 ± 10	35 ± 1	89 ± 1	17 ± 2	9 ± 0
	60	146 ± 13	33 ± 0	97 ± 6	18 ± 1	12 ± 1
	90	162 ± 24	34 ± 1	87 ± 9	18 ± 2	12 ± 2
Alkali	0	117 ± 9	39 ± 5	82 ± 2	15 ± 0	10 ± 1
	60	133 ± 8	38 ± 2	87 ± 16	16 ± 2	13 ± 2
	90	194 ± 19	38 ± 1	88 ± 6	16 ± 0	13 ± 0
Acid	0	117 ± 9	41 ± 1	87 ± 11	17 ± 1	10 ± 0
	60	146 ± 13	37 ± 2	96 ± 8	18 ± 1	13 ± 1
	90	173 ± 22	41 ± 1	95 ± 8	19 ± 0	14 ± 0

Values are mean and standard deviations of four replicates. Different letters indicate significant differences between means according to Fisher's protected LSD test ($p < 0.05$). Lack of lettering indicates absence of significant effect according to ANOVA.

There was no statistically significant difference in the interaction of biostimulant and salinity stress on Na, K and Na/K ratio (**Table 4.13**). On the other hand, Na concentration increased significantly with increasing salinity level (**Table 4.13**, **Table 4.16**). Compared to control plants, lower Na levels were detected in water-extract treated plants under low stress conditions, while higher Na levels were observed in wheat plants treated with alkaline extraction under high stress. Overall, stress applications adversely affected K concentration, causing a reduction of around 40% (**Table 4.16**). Generally, control plants exhibited the lowest K concentrations, whereas SWEs treatments elevated K levels, with the highest amounts observed in plants treated with water and acid extract. Higher K concentrations were observed in wheat plants treated with SWE in both non-stress and stress conditions. Although the Na/K ratio was statistically affected only by salinity stress, wheat plants treated with water extract under stress had the lowest Na/K ratio in both stress conditions, followed by those treated with acid extract (**Table 4.16**).

Table 4.16: Effect of SWEs (Control; Water; Alkali; Acid) and salinity stress levels (0 mM NaCl, 60 mM NaCl, 90 mM NaCl) on Na (%), K (%) and Na/K ratio of hydroponically grown wheat (*Triticum durum* cv. Sarıçanak-98).

Seaweed Extracts	NaCl (mM)	Na (%)		K (%)		Na/K	
Control	0	0.02	± 0.01	5.9	± 0.2	0.00	± 0.00
	60	3.33	± 0.38	3.5	± 0.3	0.95	± 0.11
	90	3.80	± 0.55	3.3	± 0.2	1.17	± 0.15
Water	0	0.05	± 0.01	6.3	± 0.2	0.01	± 0.00
	60	3.19	± 0.37	4.2	± 0.1	0.77	± 0.09
	90	3.83	± 0.41	3.6	± 0.3	1.06	± 0.16
Alkali	0	0.04	± 0.01	6.1	± 0.1	0.01	± 0.00
	60	3.62	± 0.90	3.9	± 0.5	0.97	± 0.38
	90	4.72	± 0.33	3.5	± 0.2	1.36	± 0.18
Acid	0	0.04	± 0.00	6.3	± 0.1	0.01	± 0.00
	60	3.22	± 0.23	4.0	± 0.3	0.80	± 0.10
	90	4.24	± 0.48	3.6	± 0.2	1.19	± 0.15

Values are mean and standard deviations of four replicates. Different letters indicate significant differences between means according to Fisher's protected LSD test ($p < 0.05$). Lack of lettering indicates absence of significant effect according to ANOVA.

The biostimulant and salinity applications as well as the interaction of salinity and biostimulants did not cause any statistically significant effect on protein concentrations (Table 4.13, Table 4.17).

Table 4.17: Effect of SWEs (Control; Water; Alkali; Acid) and salinity stress levels (0 mM NaCl, 60 mM NaCl, 90 mM NaCl) on average protein concentration (590/450) (%) of hydroponically grown wheat (*Triticum durum* cv. Sarıçanak-98) shoots

Seaweed Extracts	NaCl (mM)	Average [Protein] _m (590/450) (%)		
Control	0	3.1	±	0.9
	60	4.0	±	0.7
	90	3.5	±	0.5
Water	0	3.2	±	0.9
	60	3.7	±	0.3
	90	3.1	±	0.8
Alkali	0	4.2	±	1.2
	60	4.0	±	1.0
	90	4.0	±	1.4
Acid	0	3.5	±	0.1
	60	4.1	±	1.1
	90	4.0	±	1.0

Values are mean and standard deviations of four replicates. Different letters indicate significant differences between means according to Fisher's protected LSD test ($p < 0.05$). Lack of lettering indicates absence of significant effect according to ANOVA.

To assess the antioxidant responses of hydroponically grown wheat plants to saline stress, the total enzymatic activity of SOD, APX, GR and CAT in the shoots were quantified (**Table 4.18**). The interaction of salinity and biostimulant treatments significantly influenced total APX and SOD activities, although CAT and GR activities were unaffected by this interaction (**Table 4.13**). The total APX activity was reduced by biostimulant and salinity treatments and so the greatest values in APX activity were measured with the control plant under non-saline conditions. On the other hand, all SWE applications statistically significantly decreased the total activity of the APX enzyme under 60 mM NaCl conditions (**Table 4.18**). In general, total CAT activity tended to decrease with salt stress. It was found that all wheat plants had similar levels of CAT enzyme activity. With elevated salt levels resulted in an enhancement in total GR activity. Conversely, plants treated with water extract exhibited the highest GR activity at high salt level. The activity of the SOD enzyme, which tended to increase with salinity stress and decrease with SWEs, was typically highest in wheat plants that did not receive SWEs treatment.

Table 4.18: Effect of SWEs (Control; Water; Alkali; Acid) and salinity stress levels (0 mM NaCl, 60 mM NaCl, 90 mM NaCl) on the total antioxidant enzyme (APX, CAT, GR, SOD) activities of shoot of hydroponically grown wheat (*Triticum durum* cv. Sarıçanak-98).

Seaweed Extracts	NaCl (mM)	APX ($\mu\text{mol H}_2\text{O}_2 \text{ g}^{-1}$ FW min^{-1})			CAT ($\text{mmol H}_2\text{O}_2$ g^{-1} FW min^{-1})			GR ($\mu\text{mol g}^{-1}$ FW min^{-1})			SOD (U/g FW)		
Control	0	31.0	± 3.2	a	3.0	± 0.3		0.47	± 0.06		107	± 6	a
	60	20.5	± 9.4	b	2.5	± 0.6		0.69	± 0.10		94	± 6	bc
	90	2.8	± 0.6	d	2.5	± 0.3		0.70	± 0.15		100	± 6	ab
Water	0	11.5	± 3.2	c	2.7	± 0.1		0.55	± 0.03		79	± 9	d
	60	3.9	± 0.8	d	2.5	± 0.3		0.59	± 0.09		92	± 5	bc
	90	3.8	± 1.7	d	2.6	± 0.3		0.74	± 0.15		95	± 6	bc
Alkali	0	19.3	± 5.4	b	3.0	± 0.3		0.55	± 0.05		93	± 14	bc
	60	12.9	± 0.6	c	2.7	± 0.2		0.61	± 0.02		96	± 5	bc
	90	5.5	± 3.0	d	2.8	± 0.3		0.61	± 0.07		102	± 10	ab
Acid	0	4.0	± 1.1	d	2.8	± 0.2		0.48	± 0.04		87	± 5	cd
	60	5.0	± 0.8	d	2.8	± 0.3		0.61	± 0.04		94	± 9	bc
	90	5.6	± 0.6	d	2.8	± 0.1		0.59	± 0.03		101	± 7	ab

Values are mean and standard deviations of four replicates. Different letters indicate significant differences between means according to Fisher's protected LSD test ($p < 0.05$). Lack of lettering indicates absence of significant effect according to ANOVA.

In addition to the above total enzyme activity, the impact of SWEs on the specific enzymatic activity of wheat plants subjected to salinity stress was also assessed (**Table 4.19**). The specific enzymatic antioxidant activities did not change with the interaction of salinity and biostimulant treatments, except for APX and SOD (**Table 4.13**). Specific APX activity in unstressed plants was approximately 5 times the average APX activity of all plants exposed to high salt level. On the other hand, this enzyme activity diminished by 45% with water and alkali, 75% with acid applications, respectively. When all application groups were analyzed, it was generally seen that the control plants had the highest CAT activity, which diminished in response to salt stress. Salinity stress significantly enhanced shoot GR enzyme activity, while this enzyme activity values in hydroponically grown wheat were found to be at similar levels. The activity of the SOD enzyme, which did not change significantly with SWE-based biostimulant in both salinity levels.

Table 4.19: Effect of SWEs (Control; Water; Alkali; Acid) and salinity stress levels (0 mM NaCl, 60 mM NaCl, 90 mM NaCl) on the specific antioxidant enzyme (APX, CAT, GR, SOD) activities of shoot of hydroponically grown wheat (*Triticum durum* cv. Sariçanak-98).

Seaweed Extracts	NaCl (mM)	APX (nmol H ₂ O ₂ mg ⁻¹ prt min ⁻¹)			CAT (μmol H ₂ O ₂ mg ⁻¹ prt min ⁻¹)			GR (nmol NADPH mg ⁻¹ prt min ⁻¹)			SOD (U mg ⁻¹ protein)		
Control	0	1048	± 224	a	96	± 28		14	± 5		3.6	± 0.8	a
	60	465	± 157	c	66	± 25		18	± 5		2.4	± 0.3	c
	90	93	± 31	e	86	± 23		18	± 1		2.9	± 0.4	a-c
Water	0	669	± 170	b	90	± 25		18	± 6		3.6	± 1.1	a
	60	103	± 26	e	68	± 15		16	± 4		2.5	± 0.3	bc
	90	114	± 63	e	76	± 17		19	± 2		3.2	± 0.8	a-c
Alkali	0	426	± 24	c	75	± 19		14	± 5		2.3	± 0.8	c
	60	309	± 82	d	70	± 18		16	± 4		2.5	± 0.8	bc
	90	146	± 87	e	74	± 20		16	± 4		2.8	± 1.1	ab
Acid	0	113	± 31	e	80	± 6		14	± 1		2.5	± 0.2	bc
	60	131	± 47	e	74	± 23		16	± 5		2.4	± 0.5	bc
	90	146	± 33	e	74	± 18		16	± 4		2.7	± 0.7	a-c

Values are mean and standard deviations of four replicates. Different letters indicate significant differences between means according to Fisher's protected LSD test ($p < 0.05$). Lack of lettering indicates absence of significant effect according to ANOVA.

Salinity stress and SWEs as well as their interaction had a significant influence on the accumulation of MDA in the shoot of wheat, as displayed in **Table 4.13**. Salinity stress resulted in a rise of roughly 37% and 51% in MDA levels under low and high salt stress, respectively (**Table 4.20**). On the other hand, compared to other applications, the highest MDA concentration was observed in wheat plants with water extract applications under high salt stress conditions.

Table 4.20. Effect of SWEs (Control; Water; Alkali; Acid) and salinity stress levels (0 mM NaCl, 60 mM NaCl, 90 mM NaCl) on shoot MDA concentration (nmol g⁻¹ FW) of hydroponically grown wheat (*Triticum durum* cv. Sariçanak-98).

Seaweed Extracts	NaCl (mM)	MDA (nmol/g FW)		
Control	0	9.3	± 1.2	e-g
	60	12.2	± 1.9	b-d
	90	11.8	± 0.7	b-e
Water	0	9.6	± 1.6	d-g
	60	11.1	± 2.5	c-f
	90	16.1	± 4.8	a
Alkali	0	7.3	± 1.6	g
	60	9.7	± 1.5	d-g
	90	11.7	± 1.1	b-e
Acid	0	8.4	± 1.7	fg
	60	14.3	± 0.8	ab
	90	13.1	± 1.2	bc

Values are mean and standard deviations of four replicates. Different letters indicate significant differences between means according to Fisher's protected LSD test ($p < 0.05$). Lack of lettering indicates absence of significant effect according to ANOVA.

The proline concentration was significantly affected by the interaction of salinity and biostimulant treatments (**Table 4.13, Figure 4.17**). Under salinity stress conditions, shoot proline concentrations increased up to 25% with 60 mM NaCl and 45% with 90 mM NaCl. Significant increases in proline levels were also observed in SWE-treated plants under high salinity conditions. Increasing effects of SWE on proline levels were determined regardless of stress, and while control plants had the lowest proline levels. Under high salinity stress, proline levels increased by 80% and 115% following treatment with water and alkali extracts, respectively. The highest proline concentration was observed in wheat treated with acid extracts under both moderate and high salinity conditions. Proline accumulation in wheat plants exposed to salinity stress was significantly increased, roughly 1.5-times and 2.5-times greater than that in control plants under medium and high stress conditions, respectively.

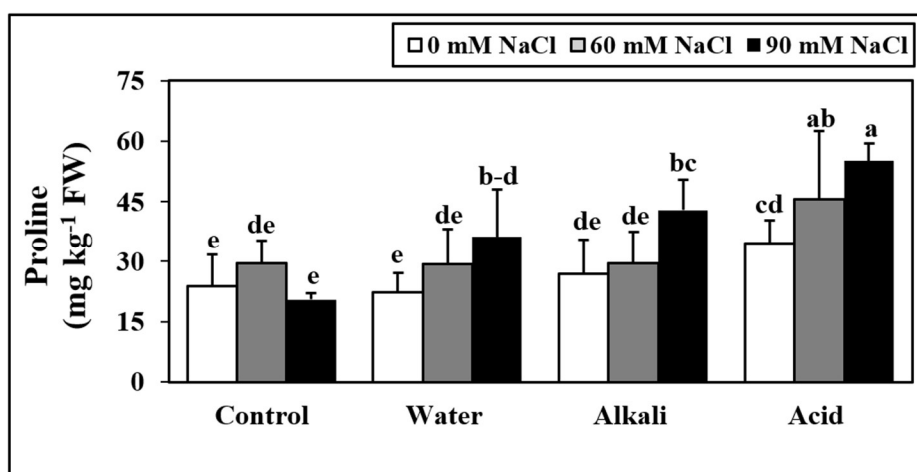


Figure 4.17: Effect of SWEs (Control; Water; Alkali; Acid) and salinity stress levels (0 mM NaCl, 60 mM NaCl, 90 mM NaCl) on shoot proline concentration (mg kg^{-1} FW) of hydroponically grown wheat (*Triticum durum* cv. Sariçanak-98).

According to ANOVA results, neither biostimulant application nor salinity stress alone had a significant effect on glycine betaine in wheat; however, their interaction had a statistically significant effect (**Table 4.13, Table 4.21**). Control plants that did not receive SWE treatment had the lowest glycine betaine concentrations, regardless of whether they were grown in the absence or presence of saline conditions. Overall, all SWEs, including alkali, water, and acid, positively influenced glycine betaine accumulation in wheat, irrespective of stress conditions. Although not statistically significant, all SWE treatments, especially water-based SWE treatments, increased glycine betaine concentration in wheat shoots under high salt stress by approximately 25% compared to control plants.

Table 4.21: Effect of SWEs (Control; Water; Alkali; Acid) and salinity stress levels (0 mM NaCl, 60 mM NaCl, 90 mM NaCl) on the shoot GB concentration (mg g⁻¹ DW) of hydroponically grown wheat (*Triticum durum* cv. Sarıçanak-98).

Seaweed Extracts	NaCl (mM)	GB (mg g ⁻¹ DW)			
Control	0	1.34	± 0.18	c	
	60	1.87	± 0.61	a-c	
	90	1.47	± 0.13	bc	
Water	0	1.74	± 0.22	a-d	
	60	1.94	± 0.35	ab	
	90	1.84	± 0.22	a-c	
Alkali	0	2.19	± 0.60	a	
	60	1.61	± 0.44	b-d	
	90	1.79	± 0.29	a-c	
Acid	0	1.76	± 0.37	a-d	
	60	1.54	± 0.20	b-d	
	90	1.82	± 0.52	a-c	

Values are mean and standard deviations of four replicates. Different letters indicate significant differences between means according to Fisher's protected LSD test ($p < 0.05$). Lack of lettering indicates absence of significant effect according to ANOVA.

4.4. Determination of the Effects of *C. barbata* Extracts on Yield and Quality Parameters of Lettuce (*Lactuca sativa* L. cv Lollo Rosso) in the Soilless Culture System

Lettuce plants grown in hydroponic medium treated with SWE had a healthy appearance with increased growth and development compared to the control plants without extract which showed the lowest biomass and less development (**Figure 4.18**, **Figure 4.19**). ANOVA findings indicated that the seaweed applications significantly influenced shoot biomass (**Table 4.23**, **Figure 4.19**). All SWE treatments resulted in a statistically significant increase in fresh biomass, whereas control plants not subjected to any SWE had the least fresh biomass (**Figure 4.19**). Moreover, higher rates of all SWE resulted in an increase in lettuce biomass by approximately 85%, 40%, and 25%, respectively. In general, lettuce plants subjected to SWE exhibited enhanced vibrancy and increased yields attributable to improved growth and development during the vegetative stage.

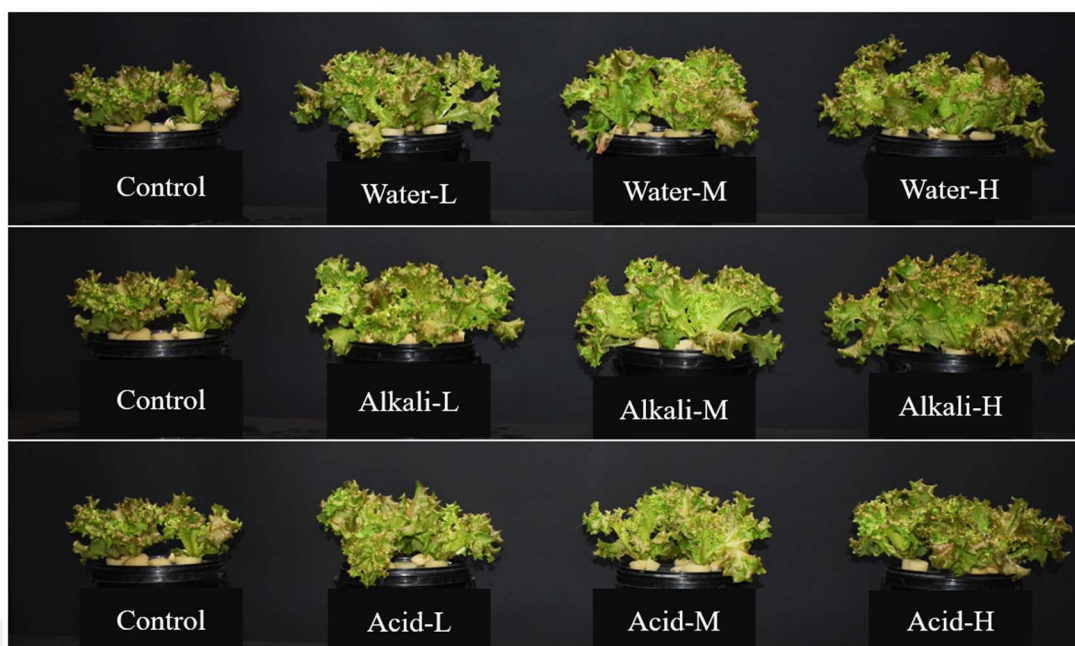


Figure 4.18: Effect of treatments (Control (Water); Water extracts (Low, Medium, High); Alkali extracts (Low, Medium, High); Acid extracts (Low, Medium, High) on hydroponically grown lettuce (*Lactuca sativa* L. cv. Lollo Rosso) plants 36 days after transplantation (DAT).

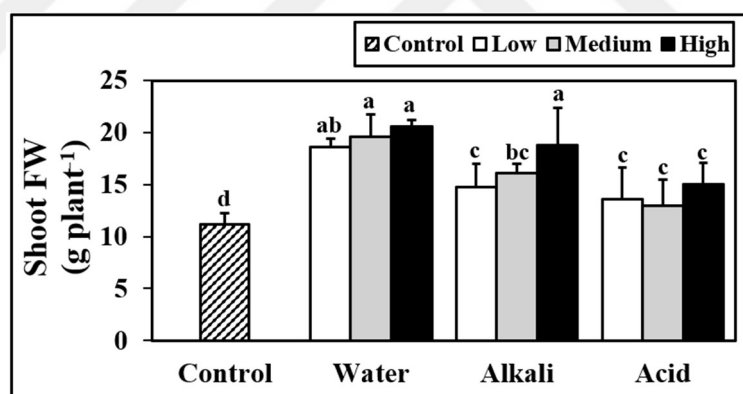


Figure 4.19: Effect of treatments (Control (Water); Water extracts (Low, Medium, High); Alkali extracts (Low, Medium, High); Acid extracts (Low, Medium, High)) on shoot FW of hydroponically grown 36-DAT lettuce (*Lactuca sativa* L. cv. Lollo Rosso).

The SWE treatments had a statistically significant effects on shoot and root DW, root to shoot ratio as well as dry matter ratio parameters (Table 4.22, Table 4.23). The lowest lettuce biomass was observed in control plants without SWE. The SWE extract applications led to a dose-dependent increase in the shoot DW, with the highest enhancement observed in water extract applications, followed by alkali and acid extracts, respectively. Among the treatment groups, lettuce plants subjected to the

highest rate of water extract had the greatest shoot DW, which was approximately two times that of the control group. Lettuce plants treated with the water extract demonstrated the greatest root biomass, similar to the shoot biomass results. Additionally, compared to the control plants, root DW increased by approximately 60% with medium rate of water extract, 40% with high rate of alkali extract, and 50% with high rate of acid extract applications (**Table 4.22**). The application of high concentrations of water and alkali extract and moderate levels of acid extract to nutrient solutions exhibited lower root-to-shoot ratio on hydroponically grown lettuce plants (**Table 4.22**). On the other hand, the application of a medium concentration of acid extract resulted in a statistically significant increase in the dry matter ratio of hydroponically grown lettuce (**Table 4.22**).

Table 4.22: Effect of treatments (Control (Water); Water extracts (Low, Medium, High); Alkali extracts (Low, Medium, High); Acid extracts (Low, Medium, High)) on Shoot DW, Root DW, Root/Shoot (%), Dry Matter (%) of hydroponically grown 36-DAT lettuce (*Lactuca sativa* L. cv. Lollo Rosso).

Seaweed Extracts	Shoot DW (mg plant ⁻¹)		Root DW (mg plant ⁻¹)		Root/Shoot Ratio (%)		Dry Matter Ratio (%)	
Control	589 ± 115	f	49 ± 1	g	8.6 ± 1.9	ab	5.26 ± 0.56	b
Water- L	989 ± 50	a-c	74 ± 15	ab	7.5 ± 1.3	a-d	5.33 ± 0.24	b
Water- M	982 ± 184	a-c	80 ± 7	a	8.4 ± 2.1	a-c	5.00 ± 0.68	b
Water- H	1112 ± 74	a	62 ± 1	c-f	5.6 ± 0.3	e	5.40 ± 0.27	b
Alkali- L	740 ± 116	e	55 ± 12	e-g	7.3 ± 0.5	b-d	5.03 ± 0.51	b
Alkali- M	884 ± 66	b-d	66 ± 10	b-e	7.5 ± 1.1	a-d	5.49 ± 0.11	b
Alkali-H	999 ± 140	ab	68 ± 8	b-d	6.9 ± 1.0	c-e	5.36 ± 0.49	b
Acid - L	825 ± 108	de	60 ± 5	d-g	7.3 ± 0.4	b-e	5.66 ± 0.27	ab
Acid - M	857 ± 46	c-e	51 ± 8	fg	6.2 ± 1.0	de	6.36 ± 1.20	a
Acid - H	824 ± 61	de	74 ± 8	a-c	9.0 ± 1.2	a	5.55 ± 0.68	b

Values are mean and standard deviations of four replicates. Different letters indicate significant differences between means according to Fisher's protected LSD test ($p < 0.05$). Lack of lettering indicates absence of significant effect according to ANOVA.

The effects of extraction type on growth- and root-system-related parameters, nutritional quality measurements, macro and micronutrient concentrations of SWEs in hydroponically grown lettuce plants, as determined by one-way analysis of variance (ANOVA), is shown in **Table 4.23**.

Table 4.23: Significance of the effects of SWEs on measured parameters of hydroponically grown lettuce (*Lactuca sativa* L. cv. Lollo Rosso) plants (36 (DAT)) according to one-way analysis of variance (ANOVA) ($\alpha = 0.05$).

Source of Variation	DF ¹	Shoot FW	Shoot DW	Root DW	Root/Shoot Ratio	Dry Matter Ratio
Seaweed Extracts	9	***	***	***	**	*
Source of Variation	DF	Phenolic	ABTS	DPPH	FRAP	
Seaweed Extracts	9	***	***	***	***	
Source of Variation	DF	Total Vitamin	Reduced Vitamin	Total Carotenoid	GSH	
Seaweed Extracts	9	***	***	*	*	
Source of Variation	DF	Root Length	Root SA	Root Volume	Root Diameter	Ntips
Seaweed Extracts	9	**	*	*	***	***
Source of Variation	DF	Ca	K	Mg	P	S
Seaweed Extracts	9	<i>n.s.</i> ²	*	*	**	<i>n.s.</i>
Source of Variation	DF	B	Cu	Fe	Mn	Zn
Seaweed Extracts	9	**	*	***	***	*

DF¹, degree of freedom; *n.s.*²: not significant; * $0.01 < p < 0.05$; ** $0.001 < p < 0.01$; *** $p < 0.001$

Seaweed treatments considerably influenced overall phenolic content and antioxidant activity (**Table 4.23**, **Figure 4.20**). Generally, a proportional rise in phenolic and antioxidant analyses was observed with escalating doses of SWE applications, with the highest levels providing greater phenolic content and antioxidant activity. The greatest phenolic and antioxidant activities were observed in lettuce plants subjected to high levels of acid among all application groups. Moreover, this application resulted in an increase of approximately 20% in total phenolic content, 124% in DPPH activity, 87% in ABTS activity, and 68% in FRAP activity. In conclusion, the acid extract

treatment had the highest effect on antioxidant activity, followed by water and alkali extracts.

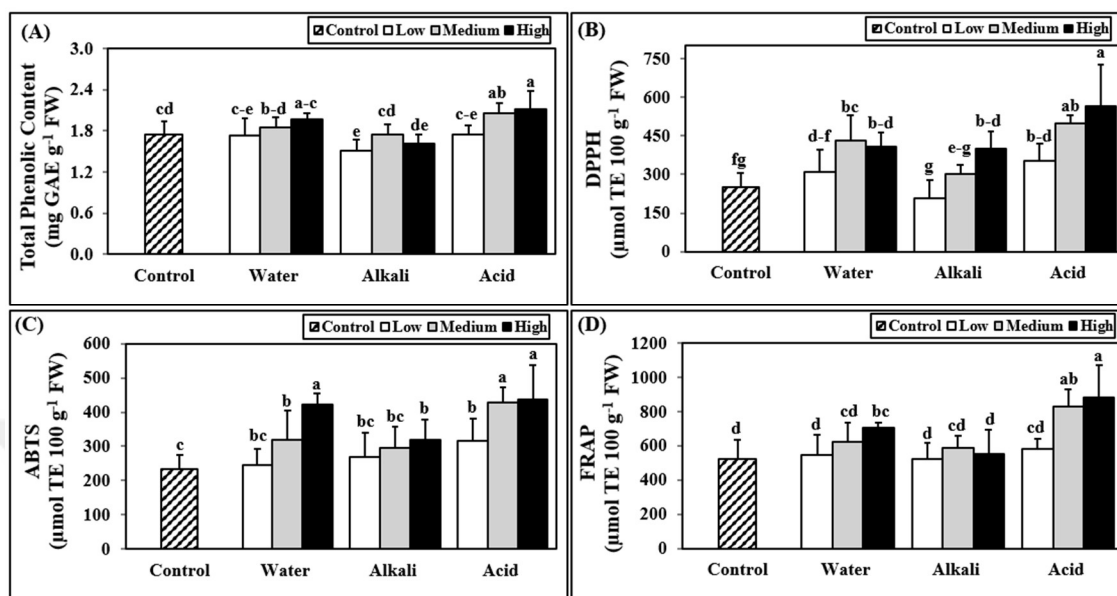


Figure 4.20:Effect of treatments (Control (Water); Water extracts (Low, Medium, High); Alkali extracts (Low, Medium, High); Acid extracts (Low, Medium, High)) on (A) total phenolic content and (B) DPPH, (C) ABTS, (D) FRAP antioxidant activity as Trolox equivalent ($\mu\text{g TE } 100 \text{ g}^{-1} \text{ FW}$) of hydroponically grown lettuce (*Lactuca sativa* L. cv. Lollo Rosso) plants 36 DAT.

According to ANOVA results, SWEs applications added to the hydroponic solution had a statistically significant effect on total and reduced Vitamin C levels in lettuce plants (Table 4.23, Figure 4.21). Water extract at medium rate, and acid extract at low and medium rates caused an increase in total vitamin C of lettuce by approximately 50% (Figure 4.21A). Moreover, there was an approximately 45% increase in the reduced vitamin C of lettuce with the high level of water extracts, compared to control plants (Figure 4.21B).

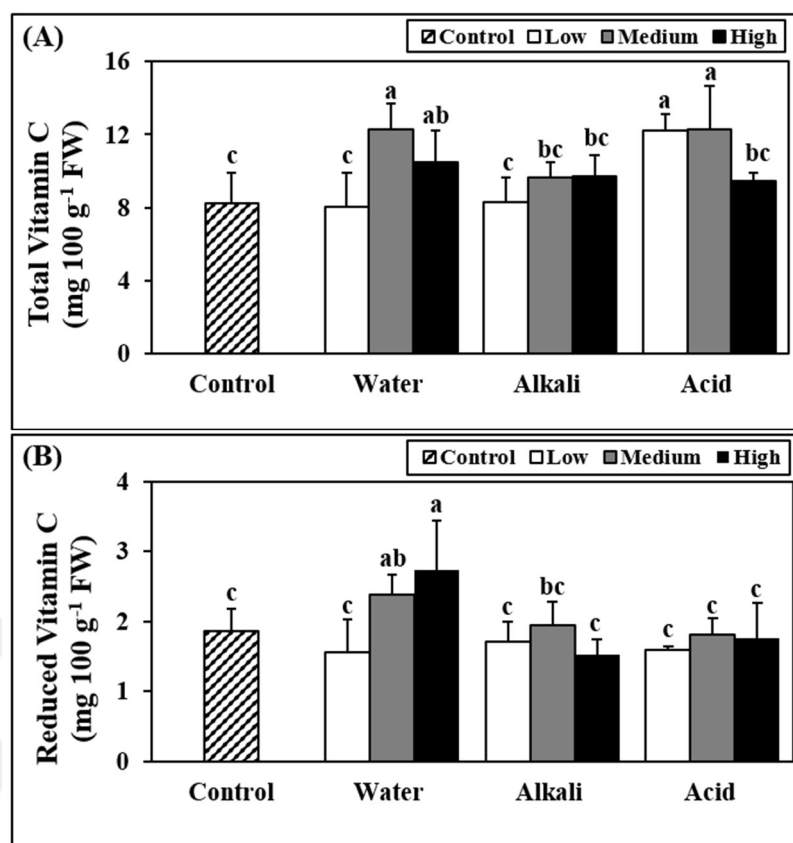


Figure 4.21:Effect of treatments (Control (Water); Water extracts (Low, Medium, High); Alkali extracts (Low, Medium, High); Acid extracts (Low, Medium, High)) on (A) Total Vitamin C and (B) Reduced Vitamin C (mg 100 g⁻¹ FW) of hydroponically grown lettuce (*Lactuca sativa* L. cv. Lollo Rosso) plants 36 DAT.

The biostimulant applications had a statistically significant effect on carotenoid concentration (Table 4.23, Figure 4.22). Compared to the control plants, the application of low- and medium-level alkali SWEs increased carotenoid concentrations by approximately 20%, while the low-level acid extract enhanced carotenoid content by 33% in lettuce plants cultivated in deep water culture systems.

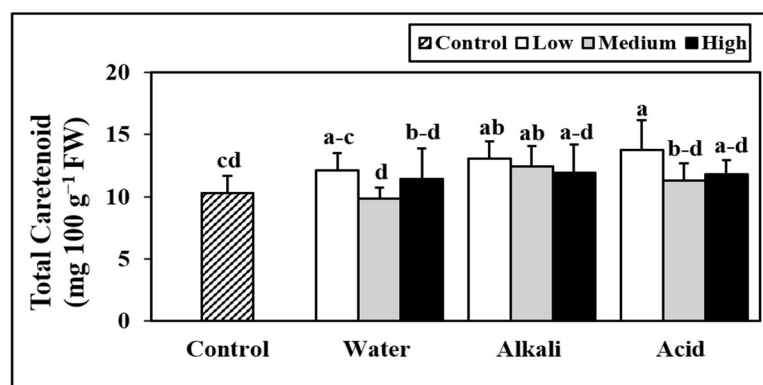


Figure 4.22: Effect of treatments (Control (Water); Water extracts (Low, Medium, High); Alkali extracts (Low, Medium, High); Acid extracts (Low, Medium, High)) on Total Carotenoid (mg 100 g⁻¹ FW) of hydroponically grown lettuce (*Lactuca sativa* L. cv. Lollo Rosso) plants 36 DAT.

The GSH concentration in hydroponically grown lettuce was affected by the tested *C. barbata* extract applications as shown in **Figure 4.23**. Lettuce plants grown hydroponically and treated with high rates of *C. barbata* acid extract had approximately 40% more GSH compared to control plants.

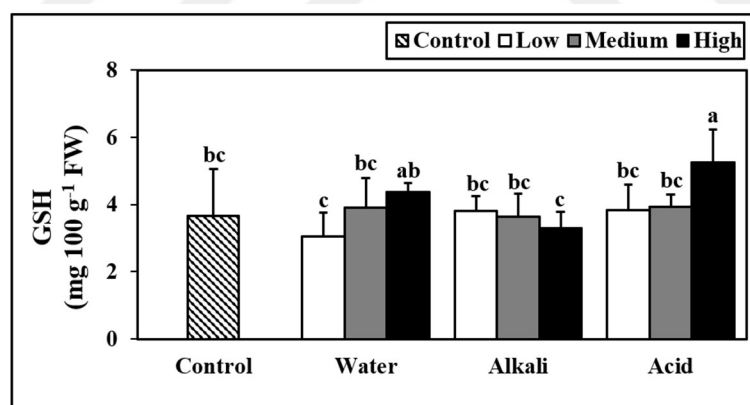


Figure 4.23: Effect of treatments (Control (Water); Water extracts (Low, Medium, High); Alkali extracts (Low, Medium, High); Acid extracts (Low, Medium, High)) on GSH (mg 100 g⁻¹ FW) of hydroponically grown lettuce (*Lactuca sativa* L. cv. Lollo Rosso) plants 36 DAT.

According to the ANOVA results, root morphology parameters of lettuce plants grown in hydroponics were significantly affected by the SWEs treatments applied at different doses (**Table 4.23**, **Table 4.24**). The greatest root length was observed in lettuce plants treated with high levels of alkali and water extracts. Moreover, the high concentration

of water extracts applied to the hydroponic solution gave the best root area, volume, and n tips results. Thanks to this application, root SA increased by about 25%, root volume was enhanced by about 50 %, and n tips were incremented 3 times. The control plant had the lowest average diameter results. Overall, all levels of water extract significantly enhanced root volume and diameter. N-tips were improved with increasing dose levels of water (except low dose) and alkali extracts.

Table 4.24: Effect of treatments (Control (Water); Water extracts (Low, Medium, High); Alkali extracts (Low, Medium, High); Acid extracts (Low, Medium, High)) on root system-related parameters of hydroponically grown lettuce (*Lactuca sativa* L. cv. Lollo Rosso) plants 18 DAT.

Seaweed Extracts	Root Length (cm)	Root SA (cm ²)	Root Volume (cm ³)	Avg. Diameter (mm)	N Tips
Control	779 ± 65 b	69 ± 11 bc	0.47 ± 0.10 de	0.27 ± 0.02 d	708 ± 189 de
Water- L	710 ± 80 b	76 ± 8 a-c	0.65 ± 0.10 ab	0.34 ± 0.02 a	557 ± 67 de
Water- M	807 ± 79 ab	82 ± 8 ab	0.62 ± 0.10 a-c	0.33 ± 0.02 ab	1186 ± 217 c
Water- H	877 ± 52 a	85 ± 7 a	0.69 ± 0.05 a	0.31 ± 0.01 bc	2242 ± 247 a
Alkali- L	796 ± 46 ab	78 ± 6 ab	0.61 ± 0.07 a-c	0.31 ± 0.01 a-c	799 ± 132 de
Alkali- M	706 ± 67 b	71 ± 19 bc	0.52 ± 0.17 b-e	0.29 ± 0.03 cd	842 ± 254 cd
Alkali-H	894 ± 65 a	82 ± 7 ab	0.60 ± 0.07 a-d	0.28 ± 0.03 cd	1643 ± 604 b
Acid - L	756 ± 58 b	63 ± 7 c	0.45 ± 0.06 e	0.28 ± 0.02 cd	489 ± 62 e
Acid - M	731 ± 78 b	71 ± 6 a-c	0.49 ± 0.08 c-e	0.29 ± 0.02 cd	528 ± 79 de
Acid - H	753 ± 43 b	68 ± 3 bc	0.49 ± 0.04 c-e	0.29 ± 0.02 cd	573 ± 95 de

Values are mean and standard deviations of four replicates. Different letters indicate significant differences between means according to Fisher's protected LSD test ($p < 0.05$).

Compared to the control group, a reduction in P and Mg concentration was detected in lettuce plants subjected to the SWEs, regardless of the applied levels (**Table 4.25**). Conversely, K concentrations in leaves from plants subjected to medium or high levels of water and high levels of alkali extracts were enhanced by roughly 17% relative to the control group. Among the measured macronutrients, Ca and S were unaffected by the biostimulant applications (**Table 4.23**).

Table 4.25: Changes in leaves macronutrient (P, K, Ca, Mg, S) (mg g⁻¹) concentrations of lettuce (*Lactuca sativa* L. cv. Lollo Rosso) in response to SWE treatments

Seaweed Extracts	P (mg g ⁻¹)	K (mg g ⁻¹)	Ca (mg g ⁻¹)	Mg (mg g ⁻¹)	S (mg g ⁻¹)
Control	10.0 ± 1.0 a	71 ± 5 cd	13.1 ± 1.6	3.8 ± 0.4 a	3.2 ± 0.3
Water-L	9.1 ± 0.3 bc	78 ± 3 a-d	11.1 ± 0.5	3.4 ± 0.1 bc	3.0 ± 0.1
Water-M	10.1 ± 1.0 a	81 ± 5 ab	12.0 ± 0.9	3.7 ± 0.3 ab	3.2 ± 0.3
Water-H	9.5 ± 0.2 ab	82 ± 3 ab	12.4 ± 0.5	3.6 ± 0.1 a-c	3.1 ± 0.0
Alkali-L	8.9 ± 0.5 bc	76 ± 9 a-d	11.3 ± 0.8	3.4 ± 0.2 bc	3.2 ± 0.2
Alkali-M	8.8 ± 0.2 bc	79 ± 4 a-c	10.9 ± 0.5	3.3 ± 0.2 c	2.9 ± 0.1
Alkali-H	9.0 ± 0.4 bc	83 ± 7 a	11.8 ± 0.6	3.4 ± 0.2 bc	3.0 ± 0.2
Acid-L	8.5 ± 0.5 bc	74 ± 7 b-d	11.4 ± 1.1	3.2 ± 0.2 c	3.0 ± 0.0
Acid-M	8.7 ± 0.8 bc	78 ± 8 a-d	11.6 ± 0.7	3.2 ± 0.1 c	3.0 ± 0.3
Acid-H	8.5 ± 0.2 c	70 ± 4 d	11.7 ± 0.8	3.3 ± 0.1 c	3.0 ± 0.1

Values are mean and standard deviations of four replicates. Different letters indicate significant differences between means according to Fisher's protected LSD test ($p < 0.05$).

Biostimulant applications had significant impacts on the micronutrient concentrations of lettuce leaves as shown in **Table 4.23**. Lettuce plants treated with elevated doses of water extracts exhibited leaves containing 37% more Fe and 30% more Mn compared to control plants (**Table 4.26**). On the other hand, the applications caused a slight decrease in other micronutrients (Zn, Cu and B) regardless of the type of SWEs.

Table 4.26: Changes in leaves micronutrients (Fe, Zn, Mn, Cu, B) (mg kg⁻¹) concentrations of lettuce (*Lactuca sativa* L. cv. Lollo Rosso) in response to SWE.

Seaweed Extracts	Fe (mg kg ⁻¹)	Zn (mg kg ⁻¹)	Mn (mg kg ⁻¹)	Cu (mg kg ⁻¹)	B (mg kg ⁻¹)
Control	257 ± 54 bc	109 ± 14 a	125 ± 21 bc	12.9 ± 2.6 a	27 ± 3 ab
Water-L	263 ± 35 bc	89 ± 5 b	113 ± 9 c-e	10.2 ± 0.5 bc	26 ± 1 bc
Water-M	305 ± 76 ab	96 ± 18 ab	134 ± 28 b	10.4 ± 1.2 bc	29 ± 2 a
Water-H	352 ± 44 a	90 ± 9 b	163 ± 14 a	10.3 ± 0.6 bc	28 ± 1 ab
Alkali-L	245 ± 13 cd	93 ± 8 b	107 ± 4 c-e	10.4 ± 1.0 bc	26 ± 1 bc
Alkali-M	197 ± 8 d	87 ± 2 b	102 ± 4 de	9.6 ± 0.3 bc	25 ± 1 c
Alkali-H	223 ± 39 cd	86 ± 12 b	117 ± 19 b-d	9.1 ± 0.7 c	28 ± 1 ab
Acid-L	192 ± 10 d	85 ± 4 b	93 ± 5 e	10.0 ± 1.2 bc	24 ± 1 c
Acid-M	197 ± 31 d	94 ± 11 b	103 ± 10 de	10.8 ± 2.1 bc	25 ± 1 bc
Acid-H	208 ± 17 cd	111 ± 12 a	104 ± 6 de	11.6 ± 1.7 ab	26 ± 0 bc

Values are mean and standard deviations of four replicates. Different letters indicate significant differences between means according to Fisher's protected LSD test ($p < 0.05$).

4.5. Comparison of Foliar Applications of *C. barbata* Extracts and a Commercial Seaweed-Based Product on Yield and Fruit Quality of Pepper

Seaweed extract treatments were found to significantly enhance both the yield and number of pepper fruits, although they had no significant effect on average fruit weight (**Figure 4.24, Table 4.27**). Commercial SWE did not appear to provide any additional benefits on fruit yield and number. On the other hand, the fruit yield increased by 35% with the water extract, by 27% with the alkali extract, and finally by 30% with the acid extract, compared to the control plant (**Figure 4.24A**). The highest number of fruits and the best fruit yield were observed in the plants treated with water extract. Application of water extract resulted in an approximate 30% increase in the number of fruits, while both alkaline and acid treatments led to a 20% increase (**Figure 4.24B**). The weight of the fruit per plant was almost the same in all the treatment groups (**Figure 4.24C**).

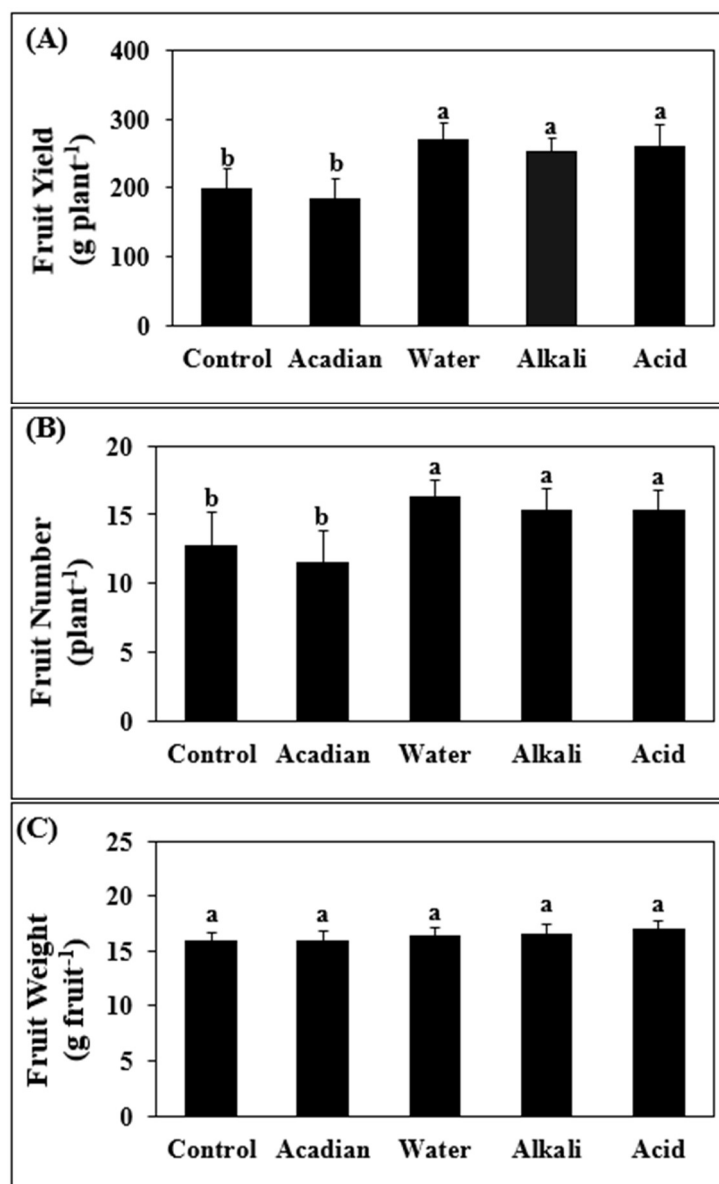


Figure 4.24: Effect of foliar applications with biostimulant (Control (Water); Acadian; Water extracts; Alkali extracts; Acid extracts) on (A) Fruit Yield, (B) Fruit Number, and (C) Fruit Weight of cocopeat grown in pepper (*Capsicum annuum* cv. Fiyaka) plants 80 DAT.

According to one-way ANOVA results, whether foliar applications of SWE to pepper plants grown in substrate culture significantly affected the measured parameters is shown in **Table 4.27**.

Table 4.27: Significance of the effects of SWEs on reported analysis of substrate culture grown pepper (*Capsicum annuum* cv. Fiyaka) plants (80 DAT) according to one-way analysis of variance (ANOVA) ($\alpha = 0.05$).

Source of Variation	DF ¹	Fruit Yield	Fruit Number	Fruit Weight per fruit	Total Phenolic	ABTS
Seaweed Extract	3	***	**	<i>n.s.</i> ²	*	***
Source of Variation	DF	DPPH	FRAP	GSH	Total Vit C	Reduced Vit C
Seaweed Extract	3	*	***	***	<i>n.s.</i>	***
Source of Variation	DF	P	K	Ca	Mg	S
Seaweed Extract	3	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
Source of Variation	DF	Fe	Zn	Mn	Cu	B
Seaweed Extract	3	**	**	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
Source of Variation	DF	Chla	Chlb	Carotenoids	Soluble Carbohydrate	
Seaweed Extract	3	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	*	

DF¹, degree of freedom; *n.s.*²: not significant; * $0.01 < p < 0.05$; ** $0.001 < p < 0.01$; *** $p < 0.001$

The levels of phenolics in the harvested pepper fruit and their antioxidant capacity, assessed by ABTS, DPPH, and FRAP assays, are shown in **Figure 4.25**. According to ANOVA results, these measured parameters were statistically significantly affected by SWEs treatment (**Table 4.27**). Although foliar sprays with water and acid extracts increased phenolic concentration in pepper fruit, this effect was not statistically significant (**Figure 4.25A**). In the ABTS assay, a significant increase in antioxidant activity was observed only in plants treated with the water and acid extract of *C. barbata* compared to the control group (**Figure 4.25B**). Furthermore, foliar applications of seaweed-based biostimulants were found to have a positive effect on the antioxidant activity of pepper fruits in other antioxidant assays as well (**Figure 4.25C-D**). Similar to the trend in phenolic analysis results, these analysis results consistently demonstrated that the application of water and acid-based extracts to the leaves increased the antioxidant activity in pepper fruits (**Figure 4.25**).

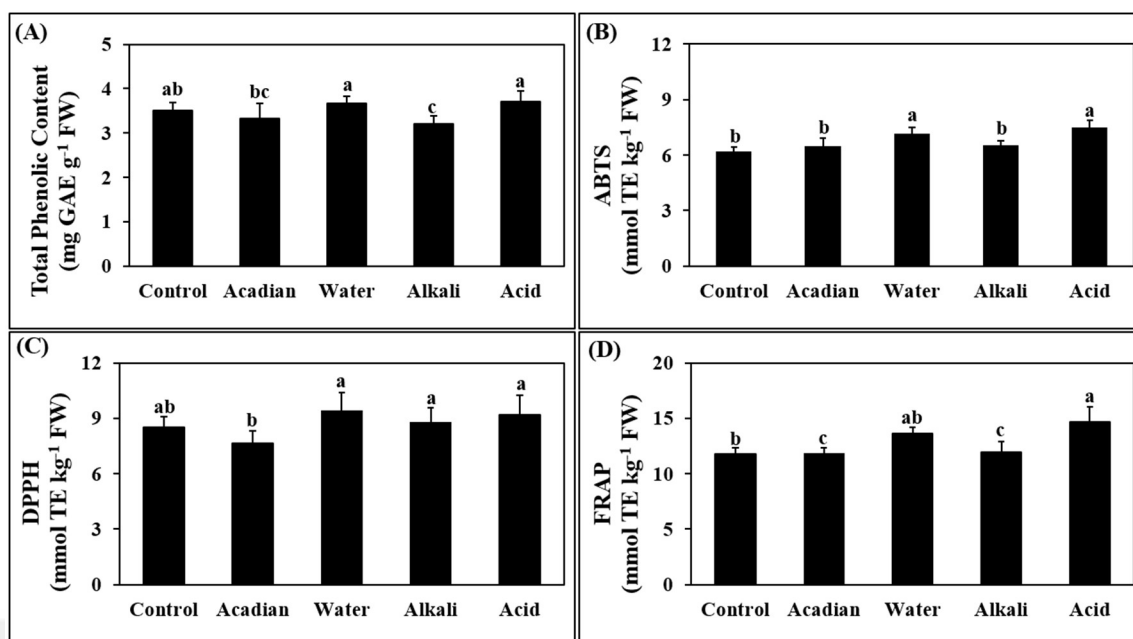


Figure 4.25: Effect of foliar application with biostimulant (Control (Water); Acadian; Water extracts; Alkali extracts; Acid extracts) on (A) Total Phenolic Content, (B) ABTS, (C) DPPH and (D) FRAP of pepper (*Capsicum annuum* cv. Fiyaka) fruit

The GSH levels in pepper fruit were markedly enhanced by foliar applications of all *C. barbata* extracts, whereas applications of the commercial seaweed-based product did not result in a statistically significant increase (Table 4.27, Figure 4.26). Significant increases of up to 45% and 25% in GSH concentration of pepper fruits were observed with water and acid extract applications, respectively. The highest GSH concentration was measured in fruits harvested from alkali extract-applied plants, exhibiting a 65% rise relative to control plants.

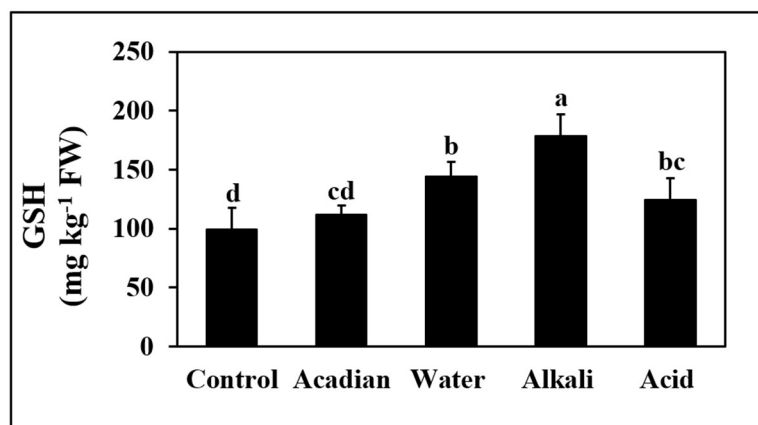


Figure 4.26: Effect of foliar application with biostimulant (Control (Water); Acadian; Water extracts; Alkali extracts; Acid extracts) on GSH of pepper (*Capsicum annuum* cv. Fiyaka) fruit

The impact of the foliar sprays with biostimulant on the activity of the total and reduced forms of vitamin C in pepper fruits is shown in the **Figure 4.27**. ANOVA results showed that total vitamin C in fruit was not significantly affected by SWEs, while reduced vitamin C was affected (**Table 4.27**). Fruits from plants treated with water extract contained the highest levels of both forms of vitamin C (**Figure 4.27**). Application of water extract increased total vitamin C by approximately 26%, although this change was not statistically significant. However, the same treatment significantly boosted the reduced form of vitamin C by around 80% compared to the control fruit.

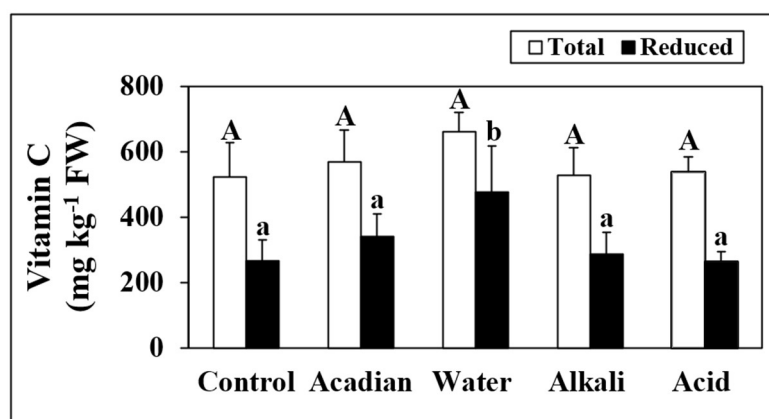


Figure 4.27: Effect of foliar application with biostimulant (Control (Water); Acadian; Water extracts; Alkali extracts; Acid extracts) on Total and Reduced Vitamin C of pepper (*Capsicum annuum* cv. Fiyaka) fruit

Neither commercial SWE nor *C. barbata* extract applications caused significant changes in fruit macronutrient concentrations of pepper plants grown in cocopeat medium (**Table 4.27, Table 4.28**). The fruits of pepper plants obtained from the different application groups had similar macronutrient concentrations (**Table 4.28**).

Table 4.28: Effect of foliar applications with biostimulant (Control (Water); Acadian; Water extracts; Alkali extracts; Acid extracts) on macronutrient concentration (P, K, Ca, Mg and S) of pepper (*Capsicum annuum* cv. Fiyaka) fruit.

Applications	P (mg g ⁻¹)	K (mg g ⁻¹)	Ca (mg g ⁻¹)	Mg (mg g ⁻¹)	S (mg g ⁻¹)
Control	2.4 ± 0.2	27 ± 2	1.4 ± 0.3	1.8 ± 0.1	2.4 ± 0.2
Acadian	2.5 ± 0.1	28 ± 1	1.1 ± 0.1	1.8 ± 0.1	2.4 ± 0.1
Water	2.6 ± 0.2	29 ± 2	1.3 ± 0.3	1.9 ± 0.2	2.5 ± 0.2
Alkali	2.8 ± 0.2	30 ± 2	1.1 ± 0.0	1.9 ± 0.1	2.8 ± 0.3
Acid	2.6 ± 0.1	29 ± 1	1.1 ± 0.1	1.8 ± 0.0	2.4 ± 0.1

Values are mean and standard deviations of four replicates. Different letters indicate significant differences between means according to Fisher's protected LSD test ($p < 0.05$).

With the exception of Fe and Zn, fruit micronutrient concentrations were not significantly affected by biostimulant application, similar to the results obtained for macronutrients (**Table 4.27, Table 4.29**). On the other hand, it was observed that the application of the alkali extract increased both the Fe and Zn concentration by about 20%.

Table 4.29: Effect of foliar application with biostimulant (Control (Water); Acadian; Water extracts; Alkali extracts; Acid extracts) on micronutrient concentration (Fe, Zn, Mn, Cu and B) of pepper (*Capsicum annuum* cv. Fiyaka) fruit

Applications	Fe (mg kg ⁻¹)	Zn (mg kg ⁻¹)	Mn (mg kg ⁻¹)	Cu (mg kg ⁻¹)	B (mg kg ⁻¹)
Control	83 ± 5 b	23 ± 1 b	17 ± 1	4.6 ± 0.5	11.9 ± 1.4
Acadian	79 ± 7 b	23 ± 2 b	17 ± 2	4.6 ± 0.5	11.8 ± 0.7
Water	81 ± 7 b	23 ± 3 b	17 ± 2	5.0 ± 0.5	12.1 ± 1.1
Alkali	100 ± 12 a	28 ± 2 a	17 ± 1	4.9 ± 0.5	13.3 ± 1.2
Acid	80 ± 3 b	22 ± 1 b	16 ± 0	4.6 ± 0.2	11.3 ± 0.2

Values are mean and standard deviations of four replicates. Different letters indicate significant differences between means according to Fisher's protected LSD test ($p < 0.05$).

As a result of foliar application with commercial seaweed or *C. barbata* extracts, no statistically significant difference was observed in the concentration of chlorophyll a, chlorophyll b and carotenoids in pepper fruits (**Table 4.27, Figure 4.28**). When compared with the control pepper fruits, it was seen that these measured values were close to each other and as a result, no positive or negative effect of the applications was determined on these values.

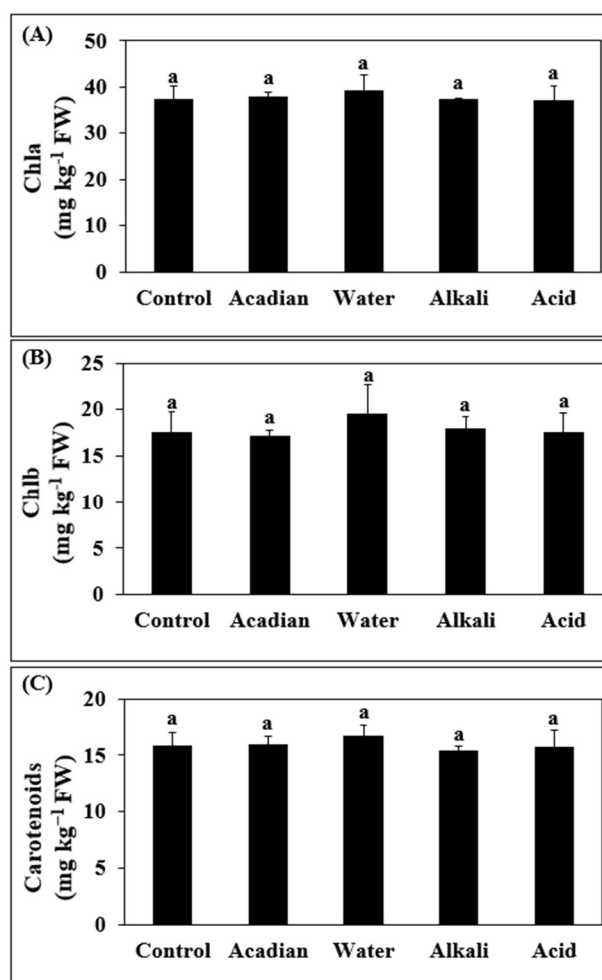


Figure 4.28: Effect of foliar application with biostimulant (Control (Water); Acadian; Water extracts; Alkali extracts; Acid extracts) on (A) Chla, (B) Chlb and (C) Carotenoids of pepper (*Capsicum annuum* cv. Fiyaka) fruit

Figure 4.29 demonstrates the substantial increase in total soluble carbohydrate concentration in pepper fruits from plants treated with Acadian or *C. barbata*-based biostimulants. ANOVA results showed that SWE applications statistically significantly affected the soluble carbohydrate in the pepper fruit (**Table 4.27**). Among the applications, both commercial and alkali biostimulant applications resulted in comparable substantial increases of approximately 20% in soluble carbohydrate content. An elevated improvement in the quantity of soluble matter in pepper fruit was attained with water and acid treatments, resulting in an approximate 30% increase relative to the control group.

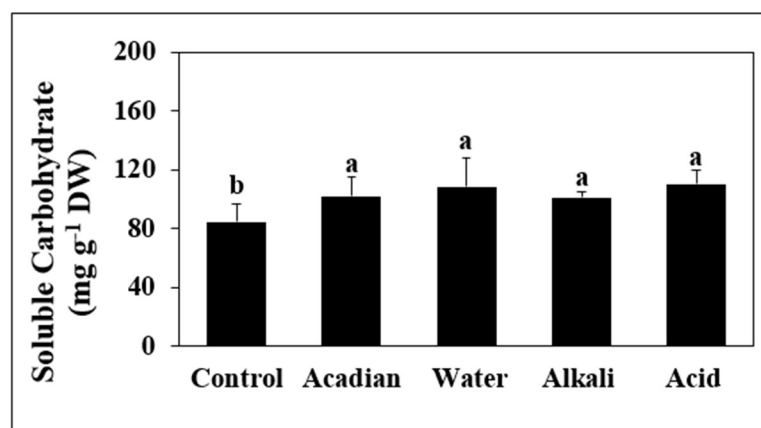


Figure 4.29: Effect of foliar application with biostimulant (Control (Water); Acadian; Water extracts; Alkali extracts; Acid extracts) on Soluble Carbohydrate of pepper (*Capsicum annuum* cv. Fiyaka) fruits

5. DISCUSSION

5.1. Biochemical Characterization of *C. barbata* Extracts

In response to diverse abiotic and biotic challenges, seaweed has developed resilient defense mechanisms and synthesize essential many important bioactive secondary metabolites [Jégou et al., 2021]. Due to their rich content of macroalgae such as hormones, polysaccharides, osmolytes, antioxidants, and mineral elements, macroalgae are increasingly valuable resources in agricultural applications for eliciting various beneficial physiological responses in plants, and they can be readily applied to seeds, growth medium, or leaves [Engel et al., 2006; Sivasankari et al., 2006; Hamed et al., 2018].

The chemical composition and bioactivity of SWEs are strongly influenced by both intrinsic biological factors including species, developmental stage, biomass age, harvest location, depth, and seasonality and extrinsic variables such as extraction and processing conditions [Domínguez, 2013; Singh et al., 2013; Shukla et al., 2019]. Even extracts derived from the same macroalgae species can exhibit substantial chemical and functional variability depending on the extraction techniques applied [Cikos et al., 2018; Wu et al., 2022]. Alkaline and neutral extracts derived from *A. nodosum*, through various methods exhibited distinct biological activities, each activating genes associated with auxin metabolism via different pathway and so this indicates that diverse gene clusters implicated in the biological and molecular mechanisms of the applied plants were significantly modulated [Goñi et al., 2016; Shukla et al., 2025].

Processing parameters critically shape the metabolite profiles of the extracts, ultimately impacting their biostimulant efficacy on target plants [Kim, 2012; Khairy and El-Shafay, 2013; Battacharyya et al., 2015]. The intricate biochemical structure of SWEs renders the comprehension of their mode of action challenging, frequently necessitating a multidisciplinary strategy because of the numerous interactions among a substantial array

of bioactive components inside the extract [El Boukhari et al., 2020]. Minor variations in the level of these compounds within extracts obtained from the identical macroalgae using different extraction techniques can give rise to much larger changes on the molecular or cellular scale, with different effects on the various metabolic pathways activated and on the plant [Goñi et al., 2016].

While the precise mechanisms by which algae-based biostimulants remain incompletely elucidated, it is widely acknowledged that they influence plant physiology, improve nutritional status, and hence foster growth and development, as a consequence of the synergistic effects of their bioactive substances [Schiattone et al., 2017].

In this context, a series of biochemical characterization analyses were conducted to understand the mechanism of action of *C.barbata* on plants. The mannitol and polysaccharide (alginic acid, fucoidan, and laminarin) concentration of the SWE were analyzed by Boğaziçi University (Table 3.1). Among the extracts, the acid extract exhibited the lowest concentrations of alginic acid and fucoidan (1 g l^{-1} and 36 g l^{-1} , respectively), while the water extract presented the highest concentrations (11.6 g l^{-1} and 45.9 g l^{-1} , respectively). Compared to other polysaccharides, fucoidan was consistently found at higher concentrations across all SWE. Laminarin content was highest in the alkali extract and lowest in the water extract. Finally, mannitol, a sugar alcohol, was determined in the SWEs at concentrations ranging between 1 and 3 g l^{-1} .

A study that compared various extraction methods for polysaccharides from the same seaweed species found that polysaccharides extracted using hot water had the highest antioxidant properties [Cheng et al. 2013]. In consistent with this study, sulfated fucans can be readily removed from the cell wall of macroalgae using hot water [Sellimi et al., 2014]. Moreover, in similar studies reported in the literature, water extraction has been found to yield the highest alginate levels among various extraction methods applied to brown seaweeds [Jayasinghe et al., 2016]. Seaweed alginates and oligo-derivatives not only activate multiple defense enzymes to induce plant antioxidant mechanisms but also enhance germination, shoot elongation, plant growth and development in diverse plants by improving N assimilation and basal metabolic processes [Merino et al., 2021; Mamede et al., 2023].

Brown seaweeds are particularly high in polysaccharides, comprising up to 80% DW in certain species; this ratio differs greatly depending on species, location, maturity and seasonal variation [Stiger-Pouvreau et al., 2016; Mohd Fauziee et al., 2021]. Seaweed polysaccharides and their derivative oligosaccharides, recognized as significant contributors to the biostimulant activities of SWEs, are primarily known for their beneficial effects on secondary metabolism and also play important roles in carbon and nitrogen assimilation, seed germination, and nutrient uptake [Holdt and Kraan, 2011; Sharma et al. 2014; Mzibra et al., 2018; Ali et al., 2019]. Moreover, these substances can stimulate signaling pathways that elicit local and systemic defense responses in plants, enhancing the production of protective enzymes that enhance resistance against diverse abiotic and biotic stressors [Klarzynski et al., 2003; Deolu-Ajayi et al., 2022; Mamede et al., 2023]. Because of the existence of phycocolloids, polysaccharides unique to seaweed, including alginate, laminarin, fucoidan, and carrageenan, missing in terrestrial plants, SWEs are believed to have distinct modes of action on plant metabolism [Khan et al., 2009; Deolu-Ajayi et al., 2022].

The concentration of mannitol, a sugar alcohol that serves as a suitable compatible osmolyte and non-enzymatic antioxidant compounds, which aids plants in enduring stress situations by enhancing osmotic adaptation, was elevated in both water and acid extracts [Battacharyya et al., 2015; Lojkova et al., 2020; Patel et al., 2023].

Although the capability of SWEs on growth-promoting effects is primarily linked to the presence of polysaccharides and also their derivatives, some research suggests that this impact could also be attributable to the existence of hormones in the extracts [Mzibra et al., 2018; Zhang and Ervin, 2004; Khan et al., 2009].

The macro- and micronutrient concentrations of three different SWE (water, alkali and acid) obtained from *C. barbata* at varying concentrations are presented in **Table 4.1**. In general, the water extract exhibited higher concentrations of P and Mg, whereas the alkali extract was characterized by elevated levels of K, S, B, Cu, Fe, and Zn. Conversely, the acid extract predominantly contained higher concentrations of Ca and Mn. Among all the elements measured, K and Fe were the most abundant mineral elements across all extracts.

Brown seaweed exhibits significant absorption and build-up capabilities for salts and minerals from their environment, with variations influenced by species, seasonal changes, and the mineral levels of the surrounding seawater [Čmíková et al., 2024]. Typically, their mineral levels approximately range from 10 % to 45 % of the DW of the seaweed [Circuncisão et al., 2018; Kergosien et al., 2023]. The abundant mineral levels render seaweed advantageous for the formulation of functional meals and nutritional supplements [Panayotova and Stancheva, 2013].

A comparable study on brown algae indicated that K had the highest concentration among macronutrients, whereas Fe displayed the largest concentration among micronutrients [Cravo et al., 2004; Derafshi et al., 2024]. The elevated Fe concentrations observed in SWE, relative to other micronutrients, may result from the necessity of Fe for the proper growth of macroalgae and the capacity of many seaweed species to biomagnify Fe from their environment [Aydın-Önen and Öztürk, 2017].

Although fertilizers contain various levels of macro and micronutrients that are naturally present in macroalgae or are generated during processing for purposes such as pH adjustment and are easily absorbed by plants, these extracts cannot replace mineral fertilizers [Hong et al., 2007; Michalak et al., 2015]. Rather, their main benefits stem from their capacity to stimulate a large number of physiological processes within the plant, leading to improved growth and development; their mechanism of action is therefore attributed not only to their mineral content but also to their biostimulant properties that fundamentally distinguish them from traditional fertilizers [Khan et al., 2009; Ali et al., 2019]. Moreover, since SWEs are typically applied at very low concentrations in agricultural practices, it is unlikely that they can supply all the mineral nutrients required by plants [Schmidt et al., 2003]. Nevertheless, seaweed-based biostimulants have been shown to enhance nutrient use efficiency in plant growth medium and can further improve plant nutritional status by stimulating the uptake of mineral nutrients [Vernieri et al., 2005; Mancuso et al., 2006]. Additionally, the use of KOH and H₂SO₄ during the preparation of the alkali extract from *C. barbata* is thought to have contributed to its higher K and S concentration compared to the other extracts.

The total phenolic concentration measured in the SWEs also exhibited considerable variation (Table 4.3). Phenolic substances levels were found to be richest in the alkali, water, and acid extracts, respectively. The use of strong bases in alkaline extraction

methods is recognized as an effective approach for extracting phenolic compounds from algae, which are known to be rich in these compounds, and the results confirm that the highest phenolic content among the extracts was observed in the alkaline extracts [Craigie, 2011; Sharma et al., 2014]. Research on the characterization analysis of *C. barbata* has revealed its abundant phenolic components, including vanillic acid, benzoic acid, salicylic acid, and ferulic acid, as well as several flavonoid compounds such as phlorotannins and quercetin, along with its derivatives [Cadard et al., 2019; Abdel-Raouf et al., 2017; Sellimi et al., 2017; Castillo et al., 2023].

In contrast to terrestrial plants, phenolics derived from seaweed exhibit greater variability in their characteristics, encompassing both simple molecules and extremely intricate, specialized compounds [Huang et al., 2019]. Polyphenolic substances exhibit potent antioxidant bioactivity and serve as beneficial secondary metabolites in seaweeds, thereby safeguarding plants from oxidative stress by mitigating oxidative processes [Freile-Pelegrín and Robledo, 2013; Audibert et al., 2010]. These substances, which typically accumulate during stress, are essential for plant growth and development since they chelate metal ions, stabilize membranes and proteins, and scavenge ROS [Kulbat, 2016; Huang et al., 2019].

Phlorotannins (eckol and dieckol), prominent polyphenolic compounds derived from brown seaweed, exhibit enhanced antioxidant bioactivity attributed to their distinct structural characteristics, including a greater quantity of phenolic rings [Venkatesana et al., 2020], and have been documented to demonstrate antioxidant activity up to tenfold greater than that of ascorbic acid and α -tocopherol [Gupta and Abu-Ghannam, 2011]. Moreover, a mixture of phenolic compounds has been documented to exhibit antioxidant properties in wheat when given externally to the leaves [Zhao and Zou, 2002].

According to the results of the total free amino acid content analysis of SWE shown in Table 4.3, the total free amino acid concentrations in both water and acid extracts were in similar ranges, while the lowest concentration was detected in the alkali extract. The varying levels of amino acids obtained from SWE using different extraction methods can be attributed to the presence of distinct solvents employed in the extraction processes, as well as the pH adjustments made, which influence amino acid solubility. Free amino acids in SWEs primarily function to shield against

oxidative stress; also, they defend cells from dehydration, salinity, and cold stress by serving as osmolytes [Conde et al., 2013].

Seaweed is particularly abundant in amino acids, notably aspartic and glutamic acids which comprise a significant percentage of the total amino acids present in various seaweed [Thiviya et al., 2022]. They contribute to the formation of nitrogenous molecules like proline, enhance nitrogen absorption, positively influence photosynthesis, and mitigate physiological damage from oxidative stress [Colla et al., 2015; Pangestuti and Kim, 2015; Rizwan et al., 2017; El-Metwally et al., 2022]. The amino acid profile of seaweed is influenced by many variables, including species, storage conditions, extraction processes, seasonal change, and the growing environment [Thiviya et al., 2022].

Reports indicate that biostimulants comprising amino acids enhance crop yields [Kocira, 2019; Hassan et al., 2020] by promoting root development through the regulation of enzymes associated with nitrogen reduction and absorption, as well as phytohormone biosynthesis, thereby augmenting the intake, transport, and accumulation of mineral microelements [du Jardin, 2015].

In studies reported in the literature, the antioxidant activities of various SWEs have been analyzed using methods including ABTS, FRAP, and DPPH [Agregán et al., 2017]. The results obtained from the three different antioxidant assays (ABTS, DPPH and FRAP) were consistent with one another (Table 4.4). According to all analyses, the antioxidant activities of the water and acid extracts were comparable, whereas the alkali extract exhibited higher antioxidant activity. When compared to other extracts, it has been found that the alkali extract with the highest phenolic concentration exhibits the most antioxidant activity. These findings demonstrate a correlation between radical scavenging activity and phenolic levels [Meenakshi et al., 2009]. This parallelism implies that the extracts' antioxidant capabilities might be improved by the existence of phenolic substances.

The superior antioxidant activity of brown algae extract among algal species is likely attributable to the synergistic combination of polyphenols and alkaloids [Kosanić et al., 2015; Hamdy et al., 2020], with red and green algae extracts following in efficacy [Harb et al., 2021; Begum et al., 2021]. Seaweed-based biostimulants can enhance plant resilience against damage caused by oxidation, either directly via their

antioxidant properties or indirectly by activating the plants' defense systems [Paradikovic et al., 2019]. The antioxidant activity of seaweed is directly associated with the presence of polysaccharides, polyphenols, carotenoids, vitamins and other bioactive substances that scavenge ROS and protect against oxidative stress [Ali et al., 2021; El-Rafie et al., 2024]. Moreover, research indicates that brown seaweeds have superior antioxidant activity compared to terrestrial plants [Chan et al., 2017; Chew et al., 2008; O'Sullivan et al., 2011].

According to the results of the maize coleoptile elongation assay, conducted using indole-3-acetic acid (IAA) as a reference to assess the hormone-like activity of the SWEs (water, alkali and acid) obtained from *C. barbata*, all extracts exhibited comparable auxin-like activity across the tested concentrations (Figure 4.1). The greater stimulation of coleoptile elongation by the alkali extract compared to the IAA positive control aligns with previous findings reporting the presence of auxin-like compounds in alkali hydrolysates of macroalgae [Buggeln and Craigie, 1971]. Additionally, it has been demonstrated that plants treated with SWEs may accumulate higher levels of auxin and auxin-like compounds [Wally et al., 2013].

Beyond auxin and auxin-like compounds, a range of phytohormones including abscisic acid, cytokinins, ethylene, and gibberellins have been detected in SWEs, with their profiles influenced by the algal source, extraction technique, and purification conditions [Stirk and Van Staden, 2014; Battacharyya et al., 2015; Lu and Xu, 2015]. Numerous studies have confirmed that auxins and other auxin-like compounds present in SWEs enhance root system morphology by stimulating lateral root and root hair development, thereby improving nutrient uptake efficiency and ultimately promoting overall plant growth and yield [Mugnai et al., 2008; Rayorath et al., 2008; Kurepin et al., 2011; Zluhan-Martínez et al., 2021].

In the present study, the increases in root growth parameters observed in early-stage wheat plants in the substrate experiment and in lettuce plants grown hydroponically following SWEs application can be attributed to the presence of hormone- and hormone-like compounds in these extracts and also their effects on the plant.

When given at prescribed quantities [do Rosário Rosa et al. 2021], the growth hormones included in the SWE may be inadequate to induce physiological changes in

plants [Górka and Wieczorek 2017]; nonetheless, the constituents of the SWE may activate inherent pathways for the production of growth hormones in plants.

5.2. Determination of the Effects of *C. barbata* Extracts on Germination and Early Growth Periods in Wheat

Pre-sowing treatments, which directly influence plant physiology in multiple ways, represent an important strategy within sustainable agricultural practices by promoting improved development and yield [Lanka et al., 2017]. Seed priming or soaking, a hydration method that stimulates certain pre-germination processes before radical emergence, can enhance seed quality and mitigate yield loss by many strategies, including osmo, biological, hydro, hormonal, and chemical priming [Salehzadeh et al., 2009; Stanley-Raja et al., 2021]. Priming has demonstrated beneficial effects on germination in various crops, including wheat, resulting in earlier emergence, enhanced seed viability index, and improved biomass and yield as well as stress tolerance [Ibrahim, 2016; Chanthini et al., 2019]. Research indicates that a portion of the biostimulant function of SWE is attributable to a priming impact that enhances plants' resilience to stress [El Boukhari et al., 2020; Fleming et al., 2019]. The application of mild stress with SWEs during the early growth stage may serve as an effective technique to stimulate defense metabolism in plants, aiding their resilience against unfavorable abiotic stress factors [Kawatra et al., 2019; Bouriou et al., 2020; Hakeem et al., 2017; Lemmens et al., 2019]. Osmo-priming with SWEs enhances the plant's ability to withstand subsequent stress by activating various stress tolerance mechanisms, including increased antioxidant activity, stimulation of signaling compounds, and synthesis of abscisic acid, thereby boosting plant performance, yield and chances of survival [Kerchev et al., 2020].

No statistically significant positive or negative effects of SWEs on the germination rate of wheat seeds were observed in either of the perlite-based experiments (**Figure 4.2, Figure 4.6**). The absence of adverse effects indicates that applied concentrations were not phytotoxic. Conversely, the lack of a stimulatory response is not unexpected, as the high-quality wheat seeds used in the study already exhibited a germination rate exceeding 90% under control conditions. This result is consistent with the results of a similar study, in which seed treatments with extracts from brown algae showed no significant effect on seed germination [Esserti et al., 2018].

The direct application of *C. barbata* extracts to the perlite medium led to an increase in biomass of early wheat seedlings (**Figure 4.3, Figure 4.4**), likely due to the existence of growth-promoting compounds such as polysaccharides, free amino acids, hormone-like compounds as well as essential mineral elements contained within the extracts.

Previous study in the literature have also demonstrated that treatments with SWEs can function as biofertilizers, thereby enhancing early wheat growth and development [**Hamouda et al., 2022**]. Moreover, numerous studies have demonstrated beneficial effects of SWEs on early growth and seedling vigor of various plants other than wheat, including sunflower [**Cabral and Castilho, 2016; Santos and Castilho, 2018**] and soybean [**Gehling et al., 2017**], cowpea [**Sivasankari et al., 2006**]. Although the mechanisms of action of SWEs are not fully understood, these positive effects can be attributed to the existence of growth-promoting compounds including macro- and micronutrients, amino acids, vitamins, hormones (auxin, gibberellin, cytokinin) and hormone-like compounds, betaines and phenolic compounds in SWEs [**Khan et al., 2009; Wally et al., 2013**].

Based on both visual observations and quantitative data obtained from the two perlite experiments investigating early wheat growth, direct application of SWEs to the growing medium was more effective in promoting biomass accumulation compared to seed treatments (**Figure 4.4, Figure 4.8**). This finding confirms that the efficacy of SWEs on plant growth can vary significantly depending on the method of application. Supporting this observation, a previous study similarly reported that foliar application of SWEs had no noticeable effect, whereas their direct incorporation into the growing medium successfully stimulated plant growth [**Crouch and Van Staden, 1993**].

The efficacy of the *C. barbata* extract may have varied greatly depending on where and for how long the plant was exposed to the bioactive compounds in SWEs. In the substrate experiment, the bioactive molecules in SWEs may have been continuously present around the roots during germination and seedling development, allowing for continuous and effective exposure throughout root and shoot development, providing stronger and longer physiological stimuli. On the other hand, a single exposure to bioactive compounds at the seed level and limited uptake prior to radicle emergence may not have been sufficient to trigger sustained physiological responses, which may have limited benefit. Furthermore, when SWEs were applied to seeds, the bioactive

compounds may have been degraded by enzymes in the seed coat, remained on the seed surface without being absorbed and were ineffective, or the seeds may have protective barriers that prevent the uptake of larger molecules such as polysaccharides or phenolics.

In the substrate experiment where *C. barbata* extracts were applied directly to the perlite medium, shoot related growth parameters increased in a dose-dependent manner, whereas improvements in root development appeared to be independent of the applied dose (**Figure 4.4, Table 4.8**). It is known that SWEs can increase the growth and development of plant roots, especially when applied in the early developmental stages [Samuels et al., 2022]. Enhancements in the root system through SWEs have been linked to the activation of genes governing nutrient absorption by roots and the modulation of endogenous plant hormone activity, facilitating improved nutrient and water uptake [Kergosien et al., 2023]. The beneficial impacts of SWEs on rooting and root development are mostly due to the presence of auxins; also, amino acids found in SWEs have demonstrated an enhancement in rooting efficacy in plants [Gomes et al., 2018]. Moreover, alginate and also their derivatives like oligosaccharides present in SWEs are recognized for their ability to promote root growth in plants [Abd Tahar et al., 2022].

In contrast, seed treatments with SWEs did not produce a pronounced effect on root system, and their impact on shoot growth was less significant compared to the substrate experiment (**Figure 4.8, Table 4.9**).

Esterase enzyme activity, one of the key enzymes involved in metabolic processes such as germination and ripening through hydrolysis of ester bonds [Subramani et al., 2011; Ertani et al., 2018], did not show a consistent response in shoots and roots through substrate and seed applications of SWE (**Figure 4.5, Figure 4.9**). Similarly, Hameed et al., [2013] reported that biostimulant applications did not consistently modulate esterase enzyme activity in wheat. Moreover, the high esterase activity observed in control plants, in the absence of stress factors, may have masked any potential positive effects of SWE applications [Lamhamdi et al., 2011; Hu et al., 2015].

Consistent with findings in literature, an increase in K and S macronutrient concentrations was observed in wheat seedlings grown in growth medium treated with

C. barbata extracts; however, no additional positive effects were detected in micronutrient concentrations (**Tablo 4.6, Tablo 4.7**). Seaweed extracts are known to enhance the accumulation of macro- and micronutrients in plants, with a particularly pronounced effect on K levels [**Rouphael et al., 2018b**]. While the water, alkali, and acid extracts contained appreciable levels of K, which may have partially contributed to the observed plant responses, the distinct variations in their biochemical and mineral ingredients combined with the lack of a direct relationship between shoot K levels and the magnitude of the effects indicate that these extracts act not merely as nutrient sources, but also as biostimulants [**Mutlu-Durak et al., 2024**].

The water extract is abundant in potassium and sulfur owing to the substantial presence of soluble substances like fucoidans in *C. barbata*. However, the elevated concentrations of potassium and sulfur in the alkali and acid extracts may result from the chemicals employed in the extraction process, specifically KOH and H₂SO₄. Besides the direct sulfur fertilizer role of the evaluated SWEs, bioactive substances, especially sulfur-containing carbohydrates like fucoidans, may improve nitrogen and sulfur assimilation via metabolic alterations in plants [**Bouissil et al., 2020; Nivetha et al., 2024; Shukla et al., 2016**].

5.3. Investigation of the Effects of *C. barbata* Extract Applications on Salinity Stress Resistance and Related Physiological Parameters in Wheat

5.3.1. Determination of the Effective *C. barbata* Extracts Application Levels on Plant Growth in a Hydroponic Environment

Seaweed-based biostimulants are known to have substantial potential to promote growth parameters, including increasing plant height, shoot and root biomass [**Craigie, 2011**]. As a cost-effective and easy-to-use practice to enhance crop production and decrease the need for chemical inputs, the addition of biostimulants from SWE as growth-promoting products through nutrient solutions in soilless growing systems can be an important approach for sustainable agricultural production [**Miceli et al., 2021**]. In contrast, studies on the direct addition of biostimulants to hydroponic systems and their mode of action are still limited in the literature [**Wang et al., 2022**]. In a study conducted on lettuce it was reported that the commercial extract of *E. maxima* applied through nutrient solutions improved numerous morphological and physiological

characteristics of the lettuce as well as plant growth and yield [Miceli et al., 2021]. Another study showed that when sprayed with seaweed liquid extracts or mixed into the nutrient solution in the medium, vegetative growth and biochemical characteristics, including protein content and chlorophyll pigment, were improved in comparison to untreated hydroponically grown bean plants [Latique et al., 2013].

In this preliminary hydroponic experiment with wheat, the direct addition of water, alkali, and acid extracts derived from *C. barbata* to the nutrient solution at three distinct concentrations resulted in a significant enhancement of biomass in both the aerial parts and the root system (**Figure 4.10, Figure 4.11**). Growth-promoting effects on shoot development were most pronounced following treatment with medium concentrations of the water extract and low concentrations of the alkali and acid extracts (**Figure 4.11A**). Similarly, enhancements in root biomass were observed with medium and high concentrations of the water and alkali extracts, as well as low and high concentrations of the acid extract (**Figure 4.11B**). Wheat plants approximately one month old exhibited a healthier, more vigorous phenotype compared to control plants that did not receive any SWEs. Overall, the greatest enhancement in both shoot and root biomass were recorded in plants treated with the medium concentration of the water extract. In contrast, wheat plants cultivated in a growth medium lacking the SWEs demonstrated weaker development, characterized by less robust shoots and root systems.

Based on these findings, for the third and main hydroponic trial, designed to evaluate the performance of SWEs under salinity stress conditions, the promising level of each extract type, which was the medium concentration of the water extract and low concentrations of the alkali and acid extracts, was selected for further investigation.

No significant changes were observed in the macroelement concentrations of shoot tissues in hydroponically grown wheat following SWEs applications (**Table 4.10A**). An analysis of nitrogen metabolism in hydroponically grown wheat indicated that direct addition of SWEs to the nutrient solution caused minor fluctuations but no substantial changes in shoot N concentrations (**Table 4.11**). Across all treatment groups, irrespective of extract type or application level, shoot nitrogen content remained comparable to that of the controls. Generally, decreases in shoot Ca and Mg concentrations were identified, but no visual symptoms of Ca or Mg deficiency were observed. Furthermore, the reduced concentrations of Ca and Mg in the wheat shoots

of SWE-treated plants might result from an antagonistic relationship among K, Ca, and Mg ions during absorption and transport [Xie et al., 2021; Hernández-Pérez et al., 2020]. On the other hand, it was observed that high-level water extract application increased the K concentration of wheat plants grown in hydroponics compared to the control group. Research indicates that the application of water-based SWE derived from *C. barbata* enhances potassium concentration in wheat plants [Mutlu-Durak et al., 2024] and tomato fruits [Arikan-Algul et al., 2025].

Similarly, SWEs treatments induced minor fluctuations in shoot microelement concentrations (Table 4.12). While Cu and Mo levels remained stable across all treatments, Zn and Mn concentrations decreased. No micronutrient deficiency symptoms were detected in any treatment group.

5.3.2. Determination of Effective Salt Stress Application Levels on Plant Growth in a Hydroponic Environment

Over 6% of the global land is impacted by soil salinization, leading to a 20%–30% reduction in agricultural productivity across all cultivated and irrigated areas [Khan et al., 2019]. Moreover, it is projected that by 2050, soil salinity would threaten nearly half of all arable land [Sahile et al., 2021]. Climate change adversely impacts water availability, quality, and distribution through modified precipitation trends, elevated evaporation and salinity, and severe weather conditions, restricting access to and usage of high-quality water in agricultural practices [İkiz et al., 2024]. Salinity, whether in water or soil, is one of the main factors that has a devastating impact on wheat production worldwide by inhibiting plant growth and development and thus reducing yields [Qadir et al., 2014].

Wheat (*Triticum durum*) is known to have moderate salt tolerance among cereal crops, with greater tolerance than rice but lower tolerance compared to barley [Munns and Tester, 2008]. While salt stress tolerance differs among species, Sarıçanak exhibits relatively greater sensitivity compared to other wheat cultivars. When the EC value reaches threshold levels of 6 dS m⁻¹, wheat yield reduces by approximately 7.1% for each unit increase in EC [Chinnusamy et al., 2005; Hasanuzzaman et al., 2017]. The negative impacts of salinity stress on wheat development have been extensively shown in various research [El Sabagh et al., 2021; Saddiq et al., 2021; Seleiman et al., 2022]. Salt stress has resulted in considerable declines in various wheat yield

metrics, including spikelet count, seeds per spike, productive tillers, thousand-grain weight, and biomass output over the long run, in both sensitive and tolerant cultivars [Afzal et al., 2008]. The detrimental impacts of salt stress by altering plant metabolic processes on wheat plants generally occur in the form of induced osmotic stress, poor germination, suppressed photosynthetic activity, inhibited plant growth, changes in enzymatic activities, overproduction of ROS, and nutritional and hormonal imbalances, which ultimately result in serious yield losses [Mittler et al., 2004; Kaveh et al., 2011; Miransari and Smith, 2019; Seleiman et al., 2022].

In this study, the effects of salt stress on hydroponically grown wheat were evaluated by supplementing the nutrient solution with five NaCl concentrations (0, 30, 90, 120, and 150 mM) (Figure 4.12). The growth-suppressing effects of salinity stress observed phenotypically in hydroponically grown wheat were corroborated by shoot and root DW measurements (Figure 4.13). In this preliminary second experiment, increasing salinity levels impaired wheat growth and development. Moreover, salinity stress progressively reduced shoot biomass by 10%, 20%, 30%, 35%, and 40% with increasing NaCl concentrations, respectively (Figure 4.13A). The observed decrease in shoot dry weight may be attributed to the direct inhibitory effects of salinity on photosynthesis [Parida et al., 2005]. As expected, control plants, grown without salt stress, exhibited superior vegetative growth and development, whereas plants subjected to higher NaCl concentrations displayed significant reductions in shoot length and biomass, along with compromised root morphology. Plants exposed to severe salinity exhibited weaker shoot and root systems compared to the controls. This experiment showed that salinity also caused a growth-reducing effect on root biomass with increasing salinity levels (Figure 4.13B). Consistent with these results, salt stress in wheat adversely affects various aspects of root system development, including the quantity, length, diameter, area and volume of root, while also diminishing both shoot and root biomass [Saboora et al., 2006; Shafi et al., 2010].

Based on these findings, 60 and 90 mM NaCl was selected for the third hydroponic experiment, as this concentration induced a 20–30% reduction in shoot and root biomass, corresponding to a moderate level of stress in hydroponically grown wheat.

Given the significance of wheat in human nutrition, it is crucial to devise sustainable strategies that enhance wheat growth and adaptability to saline conditions to boost production, particularly in salt-affected regions [El Sabagh et al., 2021]. In this

context, choosing appropriate cultivars and developing salt-tolerant wheat varieties with conventional breeding, molecular approaches is a method that mitigates the impacts of salt stress; nevertheless, this endeavor is labor-intensive, costly and requires many years [Seleiman et al., 2022]. On the other hand, plant biostimulants can be used as part of sustainable agriculture to alleviate stress conditions.

5.3.3. Effects of Selected *C. barbata* Extract Applications on Salinity Stress Resistance and Physiological Parameters

It is known that different *Cystoseira* species exhibit biostimulant properties under salinity stress conditions, reducing the harmful effects of stress on different plant species including barley [Bensidhoum and Nabti, 2021], tomato [Krid et al., 2024] and wheat [Salih and Abdulrazziq, 2024]. Moreover, in a study examining the effects of applying *Cystoseira tamariscifolia* extract at different doses (2%, 5% and 10%) on stress tolerance in tomato seedlings grown under 50 mM NaCl salt stress, the growth-promoting effects of low-dose SWE on tomato plants against salt stress were determined [Krid et al., 2024]. In contrast, the potential effects of *C. barbata* under salinity stress have not been investigated before.

In this third hydroponic wheat experiment, the effects of the most promising doses of water (medium), alkali (low), and acid (low) extracts, identified in the first hydroponic experiment, were evaluated in a deep-water culture system under both control (0 mM NaCl) and saline (60 mM NaCl and 90 mM NaCl) conditions selected from the second hydroponic experiment (Figure 4.14). Visually, it was observed that high salinity in the nutrient solution slowed down the growth of wheat shoots, but adding *C. barbata*-based extracts to the solution helped the plants grow better than those that didn't receive the extracts, both when stressed and not stressed, suggesting that these extracts might help plants handle salinity stress better (Figure 4.14). The growth-reducing effects of salinity stress were further reflected in biomass measurements, ranging from a 20-25% decrease in shoot FW observed in hydroponically grown wheat (Figure 4.15A). Numerous studies in the literature demonstrate the growth-inhibitory effects of salt stress on wheat plants [Guo et al., 2015]. This decrease may be ascribed to a direct suppression of cellular proliferation and expansion because of the osmotic stress, which restricts growth [Munns, 2002]. Notable reductions in both fresh and dry weights of wheat plants in response to salt stress demonstrate the growth-inhibitory

impact of such stress factor [Annunziata et al., 2017]. In alignment with our findings, Zou et al., (2016) reported that salinity stress (100 mM NaCl) induced by resulted in a reduction of shoot and root lengths, as well as their DW in wheat plants.

Under non-stress conditions, applying water and alkali extracts significantly increased shoot dry weight (Figure 4.15A). In contrast, under stress conditions, the water extract enhanced not only shoot biomass but also root biomass when exposed to 60 mM salt stress, while the acid extract showed an increase in shoot and root biomass at higher salt concentrations (Figure 4.15). Furthermore, under normal conditions the effects of water and alkali extracts were equally effective; however, under high salt stress conditions acid extracts were more effective than other extracts.

The application of seaweed-based biostimulants has risen in agricultural practices recently owing to their many advantageous impacts on crop production under both optimal and unfavorable situations, including salinity stress [Rouphael et al., 2022]. Seaweed extracts can have varying impacts on plant development and stress tolerance, contingent upon the extraction technology employed and the amount and accessibility of bioactive metabolites present [Goni et al., 2016]. Furthermore, the developmental and priming effects of SWEs on plant stress responses are due to the rich biochemical components of the extracts, many of which are also found in *C. barbata* extracts, and their synergistic activity as well as stimulatory abilities. They are growth-promoting hormones, polysaccharides, essential elements, vitamins, amino acids, non-enzymatic antioxidants (polyphenols) and compatible osmolytes (betaine, proline); all of which enhance plant metabolism and performance, promote growth, and increase crop production while improving stress tolerance [Yakhin et al., 2017; Khan et al., 2022]. On the other hand, some molecules in SWEs may function as signaling agents that regulate critical pathways at the transcriptional and/or post-translational levels, resulting in differential expression of crucial genes in plants that enhance plant development and resilience to stress [Deolu-Ajayi et al., 2022]. The biochemical composition of SWEs indicated a high concentration of antioxidant molecules, which are crucial for mitigating abiotic stress, inhibiting the formation of ROS, enhancing plant growth and yield by making nutrients more available and easier to uptake; and make plants more resistant to salt stress [Roy and Chowdhury, 2020]. Research indicates that polysaccharides, which are also found in *C. barbata* extracts, markedly enhanced the shoot and root height, fresh and dry biomass, and chlorophyll

concentration of salt-stressed wheat, hence augmenting the salt tolerance of wheat seedlings [Zou et al., 2018; Zou et al., 2019]. Moreover, these polysaccharides can preserve the osmotic equilibrium of salt-stressed wheat by augmenting sugar and proline levels while modulating the Na^+/K^+ ratio, and additionally, they can mitigate oxidative stress caused by salinity by reducing the levels of REL and MDA and enhancing the antioxidative enzyme activity, which scavenges ROS [Zou et al., 2019]. The many studies have shown that phytohormones and their derivatives, also present in *C. barbata* extracts, can be induced more in plants exposed to SWE under salinity stress and increase endogenous hormone levels, which can affect growth and development by regulating physiological and molecular processes related to nutrient assimilation and stress tolerance by modulating hormonal pathways [Ryu and Cho, 2015; Hashem et al., 2019]. Although biostimulants cannot provide all the essential mineral elements in the amount that the plant needs due to their nature [Schmidt et al., 2003], many macro and microelements, including those found in *C. barbata* extracts, can increase nutrient utilization efficiency by stimulating many physiological processes in the plant, and can support growth and development under stress [Mancuso et al., 2006; Latique et al., 2017].

In this study, regardless of salinity stress, the most beneficial effects on the vegetative growth of hydroponically grown wheat plants were observed in the applications of water, acid and alkali extracts, in that order. Overall, the water and acid SWE had a growth-enhancing impact on hydroponically grown wheat plants subjected to salinity stress and significantly alleviated the growth suppression caused by salt stress. These findings were consistent with previous reports demonstrating that SWE treatments enhance plant growth and development under salinity stress conditions relative to untreated controls [Hernández-Herrera et al., 2022; Rouphael et al., 2022]. In general, as in this study, water extract with the same SWEs obtained from *C. barbata* in early plant growth showed the highest development in wheat seedlings [Mutlu-Durak et al., 2024]. This positive effect may be due to the presence of higher levels polysaccharides including alginic acid, fucoidan and other compounds like mannitol in water extracts [Mutlu-Durak et al., 2024]. Moreover, positive effects of acid extract under high salt levels may be attributed to osmolyte contents, which are present in extracts [Mutlu-Durak et al., 2024], including mannitol, probably proline and GB

functions as a non-enzymatic antioxidant, mitigate stress-induced growth inhibition [Lojkova et al., 2020; Patel et al., 2023].

Plant roots are the initial organs to experience salt stress, and root development is especially susceptible to salinity in the root zone [Khan, 2003]. Prior to inducing ion toxicity, elevated salt concentrations in the rooting medium restrict water accessibility, induce physiological drought, and diminish tissue water content [Azeem et al., 2023]. In this experiment, the growth-reducing effects of salinity stress were further reflected in biomass measurements, approximately 15% decrease in root DW observed in hydroponically grown wheat (**Figure 4.15B**). Root development is essential for sustaining osmotic balance as well as ion homeostasis under salinity stress, and the enhancement of root biomass through SWEs treatments may serve as an adaptive strategy to salinity stress in wheat. Seaweed extracts are known to positively influence root system architecture, improving root size, length, biomass, and vitality [Khan et al., 2009]. Similar to shoot biomass results, wheat plants that were treated with water and acid extracts mitigated the root system inhibiting effects induced by 60 mM NaCl and 90 mM NaCl stress levels, respectively (**Figure 4.15B**). Auxin-like activity found in *C. barbata*-based water and acid extracts suggests a way to improve root development [Mutlu-Durak et al., 2024], thereby augmenting the plant's nutrient absorption efficiency and boosting its overall physiological functions under stress conditions [Ertani et al., 2018]. Moreover, the beneficial impacts of SWEs on the root system can be ascribed to elevated auxin levels induced by alginate oligosaccharides and the activation of auxin-associated genes, together with the direct or indirect influences of other chemicals present in *C. barbata* extracts on root development [Zhang et al., 2014].

A notable rise in REL was observed in hydroponically grown wheat plants subjected to salt stress in the absence of SWE treatment (**Figure 4.16**). This finding parallels previous research across other plant species, where cell membrane damage and enhanced electrolyte leakage and lipid peroxidation are associated with salt stress conditions [Ahmad et al., 2019; Cheng et al., 2020; Ahanger et al., 2020; Abdelaal et al., 2020]. On the other hand, the *C. barbata* extracts led to a reduction in REL values in wheat when exposed to 60 mM NaCl levels. Moreover, the treatment with water extracts reduced electrolyte leakage caused by stress in the wheat plants at both stress levels. This investigation demonstrated that SWE positively influences

membrane stability and can preserve membrane from the negative effects of salt stress, as SWE-applied plants exhibited decreased electrolyte leakage relative to the control.

A recent study has indicated that the extract of *Cystoseira mediterranea* has the potential to preserve membrane integrity in barley seedlings by reducing electrolyte leakage, thereby mitigating the negative effects of salinity stress (350 mM NaCl) on these seedlings [Bensidhoum and Nabti, 2021]. Another study evaluated the efficacy of SWE in mitigating salt and drought stress in wheat, revealing that the extracts enhanced growth parameters, pigments and tissue water content and reduced the REL value [Patel et al., 2018]. Moreover, Pienaar et al., (2025) have shown that the application of SWE mitigates the negative effects of salinity-induced membrane damage in salt-stressed maize plants, enhancing membrane permeability as well as stability while minimizing electrolyte leakage. The use of SWE on plants subjected to salinity stress enhanced growth metrics while reducing electrolyte leakage and so, the plants were able to tolerate the stress [Bahmani Jafarlou et al., 2023].

The enhancement in membrane integrity is due to the capacity of SWE to regulate electrolyte leakage, suggesting that SWE maintain cell membrane stability by diminishing electrolyte leakage from the cytoplasm [Zou et al., 2019; Cabo et al., 2019]. Numerous components and processes, including compounds present in SWEs that contribute to the preservation of cell membrane integrity including Ca^{2+} , Mg^{2+} , alginate, and fucoidan [Pal et al., 2024]; alongside secondary metabolites and enzymatic antioxidants that enhance antioxidant defense [Gürsoy, 2024]; accumulation of compatible osmolytes (proline, betaine, and mannitol) [Mughunth et al., 2024]; increased K^+/Na^+ balance, and hormonal modulation, are linked to the capacity of SWE to diminish electrolyte leakage in plants subjected to salinity stress.

In this respect, this positive effect, which is seen at the highest level with water extract, may be due to the content of membrane-protective compounds such as fucoidan, alginic acid, and mannitol, which are known to be found more in water extracts than in other extracts [Mutlu-Durak et al., 2024]. Moreover, the rich bioactive component content of *C. barbata* extracts, especially polysaccharide and mineral content, enhance in proline and glycine betaine accumulation in the plant, increase in K level, induction of plant defense mechanism may have contributed to the reduction of electrolyte leakage salinity-induced stress by water and acid extracts and thus the positive result on membrane stability.

The effects of SWE treatments on the concentrations of macro- and micronutrients in hydroponically grown wheat plants are summarized in **Table 4.14** and **Table 4.15**. Among the macronutrients, P concentration remained unaffected by both salinity stress and biostimulant treatments. Salinity stress, however, resulted in a reduction of Ca and Mg concentrations in the shoots of wheat plants. Because of the antagonistic impact that salinity has on the uptake and transport of a variety of macro- and micronutrients inside the plant, it has the potential to completely disrupt the nutritional equilibrium of plants [Parihar et al., 2015; Hasanuzzaman et al., 2021]. It disrupts ion homeostasis by modifying ion equilibrium, characterized by an elevation in Na^+ levels alongside a concurrent reduction in K^+ , Ca^{2+} and Mg^{2+} concentrations [Rahman et al., 2016; Parvin et al., 2019; Balasubramaniam et al., 2023]. This excessive sodium influx induces ion channel dysfunction, nutrient displacement, and membrane depolarization, resulting in irregularities in nutrient absorption and assimilation [Gaikwad et al., 2022]. Moreover, salinity can impede the absorption of crucial nutrients like nitrogen due to interactions between sodium and ammonium or chloride and nitrate, hence disrupting plant functions [Parihar et al., 2015].

Overall, control plants exhibited the lowest concentrations of Mg and S, while wheat plants treated with acid extract exhibited higher concentrations of these elements compared to other treatments, irrespective of stress. Under stress conditions, S concentrations in alkali and acid treated wheat plants were higher than those in control plants. The rise in S concentration observed in this study was also observed in wheat [Mutlu-Durak et al., 2024] and tomato [Arıkan-Algöl et al., 2025] plants in response to *C. barbata* extracts. This situation may be due to the S macroelement found in the extracts caused by the chemicals applied during the extraction method, as well as the sulfur compounds it contains, and other metabolic changes caused by them.

Salinity stress led to an increase in B, Cu, Mo, and Fe concentrations in hydroponically grown wheat plants, but had no significant effect on Mn and Zn concentrations. Under high salt levels, plants treated with alkali extract exhibited the highest Fe concentrations compared to other treatments. In general, plants treated with acid extract showed higher concentrations of Mo than those treated with other extracts. While stress raised the Cu concentration in hydroponically grown wheat plants, SWE treatment had the opposite effect. On the other hand, Mn concentrations remained similar across all treatment groups, while the concentration of Zn in wheat shoots

generally diminished with the application of SWE. While B concentration increased significantly only with salinity stress, no significant increase or decrease was achieved with biostimulant applications.

One of the major detrimental effects of salinity on wheat growth is its interference with the uptake and assimilation of essential mineral nutrients [Hu and Schmidhalter, 2005]. Wheat plants subjected to salt stress exhibited an inhibition in the absorption of Ca^{2+} , K^{+} as well as Zn^{2+} , alongside an enhance in the uptake of Na^{+} and Cl^{-} , resulting in nutritional homeostasis imbalance that adversely affected the growth and development of the wheat plant [Guo et al., 2015]. Elevated concentrations of Na^{+} and chloride Cl^{-} ions in salt-stressed wheat cultivars have been shown to inhibit the absorption of key nutrients such as K^{+} , Ca^{2+} , Zn^{2+} , and Mg^{2+} , primarily competition due to chemical similarity, potentially cause nutrient deficiencies [Munns and Tester, 2008; Guo et al., 2015]. Moreover, salinity-induced stress substantially decreases root area by limiting root hair length and density, which are critical determinants of nutrient acquisition [Arif et al., 2019]. This morphological reduction can negatively affect the uptake of nutrients such as Ca^{2+} , Mg^{2+} , Fe^{2+} , and Zn^{2+} [Robin et al., 2016; Nahar et al., 2016; El Sabagh et al., 2021].

The Na, K and Na/K ratio values presented in Table 4.16 were not significantly affected by the interaction between biostimulant and salt stress. As expected, increasing stress levels caused an increase in Na concentration and a decrease in K concentration in wheat plants (Table 4.16). On the other hand, less Na accumulation was observed in wheat subjected to water extract under low stress circumstances, whereas elevated Na levels were noted in wheat plants treated with all *C.barbata* extract under high stress conditions, but this effect was not statistically significant.

Potassium is crucial for a wide variety of essential processes which include maintaining membrane potential and turgor pressure, stimulating enzyme activity, and modulating osmotic pressure, as well as supporting stomatal movement [Laurie et al., 2002; Mekawy et al., 2015]. The lowest K levels under both stress and non-stress conditions were recorded in control wheat plants not receiving SWE, whereas SWE applications had a positive effect on K accumulation under in both stress conditions. Among the other elements, K was the predominant mineral in *C.barbata* extract [Mutlu-Durak et al., 2024]. Moreover, previous studies with *C. barbata* extracts have shown that SWE applications increased K levels in wheat [Mutlu-Durak et al., 2024]

and tomato fruit [Arıkan-Algül et al., 2025]. A study indicated that potassium levels increased in proline-applied plants [Shahbaz et al., 2013]. In this study, the increased proline level with SWEs may have contributed to the increase in K levels (**Figure 4.17, Table 4.16**). In addition to the proline and K content of the *C.barbata* extract, it can also be explained by a better nutrient uptake with the developed root system.

While the Na/K ratio was statistically influenced solely by salinity stress, the lowest Na/K ratio was recorded in wheat plants added with water-based SWE in the nutrient solution under stress conditions at both levels; this beneficial effect was subsequently observed in plants treated with acid extract, but this effect was not statistically significant. In conditions of salinity, the plant absorbs a greater quantity of Na^+ compared to K^+ when the concentration of Na^+ in the growth medium rises, resulting in enhanced K^+ outflow from the cell and an elevated Na/K ratio [Cuin et al., 2008; Rahman et al., 2016]. Keeping a minimal cytosolic Na/K ratio is essential for a plant's ability to tolerate salt and facilitates survival in saline environments [Wakeel et al., 2011; Isayenkov and Maathuis, 2019]. The brown SWEs improved the growth of plants under salt stress by sustaining a higher K^+/Na^+ ratio [Elansary et al., 2017; Di Stasio et al. 2020].

Protein concentrations of hydroponically grown wheat plants were not statistically affected by the applications of biostimulants and salinity, nor by the interaction of biostimulants and salinity (**Table 4.13, Table 4.17**). The researchers demonstrated that under moderate salinity conditions, the protein content of three distinct rice cultivars remained largely stable, and a significant increase in protein levels was observed only under severe salt stress, suggesting that moderate salinity stress does not always result in significant protein concentration changes [Yao et al., 2022]. Stress does not universally lead to pronounced changes in protein content in plants; rather, the extent of the response can vary depending on the plant species and the severity of the stress condition.

Elevated levels of ROS resulting from oxidative stress induced by salinity can cause significant cellular damage, including oxidation of carbohydrates, DNA fragmentation, peroxidation of membrane lipids, protein breakdown, inhibition of enzymatic activity, reduction in photosynthetic pigment levels, disruption of membrane integrity, and ultimately cell death [Mittler et al., 2004]. Numerous studies have demonstrated that wheat plants respond to salt-induced oxidative stress by

modulating the activities of key antioxidant enzymes, including SOD, GR, APX and CAT, as a critical component of their defense mechanism against oxidative damage [Sairam et al., 2005; Caverzan et al., 2016]. Hydrogen peroxide can be eliminated via the AsA–GSH cycle, which serves as a vital antioxidant defense mechanism for H₂O₂ detoxification [Pasternak et al., 2005; Pandey et al., 2015]. The enzymes APX and GR are essential components that significantly contribute to the operation of this cycle [Fotopoulos et al., 2010; Hasanuzzaman et al., 2019a]. SOD initially protects cells from the detrimental effects of ROS by turning O₂^{•−} into H₂O₂. The H₂O₂ generated is subsequently neutralized and convert to H₂O by CAT, and APX [Foyer and Noctor, 2011; Hasanuzzaman et al., 2020]. GR facilitates the preservation of the GSH pool by converting GSSG back to GSH and regulating the redox equilibrium in plants through the maintenance of a steady GSH/GSSG ratio [Gill et al., 2013].

In this study, the changes in the enzymatic activities of total and specific APX, CAT, GR, and SOD in the shoot of hydroponically grown wheat plants under salt stress, following SWE applications, are presented in **Table 4.18** and **Table 4.19**. Salinity stress led to an increase in total GR and SOD activities, while it resulted in a decrease in CAT and APX activities (**Table 4.18**). In addition to the total antioxidant enzyme activities, the specific activities of enzymatic antioxidants except for APX, SOD were not significantly affected by the interaction between salinity stress and biostimulant treatments (**Table 4.13**, **Table 4.18**). Except for specific CAT activity, salinity stress statistically influenced the specific activities of the other enzymes (**Table 4.13**). Total APX activity was decreased by salinity stress, whereas the highest total APX activity under high salt levels was found in plants treated with acid extract. As in this study, numerous studies have indicated that salt stress may lead to a reduction in APX enzyme activity [Tepe and Aydemir, 2015; Fardus et al., 2021]. All wheat plants had similar total CAT enzyme activity (**Table 4.18**). These results suggest that wheat plants do not experience enough stress under these conditions to induce CAT activity. Similar findings have been reported in previous studies, where salinity treatment did not cause significant changes in CAT activity in lentil [Bandeoglu et al., 2004], rice [Lin and Kao, 2000], and potato [Benavides et al., 2000].

Research indicates that diminished antioxidant enzymatic activities may result from a decrease in ROS production [Mahawar et al., 2024]. Both total and specific GR enzyme activities did not change significantly with biostimulant applications but

increased with salinity stress (**Table 4.13**). In consistent with this results, research indicates that GR enzyme activity in wheat elevates during salinity stress [**Sairam et al., 2005; Talaat and Todorova, 2022**]. Total SOD enzyme activity, which increased under salinity stress but tended to decrease with SWEs applications, was generally highest in SWEs-untreated wheat plants. Research indicates that salinity stress elevates SOD enzyme activity, while the use of biostimulants diminishes this activity [**Zuzunaga-Rosas et al., 2023**].

Salinity stress has been shown to elicit diverse responses in enzyme activity, which may increase markedly, remain relatively unchanged, or exhibit substantial inhibition [**Shalata et al., 2001; Hasanuzzaman et al., 2020**]. This emphasizes the complicated nature of plant responses to salt stress. Extensive evidence in the literature indicates that variations in antioxidant enzyme activity under salinity stress are influenced by several factors, including the salinity threshold, stress type, intensity, duration of exposure, and the species and developmental stage of the plant as well as other experimental factors [**Abogadallah, 2010; Hasanuzzaman et al., 2021**]. It is worth noting that the responses of antioxidant enzymes to salt stress are highly variable, as reported in the literature [**Jakovljević et al., 2017**].

Salinity-induced high ROS production elevates the final outcome of MDA and REL, resulting in cell membrane impairment [**Miransari and Smith, 2019; Alché, 2019; Guo et al., 2019**]. While the detrimental consequences of elevated levels of ROS are recognized [**Kerchev and Van Breusegem 2022**], it is also known that moderate levels of ROS are critical as a signaling molecule in plant priming as well as regulating and triggering both enzymatic and non-enzymatic antioxidant metabolic processes [**Hu et al., 2012**].

Biostimulant treatments and salinity stress as well as their interaction significantly influenced MDA concentrations (**Table 4.13, Table 4.20**). A rise in MDA levels was observed at both levels of salinity stress (**Table 4.20**). Among the biostimulant treatments, water extract application specifically induced a further increase in MDA concentration, suggesting a potential stimulation of oxidative signaling pathways. Interestingly, the rise in MDA levels following water extract application may not exclusively reflect oxidative injury but could also indicate an adaptive physiological response. Recent studies have proposed that MDA can stimulate regulatory genes linked to defense and growth under oxidative stress, thus functioning as a protective

or signaling molecule rather than solely as a biomarker of damage [Morales and Munné-Bosch, 2019]. These findings highlight the complex role of MDA in stress adaptation and suggest that specific biostimulant treatments may prime plants for improved stress resilience through controlled oxidative signaling.

MDA formation was temporarily elevated in plants subjected to *C. barbata* extract at 90 mM NaCl; no significant rise in MDA accumulation was noted at reduced NaCl concentrations (Table 4.20). The findings indicate that wheat, which exhibits moderate tolerance to salt stress, displayed no signs of oxidative stress under 60 mM NaCl conditions with SWE applications. Furthermore, the elevated accumulation observed with water extract at higher salinity levels, along with the resultant increase in lipid peroxidation, did not signify oxidative damage in the plant, nor did it disrupt plant growth (Figure 4.14, Figure 4.15), suggesting that the wheat plant can endure this salt stress. This non-destructive boost in oxidative stress may serve as a protective strategy for plants against moderate salt stress rather than causing damage.

The amount of MDA enhanced with salinity stress was further increased by the application of Moringa leaf extract as a plant-based biostimulant [Akhter et al., 2023]. Another study also showed that rosemary plants show an increase in MDA accumulation with salt stress (150 mM NaCl), indicating that this is part of a protective mechanism that induces tolerance rather than causing oxidative damage [Tounekti et al., 2011].

Seaweed extracts can pre-activate defense mechanisms in plants by inducing a "priming" effect, characterized by a transient and regulated oxidative stress response, marked by elevated ROS generation and a transitory rise in MDA levels. This transient stress fortifies the plant's stress tolerance mechanism and enhances its long-term resilience to environmental challenges [Islam et al., 2021]. The augmenting and priming impacts of SWEs on the primary defense against stress factors can be attributed to the collectively contributing composition of the SWE and their elicitation-inducing characteristics [Yakhin et al., 2017]. Plant priming and responses triggered by SWE share numerous common characteristics: their mechanism of action is systemic throughout plants, they enhance tolerance to various stress factors, they are not unique to certain plant species, and they lead to increased plant development [Battacharyya et al., 2015; Martinez-Medina et al., 2016; Rasul et al., 2021]. It has been reported that laminarins in SWEs can function as stimulants to trigger defense

mechanisms in a number of plant species [Shukla et al., 2021]. In addition to laminarin, the synergistic effects of mannitol, polyphenols, oligosaccharides, polysaccharides and many other bioactive metabolites can act as priming agents at the molecular level in plants, rapidly and strongly increasing stress tolerance and promoting growth [Shukla et al., 2019; Kerchev et al., 2020; Rasul et al., 2021; Sujeeth et al., 2022]. As a result, it is thought that *C. barbata* extracts may have activated a priming pathway in the formation of plant responses through osmolytes including proline, polysaccharides such as alginate, fucoidan, laminarin and other compounds, or by stimulating existing pathways in the plant.

Proline is an amino acid present in plants and plays a critical role in osmotic adjustment and antioxidant activities in response to different stress conditions [Shakeri et al., 2019]. To cope with salinity-induced osmotic stress, plants can accumulate compatible osmolytes like proline, which function as osmoprotectants, lowering water potential and so enhancing uptake [Szabados and Savouré, 2010; Hnilickova et al., 2021]. It also acts as a molecular chaperone, stabilizing protein structure and membrane stability, strengthening photosynthetic apparatus, as well as increasing various enzyme activities, consequently affecting adaptive responses [Hayat et al., 2012; Borsai et al., 2020; Mehta and Vyas, 2023]. It is also involved in the plant defense mechanism including stress adaptation, recovery and signaling pathway as a non-enzymatic antioxidant by scavenging free radicals [Jogaiah et al., 2013]. Besides controlling responses to stressors, it functions as a metabolic signaling compound that modulates redox equilibrium, sustains cellular homeostasis, trigger the expression of specific genes, and affects cell proliferation or death [Matysik et al., 2002; Kishor et al., 2005; Szabados and Savouré, 2010]. It is believed that proline's function as a ROS scavenger is more significant than its function as a basic osmolyte in stress reduction [Hong et al., 2000]. Since proline concentrations increase dramatically in plants subjected to salt stress, it has been suggested that this amino acid could serve as a marker molecule for the response to stress factor [Szabados and Savouré, 2010].

In this experiment, significant changes in proline concentration were observed in hydroponically grown wheat plants when *C. barbata* extracts were added to the nutrient solution under salinity stress conditions (**Figure 4.17**). The concentration of proline was significantly influenced by the application of both biostimulants and stress, as well as their combination (**Table 4.13**). With the increase in salinity stress, proline

concentrations in hydroponically cultivated wheat plants also rose. The increase in proline levels observed at low salinity became more pronounced at higher salinity levels. The proline concentration in wheat plants subjected to acid extract application was elevated at all levels under both control and stress conditions. In addition to acid application, significant increases in proline levels were observed with water and alkali extracts, especially under high salinity stress. These findings demonstrated that salinity stress resulted in an enhancement in proline concentration in wheat plants grown in hydroponic system compared to the control, and that proline levels are further boosted by SWE treatment in salinity stress.

When wheat plants were subjected to salt stress, proline concentrations in wheat tissues were observed to increase to protect the plant against saline damage [Ahmad et al., 2010; Al-Whaibi et al. 2012; Talaat and Shawky, 2013; Abedini, 2016]. Numerous studies have demonstrated that proline plays a crucial role in mitigating osmotic stress by compensating for the osmotic pressure of Na⁺ cations [Acosta-Motos et al., 2017; Yakhin et al., 2017]. The high level of proline in the cell wall mitigated the adverse effects of stress and enhanced tolerance in soybean plants [Phang et al., 2008]. Under situations of salt stress, there was an elevation in maize proline content and growth related parameters [Molazem et al., 2015]. Furthermore, it was noted that salt-tolerant bean varieties had elevated proline levels in comparison to salt-sensitive types [El Sayed et al., 2011]. As a result, proline accumulation is an important survival strategy for plants under salinity stress [Siddique et al., 2018]. Numerous studies in literature on various plants exposed to salinity stress show that proline not only enhances germination and root development, but also increases biomass by improving leaf water potential, chlorophyll concentration and photosynthetic activity [Ehsanpour and Fatahian, 2003; Khedr et al., 2003; Roy et al., 2016]. Salinity stress induces excessive proline production in plants, which enhances stress tolerance by preserving cell turgor and osmotic balance; mitigates electrolyte leakage through membrane stabilization; decreases lipid peroxidation, and normalizes ROS concentrations, thereby averting oxidative burst in plants [Hayat et al., 2012; Hashem et al., 2017]. Moreover, proline promotes the expression of salt stress-related genes, and activates antioxidant enzymes, all of which facilitate plant response to salt stress by augmenting antioxidant capabilities [Hasanuzzaman et al., 2014].

The observed increase in proline concentration in hydroponically cultivated wheat under salinity stress is seen as a defensive strategy utilized by these plants to alleviate osmotic and oxidative damage caused by salinity. Wheat plants treated with SWE showed an increase in proline concentration, which was thought to be associated with improved stress tolerance. Algae extracts possess elevated levels of osmolytes, including proline, which, upon application to plants, can be partially uptake and/or trigger moderate, temporary osmotic stress [Di Stasio et al., 2018], resulting in morphological and physiological adaptations that improve the plant's stress response [Fleming et al., 2019]. Consequently, SWE may stimulate the endogenous accumulation of proline, potentially acting synergistically with the proline contained in SWE, thereby enhancing plants' tolerance to osmotic stress. In line with our findings, several studies have reported the stimulatory effect of SWE on proline metabolism in plants subjected to salinity stress [Elansary et al., 2017; Jithesh et al., 2019; Patel et al., 2018]. The application of fucoidan, derived from brown algae, elevated proline levels in wheat seedlings subjected to salt stress at 120 mM NaCl relative to the control group [Zou et al., 2021].

Brown SWEs can modulate proline levels by upregulating the gene encoding for proline biosynthesis, Δ^1 -pyrroline-5-carboxylate synthetase (P5CS), and/or downregulating the gene responsible for proline catabolism, proline dehydrogenase (ProDH) [Carvalho et al., 2018]. Moreover, lipophilic constituents (sugars, polyols, terpenes) of *A. nodosum* extracts have demonstrated the ability to elicit a fast metabolic reaction, resulting in elevated proline accumulation by modulating synthesis and degradation related genes of proline [Nair et al., 2012]. Additionally, many plant hormones have important roles in regulating osmolyte accumulation during abiotic stress [Sharma et al., 2019]. Auxin- and cytokinin-induced enhancements in osmolyte accumulation and osmotic adjustment have been documented in several abiotic conditions, including salt stress [Wu et al., 2014], water stress [Gadallah et al., 2000] and drought stress [Muhammad et al., 2015]. Consequently, in plants subjected to SWE, proline production may be augmented by the modification of hormone biosynthesis pathways and/or the impact of phytohormones such as auxin, cytokinin, and gibberellin found in the extracts, so promoting plant adaptation to stress conditions.

Glycine betaine, a well-known osmoprotectant, plays a crucial role in enhancing plant tolerance to abiotic stresses and improving crop productivity [Annunziata et al., 2019]. Under salinity stress, GB alleviates adverse effects by reducing cellular osmotic potential, inducing the expression of various stress-responsive genes, and protecting cells from salt-induced damage through the suppression of ROS accumulation [Giri, 2011; Paradisone et al., 2015]. Salinity-induced osmotic stress compels plants to elevate their osmolyte levels, such as GB, proline, and carbohydrates, which enable osmotic adjustment and enhance the uptake of water and nutrients [Chun et al., 2018; Arnao et al., 2021].

In this study, in terms of the levels of glycine betaine in wheat, the ANOVA findings showed that only the interaction between biostimulant and salt stress had a statistically significant impacts (Table 4.13). Conversely, control plants that did not undergo SWE treatment displayed the lowest quantities of glycine betaine, irrespective of stress exposure. In general, SWE treatments have been shown to have an increasing effect on glycine betaine accumulation in wheat shoots (Table 4.21). In wheat shoots subjected to high salt stress, the concentration of glycine betaine increased when treated with SWE, particularly when treated with water-based SWE; however, this rise was not statistically significant.

Seaweed extracts are known to include GB [MacKinnon et al., 2010], and although not specifically analyzed, the elevated GB levels observed in wheat might be because of the GB that is probably in the *C. barbata* extract. They have been shown to increase GB levels under salinity stress, leading to improved osmotic potential, water and nutrient uptake, and stabilized photosynthetic activity [Carillo et al., 2020a, 2020b; Sariñana-Aldaco et al., 2022] and increased antioxidant defense [Khan et al., 2022]. The results are ascribed to SWEs abundant in betaines and betaine-like compounds as well as promoting the accumulation of GB in plant tissues, consequently augmenting plant tolerance to salt stress [MacKinnon and Craft, 2015; Mughunth et al., 2024].

Indeed, GB is produced and stored in young leaf tissues during extended stress, whereas proline accumulates more swiftly at the outset of stress and mostly in older leaves. This elucidates the significant elevation in proline levels seen in this short-term stress investigation [Carillo et al., 2008; Annunziata et al., 2019]. Moreover, when proline is present at elevated concentrations alongside GB, it effectively defends plants against oxidative stress, rendering antioxidant compounds and antioxidative enzymes

relatively insignificant in ROS protection in saline conditions [Carillo et al., 2011; Woodrow et al., 2017; Annunziata et al., 2019].

The results of the third hydroponic experiment demonstrate that *C. barbata*-based SWE applications were found to mitigate the growth inhibition caused by moderate salt stress caused by 60 mM NaCl and 90 mM NaCl application in hydroponically grown wheat plants. The application of *C. barbata* extract resulted in enhanced shoot and root biomass, diminished electrolyte leakage, increased proline and glycine betaine accumulation, elevated K levels. This growth-promoting action of SWE on hydroponically grown wheat may be ascribed to the existence of many bioactive compounds in the extracts. The results demonstrate that the water and acid extract of *C. barbata* regulated many metabolic, physiological and molecular mechanisms in hydroponically grown wheat, potentially enhancing salinity tolerance through synergistic effects. Based on these findings, growers employing deepwater culture systems may consider incorporating SWEs into nutrient solutions to mitigate the effects of salt stress. Consequently, it has been noted that a system of action and reaction exists inside the plant, and that the use of seaweed-based biostimulants can enhance plant growth and production by eliciting responses to stressors.

5.4. Determination of the Effects of *C. barbata* Extracts on Yield and Quality Parameters of Lettuce (*Lactuca sativa* L. cv Lollo Rosso) in the Soilless Culture System

Vegetables don't possess a long-life cycle and relatively need higher amounts of fertilizer throughout the growing season. Moreover, lettuce, compared to other vegetables, has rapid growth and develops a superficial root system; it necessitates adequate fertilization during its growing period [Sarmiento-Soares et al., 2012]. Ensuring the provision of all required mineral nutrients for maximum plant growth and productivity is a fundamental necessity in soilless cultivation systems, just as it is in traditional soil-based agriculture [Domingues et al., 2012]. In recent years, the agricultural sector has become increasingly reliant on chemical fertilizers to increase crop production, but while this dependency may provide improvements in production, it is not a sustainable approach in the long term [Quaik et al., 2012]. The overuse of chemical fertilizers correlates with a deterioration in agricultural sustainability, as well as issues related to their substantial economic costs, potential for environmental

damage, and negative impacts on human health [Ramya et al., 2015]. In response to these issues, there is increasing interest in organic input that provides more environmentally sustainable and economically viable alternatives. These inputs are increasingly acknowledged as essential elements in the shift towards sustainable agriculture production systems.

Hydroponic systems, a method of cultivating plants without soil through a nutrient-rich solution, serve as a viable alternative to conventional soil-based agriculture, ensuring a stable supply of produce [Jan et al., 2020; Velazquez-Gonzalez et al., 2022]. These systems promote faster crop ripening, higher yields and quality, and enhanced nutrient content due to the efficient delivery of nutrients to the plant roots [Sardare and Admane, 2013; Sharma et al., 2018]. Lettuce, one of the most commonly grown plants in hydroponic systems, has a crop cycle of approximately 70 days in soil, whereas this period can be reduced to 30 days in hydroponic system [Cometti et al., 2013; Sapkota et al., 2019]. For these reasons, cultivating leafy vegetables such as lettuce in hydroponic systems enhances sustainable agricultural production by augmenting water usage efficiency and enhancing production yields and profitability [Majid et al., 2021].

This study evaluated the effects of three different concentrations (low, medium, and high) of water, alkali, and acid extracts derived from *C. barbata* on the vegetative growth performance and quality parameters of hydroponically cultivated lettuce plants. Compared to control plants cultivated without the SWE, lettuce plants grown hydroponically with the addition of all SWE to the nutrient solution exhibited enhanced growth, increased yields and a healthier, more vigorous appearance (**Figure 4.18**). Especially, the maximum yield was observed at the highest concentrations of all SWEs (**Figure 4.19**). Multiple studies have shown that the use of biostimulants on lettuce significantly enhances biomass, leaf area, and overall yields [Navarro-León et al., 2022; Carillo et al., 2022; Di Mola et al., 2019; Noroozlo et al., 2019].

The results indicate that SWEs derived from *C. barbata* as eco-friendly and multifunctional plant biostimulants, supporting sustainable agricultural practices in soilless systems. The observed increase in lettuce growth under hydroponic conditions when treated with various extracts derived from the same seaweed, compared to untreated plants, can be attributed to the presence of growth-promoting compounds

including polysaccharides, hormone-like compounds as well as polyphenolic substances.

Studies on lettuce plants, in soilless cultivation systems have demonstrated that the incorporation of SWEs applications into the growth medium via nutrient solutions, as in hydroponic systems, significantly enhances morphological and physiological traits, overall plant growth parameters, yield, nutrient uptake as well as postharvest quality [Miceli et al., 2021; Wang et al., 2022; Cuchca Ramos et al., 2025; Oñez et al., 2025]. Moreover, incorporating SWEs into nutrient solutions considerably enhances the vegetative growth performance of hydroponically cultivated lettuce, owing to their intrinsic plant growth-promoting properties [Vernieri et al., 2005; Wang et al., 2022]. A recent study also indicated that the use of SWE with hydroponic solutions enhanced various growth indices of lettuce, including plant height, leaf count, length, breadth, root development, and overall yield, and these observed beneficial benefits were ascribed to the response elicited by the hormones in the SWE rather than the mineral elements in the solution [Oñez et al., 2025].

The control plant exhibited the lowest shoot and root biomass, whereas the *C. barbata* SWE treatments resulted in a significant enhancement of both dry weight (**Table 4.20**). In general, both shoot and root dry weight attained their peak values when subjected to water extract treatment. In general, lettuce plants subjected to SWE revealed a reduced R/S ratio (**Table 4.20**). The low R/S ratio, which quantifies the biomass distribution between roots and shoots, indicates that in nutrient- and water-rich environments, such as hydroponic systems, plants allocate less biomass to root development due to minimal resource looking requirements [Mokany et al., 2006]. Consequently, they invest more energy in shoot growth, enhancing photosynthesis and increasing above-ground biomass accumulation. This is regarded as a highly favorable and anticipated physiological response, indicating that under optimal and stress-free development conditions, plants are effectively utilizing available resources for productive growth [Mašková and Herben, 2018]. Moreover, the R/S ratio serves as a crucial indicator of plant adaptation to environmental resource constraints [Bonifas and Lindquist, 2006]. Particularly, under conditions of nutrient or water scarcity, plants typically allocate greater resources to their root systems to enhance nutrient and water absorption, resulting in an elevated R/S ratio and increased root biomass [Franco et al., 2011; Lopez et al., 2023].

Increased dry matter accumulation in lettuce resulting from high-dose SWE treatments may correlate with enhanced photosynthetic activity and elevated nutrient density, signifying superior physiological performance (**Table 4.20**). Consistent with this study conducted in the winter months, a study revealed that the dry matter content of lettuce was approximately 7% in the summer months, decreasing to 4% in the winter months [**Pereira et al., 2023**]. The dry matter ratio (DMR), reflecting the proportion of a plant's fresh mass comprised of structural, metabolic, and storage components as opposed to water, serves as a measure of plant growth efficiency and quality. Multiple studies indicated that 95% of plant dry matter accumulation originated from organic matter generated by photosynthesis, with the photosynthetic rate serving as the foundation for biological yield development [**Zhang et al., 2023a**]. An elevated DMR typically indicates effective nutrient use, improved texture, enhanced photosynthetic activity and increased soluble solids. For fresh leafy vegetables like lettuce, a higher DMR also provides to improved texture and longer shelf life, which is critical for preventing postharvest losses and ensuring high storage quality [**Huang et al., 2021**]. Research on lettuce has consistently demonstrated a direct correlation between nutrient accumulation and DMR [**Souza et al., 2015; Govedarica-Lučić et al., 2020**].

Seaweed applications were found to significantly influence the phenolic content and antioxidant activity of hydroponically grown lettuce plants (**Figure 4.20**). Among all treatment groups, the highest total phenolic content and antioxidant activities were recorded in lettuce plants exposed to high concentrations of the extracts. Overall, acid extract applications had the greatest effect on phenolic content and antioxidant activity in a dose-dependent manner, followed by water and alkali extracts. Phenolic substances are thought to extend the shelf life of lettuce, therefore aiding in the minimizing of postharvest loss [**Rasouli et al., 2022; Cuchca Ramos et al., 2025**]. Moreover, it has been shown that these constituents in lettuce play a significant protective role in the cardiovascular system, fight inflammation, and may even help prevent cancer [**Rashmi and Negi, 2020**]. The utilization of SWE is known to induce an increase in the total phenol content of the plant [**Basavaraja et al., 2018; Cuchca Ramos et al., 2025**] as well as antioxidant activity [**Di Mola et al., 2019**].

In accordance with this study, **Kim et al., (2018)** have shown that red-green lettuce varieties exhibit a greater average total phenolic content of 227 mg GAE/100 g FW compared to green lettuce varieties, which contain 133 mg GAE/100 g FW. High

levels of phenolic compound in plants are associated with better antioxidant activity, and the use of SWEs in plant production can increase the nutritional value and quality of plants by accumulating non-enzymatic antioxidant substances and increasing antioxidant enzyme activities [Allen et al., 2001; Kang and Saltveit, 2002; Fan et al., 2011]. These observed improved phenolic and antioxidant activity in SWE-applied lettuce plant can be attributed to polysaccharides present in SWEs function as elicitors, augmenting the activity of phenylalanine ammonia-lyase (PAL), an essential enzyme in phenolic synthesis, thereby leading to an increase in phenolic compounds [Bouissil et al., 2020; Ali et al., 2021]. Moreover, SWEs improve the absorption of nutrients, particularly trace elements like Zn, Mn, and Cu, which function as cofactors in enzymatic pathways related to phenolic metabolism [Bradáčová et al., 2016]. The enhanced phenolic levels in plants may be partly ascribed to stimulation of endogenous hormone activity with SWEs [Lola-Luz et al., 2014]. Additionally, SWEs generally include high concentrations of organic and inorganic osmolytes including proline, mannitol and glycine betaine may be partially absorbed by the plant and/or may induce mild and transient osmotic stress [Di Stasio et al., 2020] and so SWEs may have simulated mild stress, which does not harm the plant but triggers secondary mechanism reactions, including the synthesis of phenolic chemicals. Glycine betaine was demonstrated to induce phenolic accumulation and the activity of their biosynthetic enzymes [Wang et al., 2019]. Moreover, ascorbic acid and carotenoids, significant antioxidant components, enhance the overall antioxidant activity of lettuce [Nicolle et al., 2004]. Consequently, the applied *C. barbata* extracts are intrinsically abundant in phenolic compounds [Mutlu-Durak et al., 2024], which can be absorbed by the plants and/or modulate endogenous signaling pathways, hence augmenting the overall phenolic content observed in the treated plants. There is a growing consumer or market interest for vegetables or fruit with high antioxidant qualities, regarded as value-added foods that may aid in avoiding damage caused by oxidative stress [Chrysargyris et al., 2018; Rahman et al., 2021].

Vitamin C, a major water-soluble antioxidant, is highly concentrated on meristems, photosynthetic tissues, root tips, flowers, and developing fruits [Akram et al., 2017]. These levels fluctuate significantly based on genotype, environmental conditions, and the developmental phase [Gest et al., 2012]. Vitamin C participates in many important processes such as photoprotection, ROS scavenging, α -tocopherol regeneration, and

electron transferring to photosystem II [Smirnoff, 2000; Tóth et al., 2009]. It also participates in the production of substances including anthocyanins, ethylene, flavonoids, gibberellic acid, which are crucial for growth and ripening of fruit as a cofactor for certain metabolic enzymes [Smirnoff and Wheeler, 2000]. It modulates plant hormones production and, by acting as an electron donor during stress, facilitates the enzymatic elimination of ROS, thus maintaining cell balance and integrity [Hasanuzzaman et al., 2019a]. Furthermore, it can serve as a precursor for molecules such as organic acids that are found in large amounts in fruits and have effects such as flavor, nutritional value, and shelf-life extension [Shi et al., 2022].

The addition of SWEs to the hydroponic system led to an increase in both total and reduced vitamin C levels in harvested lettuce plants (**Figure 4.21**). Overall, water and acid extract treatments resulted in a significant enhancement of total vitamin C level compared to other application groups (**Figure 4.21A**). On the other hand, the highest level of reduced vitamin C was observed in lettuce plants treated with the high concentration of the water extract (**Figure 4.21B**).

Seaweed-derived biostimulants enhanced ascorbic acid levels in greenhouse lettuce [Rasouli et al., 2022], pepper [Mohamed et al., 2021], spinach [Rouphael et al., 2018b], and tomato [Arikan-Algul et al., 2025]. Ascorbic acid is recognized for its significant function in the protection of chloroplasts [La Bella et al., 2021]. The correlation between photosynthesis and ascorbic acid synthesis in plants suggests that the elevated levels of vitamin C observed with seaweed-based biostimulants may result from enhanced chlorophyll content and increased photosynthetic activity, attributable to the stimulatory effects of SWE [Patel et al., 2017; Rasouli et al., 2022]. The positive impact of SWE on vitamin C levels is ascribed to the intricate combination of amino acids, hormones, carbohydrates, phenolic substances, vitamins and lipids, which are readily taken by plants [Rasouli et al., 2022; Cuchca Ramos et al., 2025].

Augmenting the vitamin C levels in plants can have several advantages, like enhancing the nutritional value of food and augmenting resilience to abiotic stress [Wang et al., 2024]. Moreover, Saini et al., [2016] reported that elevated levels of ascorbic acid in lettuce enhanced shelf-life by maintaining phenolic components.

Humans are unable to synthesize this substance, so they must depend solely on dietary sources for their intake [Fenech et al., 2019]. Fruits and vegetables are considered the

principal sources of vitamin C, which is a significant determinant of the nutritional quality of fruits and vegetables [Medina-Lozano et al., 2021]. Moreover, ascorbic acid accounts for roughly 25% of the total antioxidant activity in lettuce, signifying that vitamin C is a principal antioxidant [Carnat et al., 2004]. The mean vitamin C concentration of loose-leaf lettuce utilized in this investigation was recorded as 10.39 mg/100 g FW [Yang et al., 2022]. Moreover, a recent study, consistent with this findings, indicated that the vitamin C in Lollo Rosso rose to 12.2 mg/100 g FW following biostimulant treatment [Stojanović et al., 2020]. In this study, Vitamin C levels in lettuce plants treated with *C. barbata* extract were similarly found to reach 12 mg/100 g. Moreover, their levels are affected by many things, such as cultivar, pigmentation, genotypic differences, growing weather conditions, farming methods, the fruit or vegetable's level of ripeness, and how it was picked, stored, and handled after harvest [Marín et al., 2004].

In consistent with our results, recent research indicated that using SWEs in hydroponic systems can enhance both the yield and nutritional quality, including vitamin C, of lettuce [Rasouli et al., 2022]. Moreover, hydroponically cultivated lettuce has markedly elevated concentrations of ascorbic acid and tocopherols than soil-cultivated lettuce [Buchanan and Omaye, 2013]. Moreover, Selma et al., [2012] reported in a study that *L. sativa* cv. Lollo Rosso grown in a soilless system had higher phytochemical levels, like vitamin C and total phenolics compounds, compared to grown in soil production system. Soilless systems are favored for their ability to enhance antioxidant levels, particularly in red-leafed genotypes, as these chemicals not only confer health benefits but also safeguard against spoiling of fresh-cut products [Hodges and Toivonen, 2008; Selma et al., 2012]. In general, the increased production of phytochemical substances in leafy greens, including lettuce, represents a notable nutritional benefit, substantially impacting human health and longevity [Rouphael et al., 2018b].

Vitamin C is especially susceptible to degradation under unfavorable pre- and postharvest conditions, like prolonged storage, extreme high and low temperatures, inadequate relative humidity, and physical harm [Tudela et al., 2002]. The reduced form (ascorbic acid), a biologically active antioxidant, is a less stable form of Vitamin C and demonstrates the plant's current antioxidant capacity. It is the bioavailable form of Vitamin C, essential for collagen formation, iron absorption, immunological

support, and the neutralization of free radicals in the human body. It is easily oxidized by ascorbate oxidase, particularly in the presence of O₂, metal ions, and elevated pH and temperatures, resulting in its conversion to dehydroascorbic acid (DHA), the oxidized form [Lee and Kader, 2000]. This oxidized form can also be converted back to ascorbic acid within cells with substantial reducing capacity, such as erythrocytes or hepatocytes. On the other hand, total vitamin C, encompassing both active and inactive forms inside the plant, is a more stable than the ascorbic acid. It indicates the maximum potential vitamin C and reveals the initial quantity present prior to processing, storage, and other factors. After consumption of lettuce, both forms of vitamin C can be digested and absorbed across the small intestinal epithelium by different mechanisms via transporters in human body [Malo and Wilson, 2000; Shi et al., 2022]. Consequently, both higher reduced and total vitamin C levels are ideal, signifying that the plant or fruit is fresh, robust, and stable.

Carotenoids are a category of lipid-soluble phytochemicals that serve as accessory pigments in photosynthesis and are essential for safeguarding plants from photo-oxidative stress [Bohn, 2019; Sun et al., 2022]. They are integral to plant metabolism, facilitating the absorption of particular light spectrum, eliminating ROS produced through photosynthesis, preserving intricate light-harvesting proteins, and protecting thylakoid membranes [Young and Lowe, 2018; García-Caparrós et al., 2019; Hussain et al., 2019]. Moreover, they are essential compounds in plants, performing a wide range of functions including contribution to pigmentation, and regulation of signaling pathways [Sun et al., 2022]. Additionally, research has demonstrated that carotenoids play a role as a precursors in the production of several phytohormones, all of which enhance plant development [Maoka, 2020].

All SWE treatments resulted in elevated carotenoid levels in hydroponically grown lettuce plants, particularly under low concentrations of acid, alkali, and water extracts, compared to plants that did not receive any *C. barbata* SWE treatment (**Figure 4.22**). Carotenoids in lettuce may rise with the usage of SWEs due to various synergistic effects induced by the bioactive components included in the extracts. Moreover, this increase may be due to the fact that SWEs contain various active compounds such as polyphenols and hormone-like substances that can trigger the antioxidant defense system of the plant, which encourages the production of more carotenoids.

The use of biostimulants commonly results in an elevation of photosynthetic pigments, notably carotenoids, which are crucial for chlorophyll synthesis [Abobaker et al., 2018; Carillo et al., 2019]. Applications of SWE can enhance chlorophyll content, therefore augmenting photosynthesis, stomatal conductance as well as water use efficiency [de Carvalho et al., 2019]. Several recent studies, including those on lettuce [Di Mola et al., 2019; Asadi et al., 2022], have indicated that the application of SWE enhances photosynthetic pigment rate, resulting in considerably greater total chlorophyll levels in plant [Castellanos-Barriga et al., 2017; Mahmoud et al., 2019]. Seaweed extract comprises high levels of plant growth regulators and betaines, which can substantially enhance plant growth, boost leaf development, and augment photosynthetic pigment synthesis as well as cytokinin, which protects chloroplasts and stimulates endogenous cytokinin biosynthesis in plants [Gupta et al., 2021; Alhasan et al., 2021; Nabti et al., 2018]. Furthermore, SWE possesses a high concentration of mineral nutrient elements including K, Mg, N and Fe, with Mg being crucial for chlorophyll formation and K contributing positively to improving photosynthesis, stimulating meristem growth, and modulating the water levels of plants [Chrysargyris et al., 2018]. As photosynthesis increases, carotenoids concurrently grow to facilitate photoprotection. In general, these favorable outcomes can be ascribed to the SWE application, which enhanced the total chlorophyll content in the leaves, hence improving the ability and efficacy of the photosynthetic as well as yield.

Glutathione, an integral component of the ascorbate–glutathione (AsA–GSH) cycle that sustains redox homeostasis, fulfills critical roles in signal transmission, SO_4^{2-} transport, and molecules conjugation [Gong et al., 2018]. Moreover, it is crucial in modulating internal defense mechanisms by effectively eliminating ROS [Lushchak et al., 2012; Kesarwani et al., 2013]. The biological activity of antioxidant compounds such as vitamins C and E and coenzyme Q10 is largely dependent on glutathione levels [Ballatori et al., 2009]. Moreover, it is purportedly implicated in the mechanism that fosters the conversion of DHA to AA, a process crucial for sustaining vitamin C levels in plants, ensuring the stability of vitamin C in tissues, and enhancing cellular health and the defense system [Paciolla et al., 2019; Hasanuzzaman et al., 2019b].

In this study, hydroponically grown lettuce plants treated with high levels of acid extract had higher GSH levels (Figure 4.23). Glutathione is present in the majority of

cells and tissues in both plants and mammals [Flieger et al., 2021; Irato and Santovito, 2021]. It is an essential nonenzymatic antioxidant necessary for the life of people, animals, and plants, existing in reduced (GSH) and oxidized (GSSG) forms [Foyer and Noctor, 2011; Al-Temimi et al., 2023]. Within the cell, almost 98% of glutathione exists in the GSH form [Ighodaro et al., 2018]. The presence of amino acids such as cysteine, which contain sulfur, is required for glutathione synthesis; as a result, it is known that sulfur deficiency causes a decrease in glutathione levels [Noctor et al., 2011; Honsel et al., 2012]. For these reason, the increase in glutathione levels observed with *C. barbata* extracts can be attributed to the presence of S element and sulfur-containing compounds such as fucoidan, amino acids, sulfate esters and organosulfur in the extract (Table 3.1, Table 4.1).

This study also investigated the effects of different doses of SWE applications on the root systems of lettuce plants. The results of the root analysis showed that the applications had a positive effect, stimulating improvements in various parameters, including root length, surface area, volume, diameter, and root tip number. (Table 4.24).

Seaweed extracts added to the nutrient solution resulted in a stronger root system in various plants, including lettuce grown in these systems [Wang et al., 2022]. It is known that high levels of phytohormones and hormone-like activity, also found in *C. barbata* extracts [Mutlu-Durak et al., 2024], have a significant effect on the developed root system by stimulating and accelerating root growth, promoting lateral root formation, and enhancing root hair elongation [Craigie et al., 2010; Ertani et al., 2018; Frick and Strader, 2018; Mukherjee and Patel, 2020; Hamouda et al., 2022], which significantly improves the nutrient acquisition efficiency of the plant, supporting increased physiological function and overall yield and developmental vitality [Calvo et al., 2014]. Moreover, these beneficial impacts of SWE on the root system architecture may be ascribed to the activation of an auxin-related gene triggered by alginate oligosaccharide, resulting in elevated auxin levels that stimulate root development and elongation [Zhang et al., 2014]. Moreover, it has been documented that certain polysaccharides including alginates and sulfated compounds can activate plant defensive mechanisms and have direct or indirect involvement in root growth and development [González et al., 2013]. Thanks to SWEs, this improvements in the

root system can be even more beneficial in stress conditions where growth and development are difficult [Khan 2009; Ali et al., 2021].

The stimulating impact of *C. barbata*-based SWEs on root system morphology likely contributed to the enhanced accumulation of macroelements like K and microelements such as Fe in lettuce plants (Table 4.24, Table 4.25, Table 4.26). This enhanced accumulation of minerals in plants applied with SWEs was attributed to the upregulation of genes encoding transporters and/or the encouragement of root cell division and the growth of lateral roots and hairs [Mugnai et al., 2008; Spinelli et al., 2010].

Mineral analysis indicated alterations in the mineral element compositions of hydroponically cultivated lettuce following treatments (Table 4.25 and Table 4.26). Compared to the control group, lettuce plants treated with *C. barbata* extracts showed similar Ca and S concentrations across all application levels (Table 4.25). However, a slight decrease in P, Ca and Mg levels was observed, along with an increase in K concentration. The reductions in Ca and Mg levels in *C. barbata* extracts-treated lettuce plants may result from the antagonistic interaction between these elements and K during uptake and translocation [Jacobsen, 1993]. Although mineral concentrations are expressed on a dry matter basis, since changes in lettuce dry matter in response to SWE treatments are known (Table 4.22, Table 4.25), it can be safely concluded that all SWE-treated plants had substantially higher K levels on a FW basis than control plants. Numerous studies have demonstrated that the application of biostimulants enhances potassium levels in lettuce plants [Carillo et al., 2022; Giordano et al., 2022]. Potassium is known to be the most abundant mineral element compared to other elements in SWEs obtained from *C. barbata* [Mutlu-Durak et al., 2024]. Numerous studies have demonstrated that the application of SWE enhances K levels in plants [Fei et al., 2017; Rouphael et al., 2018b; La Bella et al., 2021; Mutlu-Durak et al., 2024 Arıkan-Algöl, et al., 2025]. Moreover, Chrysargyris et al., [2018] demonstrated that the application of foliar sprays containing SWEs reduced the effects of K deficiency, increased antioxidant capacity, and improved the quality of processed lettuce. In leafy vegetables such as lettuce, potassium concentrations play an important role in market crop quality due to their positive contributions to nutritional quality such as TSS, acidity, health-related components and vitamin levels, in addition to visual and structural quality such as postharvest durability, texture, size and color [Mitra and

Dhaliwal 2009; Lester et al., 2010; Marschner, 2012; Chrysargyris et al., 2018].

In addition, the increase in potassium accumulation due to SWEs may be thought to be related to cell expansion, membrane traffic, auxin balance, signaling pathways and especially root development **[Sustr et al., 2019].**

In addition to macronutrient concentrations, applications of algae-based biostimulants led to a reduction in the concentrations of Zn, Cu, and B micronutrients in the leaves of hydroponically grown lettuce plants, irrespective of the type of SWE used **(Table 4.26)**. In contrast, lettuce plants treated with high concentrations of water extracts exhibited higher levels of Fe and Mn compared to other treatment groups. The augmentation of Fe in lettuce resulting from biostimulant treatment may enhance the physiological condition of the plants, as Fe is essential in the catalytic centers of numerous hemoprotein redox enzymes, such as cytochromes, peroxidases, and CAT as well as is essential for photosynthesis and nitrogen assimilation **[Hänsch and Mendel, 2009; Atero-Calvo et al., 2024].**

A recent study has shown that up to 50% of commercial fertilizer input in hydroponically grown lettuce systems can be effectively replaced with SWE, thus halving the dependency on chemical fertilizers and with a return on investment of around 100%, consequently making SWE a promising component of a more sustainable fertilization strategy **[Oñez et al., 2025]**. In addition, the application of algae-based biostimulants in soilless systems without causing yield reduction can reduce the required fertilizer input by increasing mineral uptake and accumulation, thus reducing both production costs and environmental pollution concerns **[Shahein et al., 2014; Guilayn et al., 2020]**. Moreover, biostimulants enhance nutrient use efficiency, enabling a decrease in fertilizer usage and improving the uptake of macronutrients like potassium and micronutrients such as iron, thereby elevating nutritional quality, which benefits human health and fosters sustainable agricultural practices by mitigating environmental impacts **[Navarro-León et al., 2022; Yaseen and Takacs-Hajos, 2022; Noroozlo et al., 2019]**. These results can be attributed to the modification of root morphology system by SWE, which improves plant nutrient uptake **[Table 4.24-4.26]**.

Although studies on the effects of plants grown in biostimulant-treated medium on human health are insufficient, a study has shown that consumption of lettuce grown in SWEs-treated medium has positive effects on human health **[Di Gaudio et al., 2025]**.

In addition, they suggested that SWE-enriched lettuce could be a natural alternative to iron supplements with various side effects by improving Fe homeostasis in the blood by increasing serum Fe concentration and could be evaluated as a solution to Fe deficiency or anemia, a significant public health concern, particularly among individuals adhering to vegan or vegetarian diets.

The results of this thesis study revealed that all *C. barbata* extracts improved vegetative growth and overall plant performance in hydroponically grown lettuce plants, as well as improved quality parameters such as dry matter, phenolic and antioxidant capacity, carotenoids, glutathione, nutritional properties and vitamin C concentration. Consequently, biostimulants may be an important tool for enhancing edible plant components, such as leaves and fruits, with minerals that are essential for maintaining human health [Carillo et al., 2020b; Rouphael et al., 2021]. Thus, the integration of hydroponics and seaweed-based biostimulants represents a viable eco-friendly approach to vegetable crop cultivation [Vernieri et al., 2005].

5.5. Comparison of Foliar Applications of *C. barbata* Extracts and a Commercial Seaweed-Based Product on Yield and Fruit Quality of Pepper

Substrate culture, recognized as one of the most common methods in greenhouse crop production, offers advantages such as optimized particle size distribution, improved capillary porosity, and enhanced water-holding capacity over traditional soil-based systems, as well as offering more accurate and easy control over the supply and composition of mineral nutrients and other components, thereby enabling more consistent crop performance and efficient resource management [Tuxun et al., 2025].

Biostimulants, such as SWEs, demonstrate potential as sustainable solutions that enhance plant performance and minimize inputs in pepper cultivation in both soilless and soil-based systems [Arthur et al., 2022; Ashour et al., 2021]. Many studies in the literature have shown that foliar applications of different algae extracts can increase fruit yields and quality of many different pepper varieties grown in greenhouse conditions in soilless systems [Khan et al., 2018; Melo et al., 2020].

A greenhouse experiment was conducted to assess the effectiveness of foliar applications of SWEs (water, alkali and acid) from *C. barbata* and a commercial

seaweed-based biostimulant product (Acadian) in enhancing the yield and quality parameters of pepper plants cultivated in a substrate culture system using cocopeat as the growth medium.

Foliar applications serve as an alternative or complementary strategy to soil fertilization in agricultural production, as they provide nutrients directly to specific tissues, act more swiftly on the plant, and are more precise and environmentally sustainable, with a diminished risk of environmental pollution compared to soil applications [Niu et al., 2021]. However, the transfer of foliar-applied nutrients through the epidermis and into internal tissues is a complex process characterized by varying efficiency [Fernández and Brown, 2013]. Furthermore, the efficacy of foliar sprays can be affected by several environmental (humidity, windy, rainfall, light intensity, photoperiod, temperature), physicochemical, and physiological parameters pertaining to the plant, in addition to the characteristics of the spray composition [Alshaal and El-Ramady, 2017; Hariharan et al., 2024].

The predominant and popular method of biostimulant application is foliar spraying, succeeded by application to soil or hydroponic solution and seed treatment [Wozniak et al., 2020]. Seaweed-based biostimulant can be applied through seed imbibition, direct incorporation into growth media such as soil or hydroponic systems, or via foliar spraying [Ali et al., 2021]. Foliar applications, regarded as one of the most effective and popular methods for delivering SWEs to many plants such as vegetables, cereals and flowers [Karthikeyan and Shanmugam 2016]. Seaweed-based biostimulants can be applied as a foliar spray application method, resulting in superior outcomes like rapid growth promotion due to the enhanced absorption of various bioactive compounds and direct interaction with the tissues, particularly when performed during morning hours when stomata are most active [Sharma, et al. 2019; Battacharyya et al., 2015; El Boukhari et al. 2020; Ashour et al., 2021].

In this study, biostimulant application interval is once every 2 weeks as a foliar application to pepper plants grown in substrate culture medium. Numerous studies in the literature have demonstrated that applying SWEs at optimal intervals of 10 to 14 days results in the highest levels of effectiveness in plants [Arioli et al., 2015]. The components and quantity of seaweed-based biostimulants utilized in foliar applications to pepper plants exhibit significant variability, influenced by factors like macroalga types and extraction techniques [Khan et al., 2018; Ozbay and Demirkiran, 2019;

Ashour et al., 2021; Mohamed et al., 2021; Ali et al., 2022]. Consequently, the suggested application dosage for the commercial seaweed-based biostimulant product (Acadian) featured in the study was utilized as a reference.

Although foliar applications of SWEs enhanced the total yield and fruit number of pepper plants, no significant differences were observed in fruit weight per plant, with the average fruit size remaining consistent across all treatment groups (**Figure 4.24**). The highest fruit yield was recorded in cocopeat of pot-grown greenhouse pepper plants treated with water-based SWE, followed by those treated with acid and alkali extracts, respectively (**Figure 4.24A**). Notably, no additional yield benefit was detected in plants treated with the commercial product. Among all treatment groups, water extract applications resulted in the greatest fruit production, while acid and alkali treatments exhibited comparable improvements in fruit quantity (**Figure 4.25B**). Peppers from all treatment groups were of similar fruit weight. This shows that a homogeneous sampling process was achieved by harvesting only long green peppers that reached a certain fruit size (**Figure 4.24C**). The hormonal components of SWE contributed to improved growth and fruit production in pepper plants, promoting overall development [**Khan et al., 2009**]. Recent research demonstrated that an *A. nodosum* treatment of tomato and pepper markedly stimulated the upregulation of genes responsible for the synthesis of phytohormones, indicating a potential correlation with plant growth and development [**Ali et al., 2019**]. Numerous studies have documented that seaweed-based biostimulant foliar-applied to peppers revealed substantial enhancements in vegetative growth performance and enhanced some growth parameters including plant height, leaf area, branch quantity, stem diameter, shoot dry matter, root length [**Shabana et al., 2015; Ozbay and Demirkiran, 2019; Ashour et al., 2021**], flower number, fruit yield, and fruit number, length and diameter and overall vigor and yield [**Eris et al., 1995; Ali et al., 2022**] as well as post-harvest shelf life [**Khan et al., 2018**]. Overall, seaweed-based biostimulant increase yield by promoting early flowering and fruit set in plants including pepper, and the increase in yield may be attributed to the presence of plant hormone in the extracts, as well as modulation of gene expression regulating the endogenous production of hormones such as auxin and cytokinin by seaweed components [**Kulkarni et al., 2019; Ali et al., 2019**]. Cytokinin found in SWE can increase the number of branches in the treated

plants, which is reflected in the increased fruit yield of the pepper, by stimulating the development of lateral buds [Mohamed et al., 2021].

Research indicates that pepper plants sprayed with SWE exhibited an increase in both the quantity and productivity of fruits [Arthur et al., 2003; El Boukhari et al., 2020; Mohamed et al., 2021]. Moreover, Khan et al., [2011] indicated that foliar application of commercial seaweed products exhibited cytokinin-like activity, implying that SWEs contain substances capable of stimulating endogenous cytokinin-like activity.

Positive impacts of foliar applications, particularly those involving water and acid extracts, were observed on the phenolic content of fruits harvested from pepper plants cultivated in a cocopeat medium, as well as on their antioxidant capacities as assessed by ABTS, DPPH, and FRAP assays (Figure 4.25A-4.25D). While foliar application of commercial seaweed-based products did not significantly enhance the phenolic or antioxidant activity of pepper fruits, significant improvements in both phenolic substances as well as antioxidant activity were noted in plants treated with water and acid extracts from *C. barbata*.

Phenolic substances, secondary metabolites that assist in defending plants from stress factors, are recognized for their health benefits in humans, attributed to the protective properties of dietary polyphenols against numerous chronic illness [Del Rio et al., 2013]. A robust positive association typically exists between phenolics, the primary metabolites responsible for the antioxidant activity in plants, and the overall antioxidant activity [Jacobo-Velázquez and Cisneros-Zevallos, 2009]. The color, flavor, and nutritional attributes of peppers may be influenced by their antioxidant levels; thus, understanding the phytochemical alterations during maturation and their impact on antioxidant capacity are considerable dietary considerations that may influence the consumption of various types [Paradić et al., 2011]. A recently published study showed that the extracts derived from *C. barbata* utilized in this thesis research increased fruit yield as well as enhanced the phenolic and antioxidant content in tomato fruits cultivated under greenhouse conditions [Arıkan-Algül et al., 2025]. Paradić et al., (2011) indicates that the use of biostimulants on pepper plants cultivated in substrate culture under greenhouse conditions typically results in enhanced pepper yield, nutritional content and fruit quality including, antioxidant activity, vitamin C and phenolic levels. Another study also shown that foliar

application of a commercial seaweed-based biostimulant product to hot pepper plants under greenhouse conditions not only enhanced yield but also increased antioxidant activity of the fruit, resulting in increased levels of ascorbic acid and certain phenolic compounds, including phenols and flavonoids, compared to conventional NPK treatments [Ashour et al., 2021].

While foliar applications of commercial seaweed-based products did not enhance GSH levels in pepper fruits, treatments with *C. barbata* extracts significantly increased GSH concentrations, particularly in fruits from plants treated with alkali (16.7 mg/100 g FW), water (14.4 mg/100 g FW), and acid extracts (12.4 mg/100 g FW), respectively (**Figure 4.26**). Moreover, control plants exhibited the lowest GSH (9.9 mg/100 g FW) levels. Consistent with our results, the GSH value range of sweet peppers reported in the literature is 11–14 mg/100 g FW [Endo et al., 2019; Al-Temimi et al., 2023].

The essential function of glutathione in preserving redox equilibrium in humans, along with mounting evidence of its diminished amounts in numerous chronic illnesses, has prompted researchers to look at this molecule as both a diagnostic and a prospective therapeutic target [Minich and Brown, 2019].

Sulfur is a crucial ingredient for the biosynthesis of cysteine, that, together with glutamic acid and glycine, forms the tripeptide glutathione [Li et al., 2020]. Elevated glutathione concentrations were correlated with increased sulfur levels in pepper plants subjected to alkali SWE treatment (**Table 4.28, Figure 4.26**).

Vitamin C, an antioxidant molecule, acts as a powerful free radical scavenger and co-factor of various enzymes that are essential not only for plants but also for human metabolism [Paciolla et al., 2019]. Several studies have shown that Vitamin C plays a role in post-harvest stress protection not only of photosynthetic tissues but also of organs such as fruits [Gest et al., 2012].

In this study, fruits harvested from control plants that did not receive any algae-based biostimulant treatment exhibited lower concentrations of both forms of vitamin C, whereas the highest levels of these two forms of AsA were observed in fruits from pepper plants treated with water extracts (**Figure 4.27**). Moreover, water-extracted pepper plants exhibited elevated amounts (66 mg/100 mg) of total AsA, potentially attributable to increased TSC levels, the precursor of ascorbic acid.

Vitamin C concentrations in pepper genotypes can vary from 43 mg/100 g to 247 mg/100 g of fresh fruit, potentially providing 50% to over 100% of the recommended daily intake (RDI). The current RDI for vitamin C is 90 mg per day for men and 75 mg per day for women [Wahyuni et al., 2013]. This means that one pepper fruit that 70 g can fulfill an individual's daily vitamin C requirements [Mohamed et al., 2021]. Although orange has only 50 mg per 100 g, the mean maximum vitamin C content in green pepper varieties is 120 mg per 100 g; however, this concentration might exceed 200 mg in red pepper species [Locato et al., 2013; Zhang et al., 2023b]. Alongside variations in genetic background, environmental cultivation circumstances can influence the Vitamin C level [Howard and Wildman, 2007; Aloni et al., 2008].

Research indicates that the foliar spraying of SWE to tomato plants in greenhouse settings significantly enhances quality parameters, including TSS and ascorbic acid concentrations in the fruits [Ali et al., 2016]. Foliar treatments with seaweed-based biostimulant caused an increase in ascorbic acid and total phenol content of leaves in greenhouse spinach plants [Rouphael et al., 2018b].

Applications of neither commercial SWEs nor *C. barbata*-derived extracts resulted in significant positive or negative alterations in the concentrations of macronutrients (Table 4.28) and micronutrients in pepper plants cultivated in a cocopeat medium, with the exception of Fe and Zn (Table 4.29). On the other hand, only the foliar application of the alkali extract from *C. barbata* led to an increase in both Fe and Zn concentrations in the pepper fruit. Overall, the mineral nutrient levels analyzed in this study were within expected physiological ranges, with no indications of important elemental toxicity or deficiency. Chrysargyris et al. (2018) found that foliar spraying of SWEs to lettuce plants did not boost the accumulation of any of the nutritional minerals tested.

No significant differences were detected in the photosynthetic pigment concentrations of long green pepper fruits following foliar application of either commercial algae-based biostimulant products or *C. barbata*-derived extracts (Figure 4.28). Neither commercial nor *C. barbata* seaweed-based biostimulants had a notable impact on these measured parameters. This finding indicates that fruits with comparable green color intensities were consistently harvested across all treatment groups. Likewise, foliar treatments of *A. nodosum* extract did not influence chlorophyll content in lettuce [Chrysargyris et al., 2018] and spinach [Xu and Leskovar, 2015]. Moreover, a

recent study has shown that the foliar-applied brown SWE did not considerably influence carotenoid levels in spinach leaves [La Bella et al., 2021].

Compared to control plants, foliar applications of both commercial and *C. barbata*-based biostimulants significantly increased the TSC concentration in pepper fruits (**Figure 4.29**). The highest TSC was particularly observed in fruits from plants treated with water and acid extracts. Increased TSC in fruit is due to the existence of growth-stimulating hormones and minerals in SWE that increase the photosynthesis performance and higher biomass of the plant [Fan et al., 2011]. This observed increase can be attributed to the accelerated glucose biosynthesis enhanced by foliar applications of water and acid extracts to pepper plants grown in substrate culture due to the transferred ions and organic compounds converted to glucose [Nguyen-Quoc and Foyer, 2001; Abbas et al., 2020]. Soluble carbohydrates, which enable the transport of sugars from source organs to sink organs, can suppress the expression of stress-related genes while promoting the expression of growth-related genes [Rosa et al., 2009]. Foliar application of biostimulants led to an increased levels of glucose and fructose in greenhouse-grown chili peppers [Ertani et al., 2014]. Additionally, a higher concentration of total soluble sugars was observed in many plants other than pepper treated with foliar applications of SWE [Khan et al., 2012; Kumari et al., 2011; Zheng et al., 2016]. The significant increase in TSC observed in fruits may be due to the increased biomass with increased allocation of assimilates to fruits by elevated CO₂ fixation ratio, increased photosynthetic performance with SWEs treatment, as well as the sinking power generated by the accumulation of stimulants [Liava et al., 2023; Hao et al., 2024]. The accumulation of soluble sugars, including glucose, fructose, and sucrose, predominantly found in the pericarp of fruits, is crucial in defining aroma, flavor, and sweetness, hence considerably influencing the quality of fruits and vegetables [Nookaraju et al., 2010; Zhang et al., 2023b; Guijarro-Real et al., 2023]. Recent studies on rice and tomato indicate that the administration of biostimulants improved photosynthetic activity, subsequently leading to a boost in soluble sugar concentration [Liava et al., 2023; Hao et al., 2024].

The aforementioned results clearly indicated that all *C. barbata* SWEs tested as foliar applications significantly increased fruit number and yield in pepper plants and significantly improved overall plant performance compared to the control group. In general, the results obtained from this study showed that the effects of SWEs obtained

from *C. barbata* on pepper plants were more effective than the commercial seaweed-based product. Overall, substrate culture-grown pepper plants treated with water extract maximized yield as well as various fruit nutritional quality traits that were rich in phenolic, antioxidant, vitamin C, and TSC, followed by acid extract.



6. CONCLUSION

This thesis provides a comprehensive evaluation of the potential of *C. barbata* extracts as natural biostimulants for enhancing plant growth and quality in soilless systems. This research reveals that *C. barbata* is a valuable marine bioresource with strong potential for development into effective biostimulants for soilless agriculture, as the tested extracts are highly efficient, environmentally friendly, reliable, and economically viable agents for sustainable agricultural practices.

In perlite experiments, the application of SWEs significantly enhanced early wheat growth when applied to seeds or substrate, with substrate application showing more consistent benefits. The trials using wheat as a model organism also indicate that *C. barbata*-based biostimulants can alleviate salt stress in plants grown with poor-quality irrigation water, supporting their utility in stress-prone environments, contributing to the development of more sustainable and resilient agricultural practices. Application of the extracts to nutrient solutions significantly improved lettuce yield, root morphology, mineral levels (K and Fe), and nutritional quality including phenolics, antioxidant activity, carotenoid and Vitamin C levels under greenhouse conditions. Moreover, foliar treatment of pepper plants grown in substrate culture with *C. barbata* extracts led to superior performance when compared to a commercial seaweed-based biostimulant, improving not only yield but also various quality traits including phenolic content, antioxidant activity, vitamin C, glutathione, and soluble carbohydrate levels.

The positive effects observed in this study are likely attributed to the complex and diverse chemical composition of *C. barbata* extracts. These include polysaccharides, mannitol, amino acids, phenolic, antioxidants, osmolytes, and hormone or hormone-like substances, all of which may act individually or synergistically to influence plant physiological processes. However, the rich nature of SWEs and the variable responses among plant species make it challenging to pinpoint specific mechanisms of action or the exact compounds responsible for biostimulant activity. To address these challenges, future research should focus on fractionating the water, acid, and alkali extracts to characterize

specific bioactive constituents. Identifying the most active compounds will enable the development of targeted and more consistent biostimulant formulations. Additionally, the complexity of potential synergistic effects could be better understood through comprehensive characterization studies using advanced analytical techniques.

Based on these findings, future studies should focus on the biostimulant potential of *C. barbata* extracts that can be further evaluated in other economically important crops under different abiotic stress conditions. Future studies should also investigate the extracts through a wider range of applications and long-term trials under commercial soilless greenhouses to better understand their efficacy and practical applicability. Moreover, the effects of more eco-friendly *C. barbata* extracts, such as those obtained through cold pressing or supercritical fluid extraction, can also be tested. The combined effects of these SWE, either with each other or other biostimulant substances, can be explored to assess potential synergistic interactions. Investigating the interaction between SWEs and the microbiome may also reveal synergistic effects on plant health, nutrient uptake in soilless systems.

The establishment of industrial-scale *C. barbata* cultivation systems is another critical step to protect marine ecosystems. This will ensure a consistent and sustainable biomass supply, while also preventing the ecological degradation associated with the overharvesting of wild brown macroalgae. Moreover, conducting life cycle assessments and economic evaluations will provide insights into the sustainability and cost-efficiency of integrating *C. barbata*-based products into modern agricultural systems. This thesis also supports the potential integration of *C. barbata* biostimulants into urban and controlled-environment agriculture systems, such as vertical and indoor farming systems. These systems align well with the global transition toward sustainable food production, particularly in the context of limited arable land and climate change.

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BIOGRAPHY

She achieved her bachelor's degree from Department of Bioengineering, Yıldız Technical University in 2016. She started her master's degree program at Yıldız Technical University, Department of Bioengineering and continued her education between 2016-2018. After, she continued her master's degree program as a research assistant at the Biotechnology Institute, Gebze Technical University. She completed her master's thesis titled "Willow Tree Extracts as a Novel Plant-Based Biostimulant". The articles titled "Seed treatment with biostimulants extracted from weeping willow (*Salix babylonica*) enhances early maize growth" and "Willow (*Salix babylonica*) extracts can act as biostimulants for enhancing salinity tolerance of maize grown in soilless culture" from this thesis have been published.

She registered for the PhD program at the Gebze Technical University, Biotechnology Institute on 22/07/2019. Within the scope of the TUBITAK 2244 Industry PhD Program, she served as a partial scholarship holder between 07/01/2021 and 21/02/2023 in the project titled "Development of National and Sustainable Agricultural Biotechnological Fertilizers and Biostimulants" with the project code 119C030. She conducted her thesis studies within the scope of the TUBITAK 3501 (Project number: 121Z215) project titled "Investigation of the Biostimulant Potential of Extracts Obtained from *Cystoseira barbata* Seaweeds on Wheat" and served as a scholarship holder in the project between 01/07/2023 and 15/10/2024. She is working as a researcher in the project coded 2024-A-113-16 titled "Development of a local growth capsule prototype from natural raw materials for different soilless agriculture systems and seedling cultivation". As of March 3, 2025, she is working as a scholarship holder in the project titled "Development of a Probe for Real-Time Monitoring of Nitrate Concentrations in Soils," project number 22AG045.

PUBLICATIONS AND PRESENTATIONS FROM THE THESIS

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Kutman U. B., Mutlu-Durak H., Arikan-Algul Y., Haznedaroglu B. Z., Kutman B. Y., (2022), ‘Effects of *Cystoseira barbata* extracts as a novel seaweed-based biostimulant agent on various crops’, The International Society for Nutraceuticals and Functional Foods (ISNFF), Oral Online Presentation.

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Mutlu-Durak H., Arikan-Algul Y., Kutman U. B., Kutman B. Y., (2023), “Pre-sowing treatment with seaweed extracts improve early growth of wheat”, 6th International Eurasian Conference on Biological and Chemical Sciences (EURASIANBIOCHEM 2023), Oral Online Presentation.

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