

T.R.
GEBZE TECHNICAL UNIVERSITY
GRADUATE SCHOOL

**BIOSTIMULANT EFFECTS OF *Cystoseira barbata* EXTRACTS
ON SOIL-GROWN CROPS**

YAĞMUR ARIKAN ALGÜL

**A THESIS OF DOCTORATE
DEPARTMENT OF BIOTECHNOLOGY**

ADVISOR: ASSIST. PROF. DR. BAHAR YILDIZ KUTMAN

JULY 2025

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LİSANSÜSTÜ EĞİTİM ENSTİTÜSÜ

***Cystoseira barbata* EKSTRAKTLARININ TOPRAKTA
YETİŞEN BİTKİLER ÜZERİNDEKİ BİYOSTİMÜLANT
ETKİLERİ**

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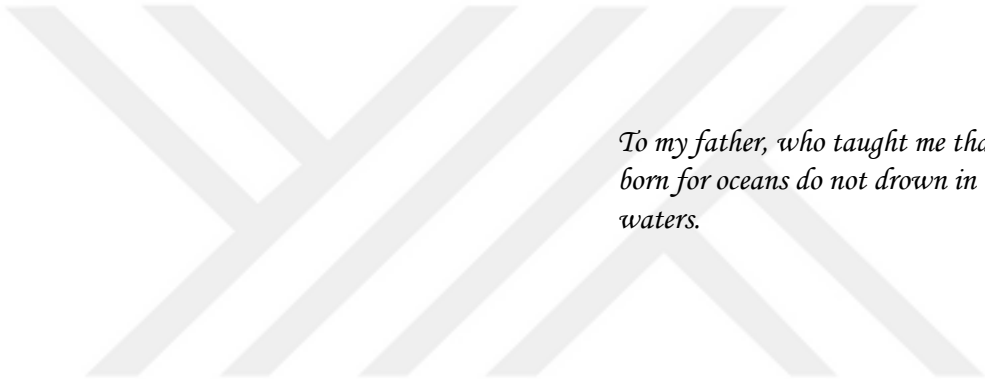
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*To my father, who taught me that captains
born for oceans do not drown in the shallow
waters.*

ABSTRACT

The escalating global demand for food, coupled with climate-related challenges and resource depletion, necessitates the development of sustainable agricultural strategies. Seaweed-derived biostimulants offer a promising approach to enhance crop growth, stress resilience, and product quality. This study is based on the hypothesis that different *Cystoseira barbata* (*C. barbata*) extracts, owing to their unique biochemical composition, would exhibit species- and application method-specific biostimulant effects on soil-grown crop plants.

To evaluate the biostimulant potential of *C. barbata*, water, alkali and acid extracts were applied to cereal crops (wheat) and horticultural crops (tomato, broccoli and pepper) in a controlled greenhouse environment. Depending on the objectives specific to each crop, applications were carried out via either soil drench or foliar spray. In wheat, soil drench treatments targeted biostimulant effects and drought-related physiological and growth responses, while foliar applications focused on the effects of biostimulants on growth, yield and differences in mineral elements. For tomatoes, broccoli and peppers, the effects of different extract types on fruit yield and quality were assessed. The pepper trial also involved a direct comparison with a commercial seaweed-based biostimulant (Acadian), enabling *C. barbata*-derived formulations to be evaluated against a benchmark.

In wheat, drench applications of water and acid extracts enhanced growth under drought conditions by stimulating antioxidant activity, osmolyte accumulation, and rhizosphere microbial activity. In tomato and broccoli, drench treatments with acid and alkali extracts significantly improved yield and quality traits, including antioxidant activity, vitamin C, and glutathione levels. Compared to drench treatments, foliar applications have less pronounced effects on wheat and tomato yield and did not significantly alter mineral concentrations. In addition, mineral analysis following drench applications revealed increased sulfur and potassium levels, likely due to improved nutrient uptake. In pepper, water and acid extracts not only increased yield relative to the control but also performed comparably to the commercial biostimulant Acadian. Moreover, these findings underscore the potential of *C. barbata* based formulations as effective and locally available alternatives to commercial products.

Importantly, this thesis provides one of the first experimental demonstrations of the biostimulant potential of a seaweed species found in Marmara Sea. Developing bio-based products by using *C. barbata* provides a sustainable agricultural solution and promotes circular bioeconomy by converting marine biomass into valuable products. Further research is required to standardize production of *C. barbata* in a sustainable way and confirm the effectiveness of biostimulants under field conditions.

Keywords: Biostimulant, *Cystoseira barbata* (*Gongoloria barbata*), Seaweed Extract, Soil-Grown, Sustainable Agriculture, Nutritional Quality

ÖZET

Artan küresel gıda talebi, iklimle ilgili zorluklar ve kaynakların tükenmesiyle birlikte, sürdürülebilir tarım stratejilerinin geliştirilmesini zorunlu kılmaktadır. Deniz yosunlarından elde edilen biyostimülantlar, bitki büyümesini, stres toleransını ve ürün kalitesini artırmak için umut verici bir yaklaşım sunmaktadır. Bu çalışma, *Cystoseira barbata* (*C. barbata*) türüne ait farklı özütlerin kendine özgü biyokimyasal bileşimleri nedeniyle, toprak koşullarında yetiştirilen çeşitli bitkiler üzerinde bitki türüne ve uygulama yöntemine özgü biyostimülant etkiler göstereceği hipotezine dayanmaktadır.

Cystoseira barbata'nın biyostimülant potansiyelini değerlendirmek amacıyla elde edilen su, alkali ve asit yosun özütleri, sera koşullarında yetiştirilen tahıl (buğday) ve bahçe bitkilerine (domates, brokoli ve biber) uygulanmıştır. Uygulamalar, bitkiye özgü hedeflere dayanarak toprak uygulaması (drench) veya yaprak uygulaması (foliar) şeklinde gerçekleştirilmiştir. Her bir ürüne özgü hedeflere bağlı olarak, uygulamalar topraktan veya yaprak püskürtme olarak gerçekleştirilmiştir. Buğdayda, toprak uygulamaları ile biyostimülant etkileri ve kuraklık koşullarında fizyolojik ve büyüme tepkilerini hedeflerken, yaprak uygulamaları ile biyostimülantların büyüme, verim ve mineral elementlerdeki farklılıklar üzerindeki etkilerine odaklanmıştır. Domates, brokoli ve biberde farklı özüt tiplerinin meyve verimi ve kalitesi üzerindeki etkileri değerlendirilmiştir. Biber denemesinde ayrıca ticari bir deniz yosunu bazlı biyostimülant olan Acadian ile bir karşılaştırması yapılmış, böylece *C. barbata* kaynaklı formülasyonların etkileri referans bir ürün ile karşılaştırılmıştır.

Buğday bitkisinde, su ve asit özütlerinin toprak uygulaması, antioksidant aktiviteyi, osmolit birikimini ve rizosferdeki mikrobiyal aktiviteyi artırarak kuraklık koşullarında büyümeyi artırmıştır. Domates ve brokolide, asit ve alkali özütlerinin toprak uygulamaları verim ve kalite özelliklerini belirgin şekilde iyileştirmiş; toplam antioksidant aktivitesi, C vitamini konsantrasyonu ve glutatyon düzeylerinde artış sağlamıştır. Buğday ve domateste yaprak uygulamaları, toprak uygulamalarıyla karşılaştırıldığında verim üzerinde daha az belirgin etkiler gösterdiği ve mineral konsantrasyonlarında anlamlı değişiklik yaratmadığı gözlemlenmiştir. Bununla birlikte, toprak uygulamaları ile kükürt ve potasyum düzeylerinin arttığı tespit edilmiştir; bu durumun besin elementi alımının iyileşmesine bağlı olduğu düşünülmektedir. Su ve asit özütleri hem kontrol grubuna göre biber verimini artırmış hem de ticari biyostimülant olan Acadian ile benzer performans göstermiştir. Ayrıca bu bulgular, *C. barbata* bazlı formülasyonların ticari ürünlere benzer etkili ve yerel olarak temin edilebilir alternatifler olma potansiyelinin altını çizmektedir.

Bu tez, Marmara Denizi'nde bulunan bir deniz yosun türünün biyostimülant potansiyelinin deneysel olarak gösterildiği ilk çalışmalardan biri olma özelliği taşımaktadır. *Cystoseira barbata* kullanılarak biyobazlı ürünlerin geliştirilmesi, sürdürülebilir tarıma katkı sağlamanın yanı sıra denizlerde bulunan biyokütlenin ekonomik değeri yüksek ürünlere dönüştürülmesini sağlayarak döngüsel biyoekonomiyi teşvik etmektedir. Bununla birlikte, *C. barbata*'nın sürdürülebilir şekilde üretiminin standartlaştırılması ve biyostimülant etkilerinin tarla koşullarında doğrulanması için daha fazla araştırmaya ihtiyaç duyulmaktadır.

Anahtar Kelimeler: Biyostimülant, *Cystoseira barbata* (*Gongoloria barbata*), Yosun Özütü, Toprakta Yetişen, Sürdürülebilir Tarım, Besin Kalitesi

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LIST OF SYMBOLS AND ABBREVIATIONS

$(\text{CO}_3)^{-2}$	: Carbonate
(HCO_3^-)	: Bicarbonate
$(\text{Na})_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	: Sodium molybdate dihydrate
$(\text{SO}_4^-)_2$	: Sulphate
Abs	: Absorbance
ABTS	: 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonate)
ACC	: 1-aminocyclopropane-1-carboxylic acid
ANOVA	: Analysis of variance
APX	: Ascorbate peroxidase
AsA	: Ascorbic acid
<i>A. nodosum</i>	: <i>Ascophyllum nodosum</i>
AWCD	: Average well color development
B	: Boron
BHT	: Butylated hydroxytoluene
Ca	: Calcium
$\text{Ca}(\text{NO}_3)_2$	: Calcium nitrate
CAT	: Catalase
<i>C. barbata</i>	: <i>Cystoseira barbata</i>
Chl	: Chlorophyll
Cl^-	: Chloride
cm	: Centimeter
CO_2	: Carbon dioxide
Co	: Cobalt
Cu	: Copper
$\text{CuSO}_4 \cdot 5(\text{H}_2\text{O})$	: Copper sulphate pentahydrate
cv.	: Cultivar
Da	: Decare
DAS	: Days after sowing
DAP	: Days after planting
DF	: Degrees of freedom
dH_2O	: Distilled water
DPPH	: 2,2-diphenyl-1-picrylhydrazyl
DTT	: Thioreithol
DTNB	: 5,5'-dithio-bis (2-nitrobenzoic acid)
DW	: Dry weight
E	: Evenness index
EC	: Electrical Conductivity

<i>E. maxima</i>	: <i>Ecklonia maxima</i>
EtOH	: Ethanol
FAO	: Food and Agriculture Organization
Fe	: Iron
Fe ⁺²	: Ferrous iron
Fe ⁺³	: Ferric iron
Fe III-TPTZ	: Ferric-tripyridyltriazine
FeCl ₃ .6H ₂ O	: Ferric chloride
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'	: Shannon index
H ₂ O	: Water
H ₂ O ₂	: Hydrogen peroxide
H ₃ BO ₃	: Boric acid
HCl	: Hydrochloric acid
HI	: Harvest index
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-P	: Potassium phosphate
K ₂ SO ₄	: Potassium sulphate
K ₂ S ₂ O ₈	: Potassium persulfate
KOH	: Potassium hydroxide
KCl	: Potassium chloride
kg	: Kilogram
KH ₂ PO ₄	: Potassium dihydrogen phosphate
KI-I ₂	: Potassium iodide - Iodine
K-P	: Potassium phosphate buffer
l	: Liter

LSD	: Fisher's least significant difference
m	: Meter
MAE	: Microwave-assisted extraction
MDA	: Malondialdehyde
MetOH	Methanol
mg	: Miligram
Mg	: Magnesium
MgSO ₄	: Magnesium sulphate
min	: Minute
ml	: Milliliter
mM	: Millimolar
Mn	: Manganese
MnSO ₄ .H ₂ O	: Manganese sulphate monohydrate
N	: Nitrogen
<i>n.s.</i>	: not significant
Na	: Sodium
NaCl	: Sodium chloride
Na ₂ CO ₃	: Sodium carbonate
Na-EDTA	: Etilendiamintetrasetik asit disodyum
NaOH	: Sodium hydroxide
NADPH	: Nicotinamide adenine dinucleotide phosphate
NADPH-Na ₄	: Dihydronicotinamide adenine dinucleotide phosphate tetrasodium salt
NBT	: Nitro blue tetrazolium chloride
NH ₄ ⁺	: Ammonium
N	: Nitrogen
NEM	: N-ethylmaleimide
NO ₃ ⁻	: Nitrate
O ₂ ⁻	: Superoxide radical
OH ⁻	: Hydroxyl radical
P	: Phosphorus
prt.	: protein
PDB	: Plant derived biostimulants
PGPR	: Plant growth promoting bacteria
pH	: Power of hydrogen
PH	: Protein hydrolysates
PLE	: Pressurized liquid extraction
ppm	: parts per million
PH	: Protein hydrolysates
REL	: Relative electrolyte leakage

ROS	: Reactive oxygen species
S	: Sulfur
SAWCD	: Substrate average well color development
Se	: Selenium
SFE	: Supercritical fluid extraction
Si	: Silicon
SOD	: Superoxide dismutase
Sp	: Specific
SR	: Substrate richness
Seaweed extract	: SWE
TBA	: Thiobarbituric acid
TCA	: Trichloroacetic acid
TE	: Trolox equivalent
TPC	: Total phenolic content
TPTZ	: Tripyridyl triazine
Trolox	: 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid
WHC	: Water holding capacity
WHO	: World Health Organization
UAE	: Ultrasound-assisted extraction
UV-Vis	: Ultraviolet–visible
μM	: Mikro molar
μl	: Mikro liter
μS	: Mikro siemens
Zn	: Zinc
ZnSO ₄ ·7H ₂ O	: Zinc sulphate heptahydrate
w/v	: weight per volume

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1. INTRODUCTION

1.1. Global Problems

One of the biggest obstacles to overcome in the coming years will be sustainably meeting the nutritional needs of an expanding global population [Springmann et al., 2018]. The human population is expected to rise to 9.7 billion by 2050, potentially reaching approximately 11.2 billion by the 2100s [Web 1, 2024]. It is anticipated that the expansion of the global economy will result in an increase in income levels for the majority of the world's population, which will lead to an overall increase in food consumption as well [Clark et al., 2020]. Food security is defined as having access to food on a domestic, national, and international scale [Paudel et al., 2023]. It is a primary focus of the United Nations' Sustainable Development Goals, considering that nearly 2.4 billion people in urban and rural areas are classified as undernourished or as not having access to nutrient-rich foods [De Vries., 2021; Ashraf et al., 2024].

Global agricultural production has been challenged by the overexploitation of Earth's resources [Bisht and Chhabra, 2024]. The widespread use of chemicals and the high production of greenhouse gases have increased, leading to global warming and environmental pollution, which are primarily linked to climate change and have a direct impact on agriculture [Layek et al., 2018]. For example, modern farmers use huge amounts of fertilizers to achieve a high yield and quality of production, whether it can cause ecological problems or not [Sebilo et al., 2013; Mengistie et al., 2017]. Increasing the amount of nutrients in the soil through frequent fertilizer applications does not always lead to better-quality goods; rather, it may make them more susceptible to stress and environmental problems [Bulgari et al., 2015]. Furthermore, soil structure, deforestation, and unsustainable farming practices negatively impact agricultural lands, affecting about 23.2 million hectares in Türkiye [Web 2, 2022]. In the upcoming years, the switch to sustainable resources, such as moving from fossil fuel-based to bio-based production, is seen as essential due to the growing competitiveness in the agricultural goods industry [Clark et al., 2020].

A steady decline in precipitation across the world as a result of climate crisis is causing an increased incidence of drought stress, which is also negatively affecting agricultural yield and food security [Francini and Sebastiani., 2019]. Water scarcity is a critical factor in agricultural production and food security, as nearly 20% of the world's irrigated agricultural land provides 40% of the world's food [Molden et al., 2010; Hanjra and Qureshi., 2010]. Given to the vulnerability of the Mediterranean region to climatic crises like drought and extreme heat, together with the essential contribution of products grown locally to the region's nutrition, the challenges confronting agricultural production have become ever more apparent [Bonofiglio, 2022; Cos et al., 2022].

The convergence of consumer demand for sustainable, affordable and nutritious food with rising production costs has created a compelling economic motivation to reduce inputs and streamline agricultural production [Zulfiqar et al., 2020]. Furthermore, efforts should be made to increase the nutritional value of products in a sustainable manner [Scharff et al., 2022]. Malnutrition and hunger remain the greatest challenges with 733 million people worldwide classified as undernourished, meaning they are not getting enough calories [Hefferon, 2016; Web 3, 2023]. Moreover, people living in the countryside cannot reach the recommended daily fresh fruit intake which is essential for a healthy diet [Peltzer and Phaswana-Mafuya, 2012]. This is the reason why there is a notable rise in public awareness of foods that are rich in nutrients, have functional properties and are classified as nutraceuticals. [Farina et al., 2020]. Farmers seek out biological solutions to improve fruit quality, thereby producing the most nutritious and largest fruits that will attract consumers [El-Nemr et al., 2012]. In recent years, various technologies have emerged to improve the productivity and quality metrics of crop production with the aim of diminishing dependence on fertilizers and pesticides and to offer innovative alternatives [Calvo et al., 2014; Punia et al., 2020]. It is as important to improve the quality of agricultural products as it is to increase harvest yields, so conventional techniques should be replaced by sustainable methods that maintain product yield and quality while being environmentally friendly [Sahota et al., 2009; Mannino et al., 2020]. Due to the reasons explained above, there is a pressing need to discover safe, eco-friendly, and more powerful sustainable solutions in modern and sustainable agricultural practices [Shubha et al., 2017].

1.2. Biostimulants

Biostimulants refer to specific chemicals or mixtures of substances that improve mineral efficiency, mitigate stress conditions, and enhance quality traits of crops [du Jardin et al., 2015]. Regulation of European Union 2019/1009 defines biostimulants as products that, irrespective of their nutrient content, stimulate plant nutritional processes with the following attributes of the plant or rhizosphere: increase in mineral nutrient utilization efficiency, biotic and abiotic stress tolerance, quality characteristics and accessibility of restricted mineral nutrients in soil are influenced by the root system [Web 4, 2019]. Plant biostimulants have attracted major interest from farmers, agricultural companies, and plant scientists seeking to develop more sustainable crop solutions [Rouphael and Colla., 2020]. The updated definition regards biostimulants as additional regulatory agents of plant metabolism that enhance the effects of other formulations used in agriculture, and their biologically active effects can be explained with simply nutrient absorption and hormonal effects [du Jardin et al., 2020]. Biostimulants can be applied to foliage, delivered to the root zone, or used in combination to enhance the plant's physiological and morphological functions [Yakhin et al., 2017].

The biostimulants can be categorized into seven distinct groups: seaweed extracts (SWE), plant biostimulants, chitosan, humic acid, fulvic acid, inorganics, and bioactive microorganisms [du Jardin., 2015; Xu et al., 2018]. Biostimulants can originate from natural [amino acids, humic and fulvic substances, plant or algae extracts, chitin, chitosan, or beneficial microbes] or synthetic [growth regulators, phenolic compounds, inorganic salts, and beneficial nutrients] sources and contain organic and inorganic compounds [Calvo et al., 2014]. Biostimulants can affect soil nutrient availability and uptake by enhancing soil structure, increasing soil exchange capacity and pH, improving mineral solubility, and reducing mineral leaching [Rouphael and Colla, 2018].

Biostimulants can be produced in liquid solution, powder, or granule form and applied directly to the soil on an agricultural scale, either as a spray on the leaf or as a seed application [Kocira et al., 2018]. Soil-applied biostimulants improve root properties and increase nutrient absorption, whereas foliar applications protect the plant from stress; however, plants' circadian clock should be considered, and applications should

be made when stomata are open and assimilation rates are highest [**Battacharyya et al., 2015** **Goñi et al., 2018**].

Biostimulatory agents are considered environmentally friendly due to their minimal adverse effects and their ability to enhance crop yields, making them an attractive option for farmers [**Álvarez-Gil et al., 2023**]. Biostimulants as commercial products represent a significant segment of the global agricultural economy, reflecting upward trends [**Sun et al., 2023**]. Biostimulants can provide economic benefits by promoting more efficient water use, enhancing productivity and crop quality, improving stress tolerance, and supporting post-harvest handling [**Salvage et al., 2024**]. As a nascent and rapidly expanding market, the global trade in plant biostimulants is projected to reach 3.8 billion USD in 2023, with an anticipated annual growth rate of 11%, reaching approximately 8.0 billion USD by 2030 [**Web 5, 2024**]. For example, Stimulate[®], a commercial biostimulant that affects plant metabolic pathways, increases carbon assimilation, stomatal conductance, root development and maintains soil water-holding capacity [**Li et al., 2019**]. Conducting comprehensive investigations is essential to determine the financial feasibility and potential market size of biostimulant treatments [**Mughunth et al., 2024**].

1.2.1. Humic Substances

Among biostimulants, humic compounds, consisting of humic acid (HA), fulvic acid (FA), and ulmic acid (UA), have been found to boost plant growth, production, and nutritional value while also making them more resistant to environmental stress [**Rathor et al., 2024**]. Humic substances are organics produced in soil by microbial metabolism as well as through the chemical and biological transformations of organic residues [**Canellas et al., 2015**], including peptides, carbohydrates, lipids, aromatic and aliphatic rings [**Schiavon et al., 2010**]. Numerous studies indicate that humic substances derived from various sources enhance plant growth and yield across diverse species, including tomato, maize [**Malik et al., 2023**], wheat [**Zhang et al., 2024**], and rice [**Rathor et al., 2024**].

1.2.2. Protein Hydrolysates

Protein hydrolysates (PH) represent a further significant category of biostimulant, consisting of products derived through chemical or enzymatic processes involving the breakdown of peptide bonds in proteins, resulting in the generation of bioactive peptides with varying sizes and free amino acids [Schaafsma, 2009; Liceaga and Hall, 2019]. Modifications to protein structures have been demonstrated to influence their physical and chemical characteristics, as well as their biological functionality [Gupta et al., 2021]. Protein hydrolysates are predominantly generated through the biological or chemical decomposition of proteins present in agricultural waste derived from plants or animals, in addition to protein-rich crops such as legumes [Colla et al., 2015]. They can indirectly influence a plant by modifying the microbiome, supplying an optimal nutrient source for microbes, altering the plant's hormonal profile, or affecting its utilization of carbon and nitrogen (N) [Colla et al., 2017].

1.2.3. Microbial Fertilizers

Biofertilizers, also known as bioinoculants, are beneficial microorganisms that can be applied to plant organs or directly to the soil and stimulate plant growth through a diverse range of mechanisms, including boosting plant growth parameters, providing mineral nutrients, and enhancing nutrient uptake [Vessey, 2003; Sun and Shahrajabian, 2023]. Various types of plant growth-promoting bacteria (PGPR), such as *Bacillus*, *Arthrobacter*, *Pseudomonas*, *Azospirillum*, and *Rhizobium*, can be considered microbial biostimulants, offering an advantageous and cost-effective solution in agricultural systems [Kaushal et al., 2023]. Microbial fertilizers enhance plant growth by solubilising essential minerals, increasing N-fixation, supplying plant hormones, additional metabolites, enzymatic agents, volatile inorganic and organic substances, sugars, terpenoids etc. [Lephatsi et al., 2021]. They can modulate the physiological performance of plants by colonising aboveground parts of plants, by growing in tissues or by interacting in the rhizosphere [Abou Jaoudé et al., 2024]. PGPRs modify their environment by initiating metabolic processes that break down organic residues in the soil and solubilise essential minerals for plants [Priyanka et al., 2020]. Coating maize seeds with a *Pseudomonas* bacterial suspension enhances growth and development while significantly improving the absorption of mineral

nutrients such as N and phosphorus (P) compared to the prescribed chemical fertilizer usage [Adoko et al., 2022]. The application of *Bacillus subtilis* as rhizobacteria altered the composition of the soil's microbial community and soil characteristics and exhibited anti-pathogenic effects, enhancing organic compounds, and enhancing root growth of cigar tobacco seedlings. [Shang et al., 2023].

1.2.4. Plant Derived Biostimulants

Plant derived biostimulants (PDB) consist of plant secondary products, entire plants or certain organs, isolated compounds and cell cultures, together with plant growth promoters such as phytohormones, proteins, and minerals. It may be used alone or in admixture with other active ingredients [Zulfiqar et al., 2020; Martínez-Lorente et al., 2024]. Moringa leaf extract, as an example of PDB, positively affected growth and yield, as well as nutritional quality, and the stimulating effect was attributed to the phytohormonal activity of the extract [Elzaawely et al., 2017]. Willow extracts can improve early growth and salinity stress tolerance in maize through biostimulatory effects derived from the rich bioactive ingredients such as salicylic acid, salicin, beneficial salicylates and phenolics [Mutlu-Durak and Kutman, 2021]. Commercial plant-based biostimulants have been shown to enhance photosynthesis, optimize N and P levels, increase yields, and mitigate stress through osmotic modification and the synthesis of sterols, aromatic compounds, and glucosinolates [Lucini et al., 2015].

1.2.5. Inorganics

These are some beneficial elements that plants need but not essential in order to grow and develop: aluminum (Al), cobalt (Co), sodium (Na), selenium (Se), and silicon (Si). They are reported to stimulate plants handle stress better, grow vigorous, and mitigate toxicity [Pilon-Smits et al., 2009]. These chemical elements can have fundamental effects, such as the deposition of silica in the cell wall for resistance to stress conditions [Franzoni et al., 2022]. Silicon can act as a signaling compound and trigger the stress response in some plants by activating defense enzymes such as peroxidases and increasing phenolics against microbial compounds, as well as elicitors such as salicylic acid [Pilon-Smits et al., 2009]. It's also been reported that the biostimulant activities of Se are most likely achieved through increased synthesis of proteins with catalytic

or non-catalytic function, phenolic components, glucosinolates, several vitamins and pigments, as well as metabolism of associated sulfur and N [Medrano-Macías and Narvaéz-Ortiz., 2022].

1.2.6. Seaweed Based Biostimulants

Seaweeds are macroscopic marine algae that comprise about 20,000 species globally and used in food, cosmetic, animal feed, and agricultural industries thanks to their rich content [Rao et al., 2018]. Seaweeds are photo-assimilating organisms that contribute to nearly half of the world's photosynthesis [Lowe et al. 2017]; Seaweeds are photo-assimilating organisms that contribute to nearly half of the world's photosynthesis [Lowe et al. 2017]; as soon as sunlight can reach them, can sustain their proliferation [Khan et al. 2009; Nature et al. 2013]. They are also recognised as species that can adapt to extreme habitats, such as extreme temperatures, toxic gases, etc. [Craigie, 2011]. Seaweeds are classified into three primary groups: Chlorophyceae as green algae, Phaeophyceae as brown algae, and Rhodophyceae as red algae, based on criteria including color, cell architecture, and other distinctive attributes [Joshi et al., 2015]. Brown algae possess the second-largest biomass among all algal types globally and can grow to lengths of almost forty-five meters [Khan et al., 2009]. Marine algae contain many beneficial nutrients, including nitrogenous compounds, proteins, carbohydrates, enzymes, and beneficial fatty acids [Okolie et al., 2018]. They produce a variety of stress-related compounds to cope with different stress factors throughout their life cycle [Shukla et al., 2016], making seaweeds useful plant biostimulants to boost crop production for their valuable content [Khan et al., 2009]. Seaweeds possess a broad array of phenolics, carbohydrates, enzymes, vitamins, vital minerals, lipids, and plant hormones, including cytokinin and auxin, and their extracts promote crop growth, productivity, and physiological activities due to this complex composition of bioactive substances [Cragie, 2011; Pereira et al., 2020; Villa e Vila et al., 2023].

Seaweed extracts, a type of plant biostimulant, can cause a variety of physiological reactions in plants, including increased growth, improved blooming and production, quality, and shelf life; they are utilized far more frequently [Battacharyya et al., 2015]. They have positive effects on the water holding capacity (WHC) of soil, remediation, and soil microbiota, as well as acting as a source of essential minerals and having hormonal-like effects [du Jardin et al., 2015]. The results of previous

studies indicate that the application of brown SWE has a positive effect on the vegetative development and fruit yield of tomato and pepper plants, as well as on the beneficial bacteria population in the rhizosphere [Renaut et al., 2019]. The drench application of SWE resulted in an increase in the number of leaves, the diameter of the stem, and the total leaf area of broccoli, primarily due to differences in nutrients availability, mobility and the induction of production of organic compounds in the soil [Mattner et al., 2013]. The foliar application of an *Ascophyllum nodosum* (*A. nodosum*) based biostimulant has been demonstrated to increase the expression of related genes, which in turn leads to the production of phytohormones. This resulted in enhanced growth parameters, including plant height, leaf number, dry weight, and fruit yield in pepper crops [Ali et al., 2019].

The macroalgae sector's total production of approximately 32 million tons has the potential to generate \$13.3 billion, establishing the global seaweed sector as a multi-billion-dollar industry [Blikra et al., 2021]. Seaweed extracts represent approximately one-third of the global marketplace for biostimulants [Eef et al., 2018]. To give an example, the majority of commercially available SWE with biostimulant properties are derived from brown algae, particularly *A. nodosum*, which has been the subject of extensive research [Craigie et al., 2011].

The application method used for SWEs, whether through irrigation, soil injection, or drip fertigation, significantly influences how well it works and how plants react [Ali et al., 2021a].

1.2.6.1. Biologically Stimulatory Activities and the Mechanisms of Actions

Seaweed-based biostimulants have been shown to increase mineral nutrient uptake and utilization, as well as crop growth and vigor [Ali et al., 2021a]. The application of brown SWE to rapeseed plants has been observed to induce N and sulfur (S) assimilation, as evidenced by the upregulation of genes involved in the uptake of N and S from the rhizosphere [Billard et al., 2014]. The use of brown SWE led to improved grain yield of wheat and increased uptake of N, P and potassium (K) [Stamatiadis et al., 2015]. Applying SWE to spinach has been demonstrated to elevated K and Mg levels through up-regulation of genes coding for membranes uptake of nutrients, the improving of the root system, and the expression of signaling

compounds [Ali et al., 2021a]. The use of commercial brown SWE increases the production of key photosynthetic enzymes, including Rubisco, which in turn increases nitrogen uptake from the roots and its utilization in the plant [Jannin et al., 2013]

The application of SWE, whether via drench or foliar, has been demonstrated to exert a beneficial modulating effect on the microbiome of plants and soil, which may in turn facilitate enhanced plant growth [Ali et al., 2021a]. A broad spectrum of bacteria engages in enhanced mineral nutrient availability, breaks down organic substances in the soil, and improves soil structure and its water retention capacity [Singh et al., 2011]. The application of brown SWE to tomato found to enhance the abundance of beneficial bacteria, available N, and the diversity of the microbiome [Hussain et al., 2021]. This suggests that organic matter can be metabolized and facilitates the binding of minerals and colloids with the assistance of different microbiome [Neal et al., 2020]. The exploitation and management of soil composition and physico-chemical properties can be optimized to enhance crop output, thereby facilitating the implementation of sustainable agricultural practices [Singh et al., 2011]. Applying brown SWE to carrot plants promotes yield and root volume, attributed to elevated microbial activity and diversity in the rhizosphere, which provides an energy source for microorganisms [Alam et al., 2014].

Alginate, laminarin, and fucoidan make up the rich carbohydrates profile of brown macroalgae [Goñi et al., 2020]. Complex carbohydrates such as laminarin, degrade over time into smaller molecules such as glucose, providing resources for more durable or slower developing microbiota [Yu et al., 2022]. Hydrophilic colloids and the network of cross-linked polymers present in SWEs retain water in the soil, increasing its hydraulic capacity and improving aggregation stability [Battacharyya et al., 2015; Hellequin et al., 2020]. Polysaccharide extracted from a brown seaweed was found to include sugar monomers of mannose, fucose, and xylose and to demonstrate tolerance under saline conditions due to their lower molecular weight and higher sulfate content [Zou et al., 2019]. In addition, seaweed alginate helps to chelate iron (Fe) by acidifying the rhizosphere, facilitating its mobilization by plants, thus showing potential as a biostimulant [Spinelli et al., 2010].

Osmotic substances such as betaines and their counterparts are crucial in regulating water pressure in cells, preventing mineral toxicity and dehydration of cellular organelles, and have also been identified in brown SWEs [Battacharyya et al., 2015;

Singh et al., 2022]. The application of brown SWE to spinach increases biomass through enhanced expression of genes related to N-assimilation and elevated chlorophyll content linked to the upregulation of genes in the betaine production pathway **[Fan et al., 2013; El Boukhari et al., 2020]**. The biostimulant effect of foliarly applied SWE on wheat is associated with the presence of phenolics, antioxidants, and compatible solutes such as amino acids and ammonium compounds **[Ali et al., 2022]**.

Phytohormones and plant growth regulators, including jasmonic and salicylic acids found in seaweed, are a variety of biologically active ingredients that promote flowering, fruit, and vegetative growth **[Stirk and van Staden, 2014; Araújo et al., 2021]**. The application of *Ecklonia maxima* (*E. maxima*) extracts led to an increased accumulation of plant hormones in spinach **[Digruher et al., 2018]** and boosted shoot growth in willow plants, together with an elevated endogenous phytohormone reserve **[Nanda et al., 2022]**. In addition, brown seaweed has been shown to produce ethylene, with its precursors, 1-aminocyclopropane-1-carboxylic acid (ACC), identified in the extracts **[Broadgate et al., 2004]**. Cytokinin-like activity has been observed in the seaweed, associated with growth circumstances and developmental stages **[Stirk and van Staden, 2014]**. Plants exposed to seaweed-based biostimulants, as well as the seaweeds themselves and their extracts, exhibit cytokinin and auxin-like substances and similar effects **[Crouch and Van Staden., 1993]**. The mode of action of brown SWEs is based on the regulating of phytohormones, which control the induction of expression of genes involved in the production of bioactive compounds, the transmission of hormonal signals and the production of certain enzymes involved in the plant's defense system **[Villa e Vila et al., 2023]**.

1.2.6.2. Seaweed Extraction Methods

Technologies utilized for SWE should effectively manage the crude seaweed complex while maintaining the integrity of the bioactive components **[Ali et al., 2021b]**. The diverse characteristics of the ingredients within the mixtures of biostimulant components make the modes of action mainly ambiguous, making it difficult to identify the exact components responsible for the biological activity in plants **[Yakhin et al., 2017]**. It was listed that biochemical substances such as sugars, N-containing compounds, lipids, several pigments, phenolics, vitamins, mineral nutrients,

antioxidants, and hormone-like activities are present in SWEs in lots of studies [Khan et al., 2009; Kadam et al., 2010; Craigie et al., 2011; Michalak and Chojnacka, 2015; Yakhin et al., 2017].

Water is the primary agent in extractions of living organisms, offering numerous advantages such as simplicity, sustainability, ability to rapid environmental degradability, and nontoxicity [Jang and Kuk, 2019]. Dried seaweed mass can be extracted with water to produce a potent biostimulant with high phytohormonal activity [Sharma et al., 2014]. Algal proteins can be extracted using aqueous, acidic, and alkali methods [Bleakley and Hayes, 2017]. Brown SWEs that undergo acid hydrolysis generate sulfated polysaccharides such as fucose [Ale et al., 2012; Flórez-Fernández et al., 2018]. Pre-treating seaweed with sulfuric acid at 45°C for half an hour removes phenolic compounds and increases the breakdown of polymers to make alkali extraction efficient, while calcium alginate is converted to alginic acid, which KOH can better extract [Shukla et al., 2019]. Polysaccharides such as alginate oligosaccharides and polyphenols extracted from macroalgae have been shown in certain studies to act as elicitors [Khan et al., 2012]. The alkali extraction method can yield substances through the processes of breaking, remodeling, condensing, and catalyzing reactions that are not inherently present in the first version of seaweeds [Baardseth et al., 1970; Dierick et al., 2009]. Alkali extraction consists of initiating the process under 70–100 °C with brown algae under basic conditions to break down complex carbohydrates into smaller molecules and generate new compounds [Craigie, 2011; Shukla et al., 2019].

New extraction methods, like ultrasound-assisted extraction, enzyme-assisted extraction, microwave-assisted extraction, supercritical fluid extraction, and pressurized liquid extraction, make it possible to get bioactive compounds without changing how they work [Michalak and Chojnacka, 2015]. Microwave-assisted extraction is an innovative, environmentally friendly technique for seaweed extraction that uses radiation to break hydrogen bonds, allowing ionic movement and increasing solvent penetration into the matrices for the extraction of fatty acids, pigments such as carotenoids, and phenolic compounds [Michalak and Chojnacka, 2015]. High-frequency ultrasound waves spread through various media by accelerating mass transfer and the release of biologically active compounds like laminarin from brown seaweed in the extraction solution [Kadam et al., 2013; Kadam et al., 2015]. This

method has been effectively utilized to isolate chlorophyll, carotenoids, phenolic ingredients, and polysaccharides from several seaweed varieties [Carreira-Casais et al., 2021]. Enzyme-assisted extraction is a technique that is both gentle and eco-friendly, as it releases the structures inside the cell by disrupting the membrane components; nevertheless, it takes more time comparing other extractions [Kumar et al., 2017a]. This technology can be used to produce a biostimulant from seaweed in a sustainable manner, preserving complex carbohydrates from breaking down and including a high concentration of proteins and sulfated polysaccharides [Spain et al., 2024]. Supercritical fluid extraction appears to be a promising method for the extraction of various bioactive ingredients from seaweed biomass collected from the sea in a condensed and biologically non-destructive form, such as plant hormone-rich biostimulants [Michalak et al., 2016]. Pigments, polyunsaturated fatty acids, and phenolics can be extracted by utilizing SFE in seaweeds [Mena-García et al., 2019]. The growing interest in SFE is due to the fact that the resulting extract is a concentrated form of biologically active compounds, produced in a non-solvent environment [Joanna Gil-Chávez et al., 2013]. Pressurized liquid extraction, which is based on a solid matrix in a liquid medium, has the ability to extract a wide range of organic pollutants from biological organisms using organic solvents [Usenko et al., 2013]. This ecologically safe approach effectively extracts a range of biologically active components, including oligosaccharides, polypeptides, and fatty acids, with high efficiency [Perez-Vazquez et al., 2023]. It was successfully used to extract fucoidan, sulfated polysaccharide, from brown seaweed species [Saravana et al., 2016].

1.3. *Cystoseira barbata*

The brown algal genus *Sargassum* contains 360 species, which belong to the class Phaeophyceae, order Fucales, and family *Sargassaceae*, mostly found in the Mediterranean, Marmara, and Aegean Seas in Türkiye, as well as in the Atlantic, Pacific, and Indian Seas [Yip et al., 2020; Guiry and Guiry., 2021; Web 6, 2024].

The genus *Cystoseira* is endemic organisms in the rocky reef in the coastal zone of the Mediterranean Sea [Falace and Bressan, 2006; Taşkin et al., 2012]. *Cystoseira barbata*, commonly known as *Gongolaria barbata*, is a perennial brown alga that lives in the shallow seas (0.5–1 m depth) in the Mediterranean Sea. It is classified under the

genus *Cystoseira* sensu lato (the *Cystoseira* complex) under the *Sargassaceae* family. [Savonitto et al., 2021; Blanford et al., 2025].

Many important biologically active chemicals such as terpenoids, fatty acids, glycerols, tannins, steroids, and long chains of carbohydrates have been detected in various *Cystoseira* species [de Sousa et al. 2019]. Alginate has been identified in *C. barbata* obtained from the Black Sea [Trica et al., 2019]. *C. barbata* extract, which is rich in polysaccharides, proteins, sulfated compounds, and phenolics, can detoxify ROS and chelate minerals [Sellimi et al., 2017]. Laminaran extracted from *C. barbata* has been shown to have antioxidant and antibacterial activities [Sellimi et al., 2018]. The massive biomass of seaweeds present in marine environments can be utilized in the manufacturing of growth-promoting biostimulants in agriculture [Calvo et al., 2014].

1.4. Drought Stress

Drought is a widespread environmental risk that can disrupt the water cycle, reduce water availability, and have detrimental effects on the environment, national economies and global community [Li et al., 2022a; Xiang et al., 2023]. The effects of climate change have resulted in extreme temperatures and imbalances in precipitation, leading to instability in agricultural yields and a 21% decline in total agricultural production since 1961 [Ortiz-Bobea et al., 2021]. It can be detrimental if water availability is halved due to droughts around the world, which could reduce wheat production by up to 40% and maize by 20% [Daryanto et al., 2016]. The lack of rainfall in the soil, which leads to agricultural drought, is a significant impediment to crop productivity [Kumar et al. 2017b]. Crop production is becoming more vulnerable to severe water stress as unprecedented droughts intensify due to global climate change [Trivedi et al., 2018]. In response to a water deficit, plants initially restrict growth, with a greater impact on shoot growth and a lesser impact on root growth, which is followed by an increase in stomata closure. As a result, transpiration and photosynthesis rates are restricted, limiting carbon assimilation [Navarro-Morillo et al., 2024]. The formation of reactive oxygen species (ROS) as a result of secondary stress of water deficit have detrimental effects on biological components like nucleic acids, peptides, fatty acids etc. [Zhang et al., 2022].

Incorporating biostimulants into agricultural systems is a sustainable approach to managing plant growth, particularly in the face of ongoing stresses caused by climate change [Bisht and Chhabra, 2024]. The beneficial biostimulatory effects may result from the activation of a complicated signaling cascade that produces appropriate responses to stress conditions [Drobek et al., 2019]. The application of biostimulants can induce plant responses by activating the antioxidant defense system or enhancing the production of osmoregulatory under various abiotic stress conditions [du Jardin, 2015; Zulfikar and Ashraf, 2021]. Plants have two different systems for dealing with oxidative stress; one is an enzymatic antioxidant response that prevents the accumulation of ROS by reducing them, and the other is a nonenzymatic compound that protects photosynthetic membranes [Impa et al., 2012]. Various biostimulant agents have also been shown to alter drought stress conditions by improving photosynthesis, decreasing stomatal closure, and lowering ROS and ethylene production. This is due to increased production of non-enzymatic antioxidants like proline and carotenoids [Navarro-Morillo et al., 2024]. In other plants, biostimulants exhibited multiple mechanisms to mitigate water stress, such as restoration of foliar gas exchange, increased stomatal number, reduced peroxide levels, increased catalase (CAT) production, increased non-enzymatic antioxidants, and also increased growth rate and yield [Agliassa et al., 2021]. Research has also shown that the use of biostimulants reduces the activity of antioxidants such as CAT, peroxide (POD) and superoxide dismutase (SOD) in tomato seedlings suffering secondary oxidative stress due to drought. This in turn helps to mitigate ROS generation, ultimately reducing the loss of membrane integrity [Turan et al., 2023].

Stomatal regulation involves assessing the extent of the aperture of stomatal in relation to water availability, a crucial response of plants subjected to drought stress [Diatta et al., 2021]. The acidic extract of brown seaweed has been reported to reduce stomatal carbon dioxide transport by downregulating the expression of key genes [Santaniello et al., 2017]. Brown SWE has been shown to improve soybean resistance to drought stress by regulating cell turgor pressure, optimising stomatal closure and upregulating drought-related gene expression [Martynenko et al., 2016]. Plants treated with *Ascophyllum nodosum* extract had higher water status in cells and increased gas exchange through stomata. The increase in scavenging ROS activity was also found to be higher in the treated plants. [Shukla et al., 2018]. The scarcity of water has an

impact on carbon assimilation, which is influenced by alterations to the photosynthetic pigments and the associated enzymes involved in this process [Bakry et al., 2019]. The application of SWE to rice subjected to drought stress resulted in enhanced plant growth and the regulation of chlorophyll concentrations, osmolytes, hydrogen peroxide (H₂O₂), lipid peroxidation as well as phenolics in the rice [Alshegaihi and Helal, 2024]. The application of brown SWE to maize plants, due to its beneficial ingredients, may confer a yield benefit under drought conditions, as indicated by increased chlorophyll content, carbon assimilation, and less photoinhibition resulting from improved protection against ROS [Trivedi et al., 2018]. Seaweed-based biostimulants have been shown to alleviate severe abiotic stress by improving root structure, accumulating functional sugars that serve as energy stores, accelerating metabolic pathways, providing osmoregulation, and producing compatible solutes [Dalal et al., 2019]. In addition, the levels of osmolytes were significantly higher in the seaweed-treated tomato plants, as was the up-regulation of related genes encoding dehydrin proteins, which are also involved in osmoregulation [Goñi et al., 2018]. Seaweed extracts can modify plant physiological processes by improving osmoregulation, root morphology and development, ultimately leading to increased crop yield [Salvi et al. 2019; Petropoulos et al., 2020]. The deleterious effects of water limitations have been mitigated by the application of brown SWE, which enhances the concentration of compatible solutes, including carbohydrates and soluble proteins, in wheat [Alharbi et al., 2022].

1.5. Quality

Yield is defined as the number of edible parts of plants produced from a given area of agricultural land and can be affected by the type of biostimulant used, the method of its application, as well as the plant species [Milić et al., 2018]. An increase in yield parameters is also linked to an improvement in quality [Drobek et al., 2019]. The quality of crops is determined by both organic and inorganic nutritional content, alongside agronomic traits including yield, fruit weight, stress resistance, and organoleptic attributes such as shape, hue, and texture [Di Vittori et al., 2018]. Once the plant has reached a sufficient level of growth, completed its developmental phase, and achieved a stable metabolic rate, it will begin to accumulate nutrients in its reserves, which in turn define the quality of the products that it will yield [Pask et al.,

2012]. Seeds determine yield parameters for some crops and are main accumulators of nutrients, so the filling stage affects both grain size and nutritional quality [**Reynolds et al., 2021; Houshmand et al., 2022; Burgess et al., 2023**]. Grain weight and size, starch, protein, fatty acids and fiber composition determine the quality and economic value of cereals [**Jayas and Singh 2012**].

The assessment of quality is of paramount importance in these processes, which include sink-source transport in plants, the transfer of photosynthetic products to consumable organs, the optimization of metabolic activities, and the remobilization of nutrients [**Havé et al., 2017**]. The growth and development of plant organs is also dependent on several internal and external factors, including the amount of light, the transport of carbohydrates, the availability of water and essential minerals [**Hilty et al., 2021**]. For example, the fruit's quality and marketability can be affected by nitrogen content, which contributes to plant growth and development by participating in photosynthesis and building blocks of nucleic acids and proteins [**Jämtgård et al., 2010**].

Biostimulant applications increase yield and stress resilience while improving the nutritional and nutraceutical quality of fruits, facilitating accelerated ripening and enhanced marketability [**Mannino et al., 2020; Tejada Moral et al., 2018**]. The shape characteristics of fruit, such as length and diameter, as well as their color, which appeals to consumers, can be altered by biostimulant applications [**Tarantino et al., 2018**]. Furthermore, the flavor and taste of the fruit can be enhanced by improving parameters such as dry matter content, fruit acidity, nutritional value, brix scores which define soluble solutes, and other chemical constituents through the application of biostimulants [**Grajkowski and Ochmian, 2007**]. Biostimulants affect fruit firmness by strengthening cell walls and reducing extensibility; increased cell wall flexibility also contributes to longer shelf life and easier storage [**Tarantino et al., 2018**]. Seaweed based biostimulant treatment to potato has been reported to increase quality traits via mineral nutrient uptake and increases vitamin C content and decreases sugar ingredients, which gives them an economic advantage [**Garai et al., 2021**]. As another example, brown SWE applied to strawberries has been shown to improve fruit quality by increasing soluble sugars, organic acids and carbohydrates, as well as increasing nutraceutical compounds such as quercetin, which is known to promote human health [**Kapur et al., 2018**].

The impact of stress conditions on plant yield is direct, as the plant's available reserves are allocated to coping with the stress, thereby limiting the potential for yield and quality increase [Drobek et al., 2019]. Drought stress reduces the viability of pollen grain and adversely affects flower pollination, leading to decrease nectar production, delayed seed formation, and the generation of smaller, lower-quality seeds [Alqudah et al., 2011]. The effects of drought, particularly during the grain filling period, have been observed to reduce the high starch content of cereals [Wang and Frei, 2011]. The negative effects of drought also vary according to the developmental stage of the plant, e.g. during the reproductive stage it directly impairs seed formation and causes seed desiccation [Fábián et al., 2011]. Drought stress diminishes the carbon assimilation capacity, resulting in modified sugar synthesis and translocation; hence, abbreviating the grain filling duration and compromising grain quality [Sehgal et al., 2018]. It has been reported that levels of K, an important mineral for fruit flesh and firmness, have decreased in banana plants because drought also reduces mineral uptake [Martínez-Ballesta et al., 2010].

Harvest success, both in terms of yield and quality, is dependent on how well plants use the nutrients that are already in the soil [Ostmeyer et al., 2020]. For instance, low S levels have an impact on the production of storage proteins and a reduction in the quality of wheat [Zhao et al., 1999]. Sulfur-containing metabolites, known for their aromatic properties and defined quality in fruits, are abundant in the Brassicaceae family [Rengel et al., 2022]. N fertilization not only optimized soluble sugar concentration and acidity but also enhanced tomato output and size of fruit [Kuscu et al., 2014]. K fertilization increased total and marketable tomato yield, with better color and was found to mitigate the effects of biotic stress under field conditions [Hartz et al., 2005]. Elevated levels of organic acids and their anions contribute to the acidity of fruits, a key flavor attribute and quality parameter that can be affected by K concentration [Villette et al., 2020]. The increased K content in tomatoes correlates with elevated levels of sugars and compatible solutes, which contribute to enhanced water retention and firmness in fruit [Chapagain and Wiesman, 2004; Rengel et al., 2022]. Calcium, as an essential mineral, plays a critical role in the cell wall by facilitating the formation of cross-linking pectin, which contribute to the stiffness and overall quality of the fruit [Martins et al., 2020].

1.6. Wheat

Wheat (*Triticum aestivum* L.) is a staple crop belonging to the grass family of Poaceae, grown for grain in both the spring and winter growing seasons [Asseng et al., 2018]. Worldwide, there are 219 million hectares of wheat under cultivation, and in Türkiye alone there are 6.6 million hectares [Web 2, 2022]; thus, in order to feed the growing number of people, it is necessary to increase grain yield while simultaneously enhancing grain quality [Curtis and Halford et al., 2014]. Wheat provides about twenty percent of the average daily calories and protein intake worldwide, and in certain regions, this contribution can be as high as seventy percent [Poursarebani et al., 2014; Timmer 2014; Cakmak and Kutman, 2018]. According to reports from 2017, the world's consumption of wheat and its products contributed 16 g of protein every day, equivalent to 65 g of food consumption per individual annually [Poutanen et al., 2022]. Wheat is one of the most important staple crops in Türkiye, in terms of its national economy, nutrition and workforce, and plays a critical role in global food security along with other major grains [Altay, 2012].

While the majority of durum wheat is cultivated under rainfed circumstances, water requirements escalate, particularly during anthesis, making irrigation optimization essential for attaining greater yields [Khadka et al., 2020; De Santis et al., 2021]. Plants growth and yield parameters, morphology, physiological and biochemical processes are seriously affected from water limited conditions [Grosse-Heilmann et al., 2024]. Recent years have demonstrated that rising average temperatures and their adverse stress factors, such as limited water availability, rising CO₂ concentrations and agricultural practices, are already having a detrimental effect on global wheat growth and yields [De vita et al., 2019; Grosse-Heilmann et al., 2024]. It is anticipated that a one-degree of Celsius rise in a day average temperature could lead to a rate of 2.5 reduction in durum yield [Cetin et al., 2022]. Rain-fed agriculture is utilized for spring wheat, the primary food supply in the world's poorest region, and constitutes a significant share of worldwide wheat production [Braun et al., 2010; Asseng et al., 2017]. Furthermore, it has been documented that drought-related yield losses account for up to 40% of the global reduction in wheat yield, with other abiotic stress factors also contributing to this decline [Daryanto et al., 2016; Fahad et al. 2017]. In addition, the prolonged war between Russia and Ukraine has also reduced accessibility

of wheat, potentially putting an additional 19 million people at risk of severe hunger by 2023, in addition to the 193 million people who are already facing food security challenges [Web 7, 2022]. The present and anticipated demographic and economic expansion indicate that global demand for wheat will persistently rise in the coming years [Tilman et al., 2011].

1.7. Tomato

Tomato (*Solanum lycopersicum* L.), a member of the Solanaceae family, is a widely consumed, and economically valuable fruit, rich in nutrients and cultivated all over the world [Campobenedetto et al., 2021; Subramaniyan et al., 2023]. Tomatoes have a global annual production of approximately 182 million metric tons [Mzibra et al., 2021]. With approximately 13 million metric tons, Türkiye holds the fourth position in the world's vegetable production, accounting for 50% of the total fruit production. [Web 2, 2022]. It is required to optimize nutrient management practices, drastically reduce our environmental footprint and increase agricultural output to ensure future food security and sustainability on a global scale [Wang et al., 2020].

The nutritional value of tomatoes is due to their high content of carbohydrates, soluble sugars, dietary fiber, vitamins, proteins and lipids [Liava et al., 2023]. Tomatoes contain high concentrations of antioxidants including polyphenolic compounds, carotenes and their derivatives, which have anti-cancer and anti-infective properties, and help in the treatment of autoimmune diseases, benefiting human health [Ali et al., 2020]. Sweetness degrees and alterations in the pH of fruits are two major factors that affect flavor and taste [Hudson et al., 2018]. Tomatoes are abundant in lycopene, comprising up to 90% of total carotenoids, and serve as the principal contributor to their vibrant red color [Li et al., 2024]. Being a precursor to vitamin A, which is vital for human nutrition, β -carotene deficiency can cause blindness. These pigments work as antioxidants, lowering ROS generation and cancer cell proliferation. They are also important for eye health since they protect against degenerative diseases [Demiray et al., 2013; Giorio et al., 2013; Ali et al., 2020]. In addition to a wide range of beneficial nutrients, tomatoes provide essential minerals such as calcium (Ca), magnesium (Mg), P, and K [Perveen et al., 2015].

1.8. Broccoli

Broccoli (*Brassica oleracea* var.) is a highly valued and widely consumed crop due to its rich in nutritional content, which helps to prevent carcinogenesis and improve health [Latté et al., 2011]. It is an herbaceous plant that produces consumable bunches of flowers, known as heads, in a single growing season [Ilahy et al., 2020]. Broccoli belongs to the order Brassicaceae and contains significant amounts of biologically active chemicals, such as sulfur and N-containing constituents [Seo et al., 2016]. Sulfur-containing compounds and phenolics are linked to health-enhancing effects generated by the broccoli defense mechanism, which initiate the production of major bioactive compounds by adverse conditions [Seo et al., 2016; Flores et al., 2021]. Broccoli is a nutraceutical plant notable for its elevated levels of essential mineral nutrients such as P, K, and S, as well as selected vitamins, dietary fibers, antioxidative chemicals, and glucosinolates [Moreno et al., 2006; Ilahy et al., 2020]. Secondary metabolites found in broccoli exhibit powerful capabilities of antioxidative traits and the chelating of metallic ions [Kasote et al., 2015].

It has been demonstrated that the most effective treatments for increasing the levels of glucosinolates and phenolics in broccoli were the application of alginic acid and mannitol containing brown SWE [Flores et al., 2021]. The concentrations of phenolic compounds, total flavonoids, and isothiocyanates were elevated in the brown SWE treated both broccoli varieties cultivated in the field trial [Lola-Luz et al., 2014]. The application of brown SWE was found to significantly enhance the total phenolic compounds and phytochemicals in broccoli, including hydroxycinnamic acid and quercetin [Kałużewicz et al., 2017].

1.9. Pepper

Sweet pepper (*Capsicum annuum* L.) is a member of the nightshade family (Solanaceae) and can yield an annual crop of significant commercial and social importance [Melo et al., 2020]. Sweet long peppers possess significant economic potential globally, exhibiting higher production capabilities, with Türkiye recognized as a crucial genetic resource for peppers [Akyavuz et al., 2018]. Pepper fruit contains health-promoting compounds such as ascorbic acid, vitamin E, pigments, antioxidants and capsaicin [Darko et al., 2022], and can be used for both fresh and processed food, as well as for therapeutic purposes such as the treatment of chronic lung disease [Wahyuni et al., 2013]. Its mineral nutrient composition also rich in P, Mg, Ca, Na, Fe, and zinc (Zn) [Rubio et al., 2002]. It is a common crop in the Mediterranean food tradition and contains a variety of medicinal phytochemicals, including capsaicin, phenol- and alkyl glycosides [Ríos and Andújar, 2020].

The biostimulant treatments have been demonstrated to enhance the production of secondary metabolites in plants, including increased levels of color pigments with antioxidant activity and phenolic compounds, which accumulate in the harvestable part of the pepper [Bonini et al., 2020]. On the other hand, biostimulant treatments can improve photosynthetic activity, mineral nutrient concentrations, growth parameters such as plant height and fruit number, and nutritional quality such as vitamin C activity in pepper [Ávila-Pozo et al., 2023]. The addition of SWE boosted vitamin C, chlorophyll, phenolic compounds, minerals, and overall plant development and yield of pepper [Melo et al., 2020; Ashour et al., 2021]

2. PURPOSE OF STUDY

This thesis aims to assess the biostimulant potential of *Cystoseira barbata* extracts in plant growth performance, yield, stress tolerance, and food quality in various soil-grown crops under greenhouse conditions. Three types of *C. barbata* extracts (water, alkali, and acid) with known biochemical characterization were evaluated for the diverse effects on plant physiology resulting from changes in bioactive ingredient profiles caused by different extraction methods. It was hypothesized that each *C. barbata* extract would have a distinct biostimulant effect on plants depending on their unique compositions.

The extracts were applied to durum wheat (*Triticum durum* cv. Sarıçanak-98) via soil drenching at three different levels to examine their impact on growth performance, and subsequently at selected levels to examine their impact on drought stress tolerance. Biomass differences, compatible solutes such as proline and glycine betaine, antioxidant enzyme activities, lipid peroxidation, and rhizosphere microbial community were examined under both control and drought-stressed conditions. Additionally, foliar applications were conducted to evaluate the impact of extracts on growth and yield of wheat.

Additional greenhouse experiments were conducted on dicots like tomato (*Solanum lycopersicum* L.), broccoli (*Brassica oleracea* var.), and sweet long pepper (*Capsicum annuum* L.). All plants applied soil drenching to examine the impact of the *C. barbata* extracts on the yield and nutritional quality of edible organs. Quality parameters included lycopene and β -carotene in tomatoes, carotenoids content in broccoli, and the levels of GSH, soluble carbohydrates, total phenolic content, total antioxidant capacity, and vitamin C concentration. Tomato plants were subjected to foliar treatment to assess the effects of extracts on fruit yield and mineral analysis. A separate study assessed the efficacy of a commercial product (Acadian) compared to a drench application of *C. barbata* extract on sweet long pepper.

Although the increasing use of brown SWEs as biostimulants, the genus *Cystoseira*, *C. barbata*, has not been extensively studied in this context. This research represents the first systematic study of the effects of *C. barbata* extracts on various crop species, using both foliar and soil drenching techniques on soil grown plants under controlled greenhouse conditions. The primary novelty consists of a comparative analysis of three distinct extracts and their varying effects under control and water-deficit conditions. By integrating agronomic, physiological and biochemical assessments across different crop models and by including a practical benchmark comparison, the purpose of this thesis study is firstly, to address a significant gap in the extant literature on biostimulants, and secondly, to support the development of sustainable and standardized products derived from local marine resources.

The findings of this study may establish a foundation for the transformation of *C. barbata* extracts into commercially viable products, contributing to the bioeconomy and offering strategic value for sustainable agriculture.

3. MATERIALS AND METHODS

3.1. Materials and General Information

3.1.1. Plants Materials Used in the Experiment

Sarıçanak-98 was officially registered in 1998 after being bred by GAP International Agricultural Research and Training Centre. This cultivar exhibits a medium length, a robust and waxy stem with large curving leaves. This variety stands upright, adorned with filthy white and yellow heads and thorny brown spines. The grains are amber-hued, thick, firm, and polished. The cultivar ranges in 1000-kernel weight from 35 g to 45 g. Sarıçanak-98, a cultivar with a strong tillering pattern, is categorized as mid-early. The average yield under optimal circumstances is approximately 500 kg per decare with a potential yield of up to 800 kg per decare [Web 8, 2024].

Tomato seedlings (*Solanum lycopersicum* L. cv. Cuma F1) were utilized and chosen as a representative highly valuable crop to investigate fruit growth and quality [Klee and Giovanni, 2011]. The cultivar is characterized by a robust constitution, an early maturation phase and high productivity. The fruit weighs approximately 300 grams, is firm in texture, dark red in coloration, slightly flattened in shape, and of good quality [Web 9, 2024].

The Marathon broccoli (*Brassica oleracea* L. var. Italica) a standard variety that has been adapted to a variety of conditions and is highly versatile. It is reliable and produces heads of a high quality. It can be harvested over a long season, including during extreme winter sowing. It reaches maturity 75-80 days after planting and can be harvested all season. The color is medium green [Web 10, 2024]. The Fiyaka Pepper Seeds (*Capsicum annuum* L.) is a robust and straightforward variety to cultivate. It displays remarkable resilience to summer conditions, exhibiting an early variety with a continuous yield throughout the season. The fruit is aesthetically pleasing, exhibiting bright, light hues. It can be safely cultivated in all regions of Türkiye with autumn and spring planting seasons. The average harvest time is 65 85 days. Fiyaka F1 is an early pepper and has a sweet taste.

The research and development greenhouse, where the all experiments were conducted, is a Venlo-type glass structure automated by computer, equipped with central heating and evaporative cooling systems. The temperature is maintained at approximately $25 \pm 5^{\circ}\text{C}$ throughout the day and $20 \pm 5^{\circ}\text{C}$ throughout the night.

3.1.2. Chemical and Physical Properties of Experimental Soil

The soil utilized in the wheat trials was obtained from Ömerli, Gebze, Kocaeli, was found to have a loamy texture (**Table 3.1**). The soil exhibits a low organic matter content and a limited amount of lime. The concentrations of K, Mg, and P were found to be low in the soil. The quantity of extractable Ca was determined to be sufficient. It exhibits a high concentration of extractable Fe and Zn, while the manganese (Mn) concentration is found sufficient.

The soil used in the tomato trials was taken from Kaşıkçı, Derince, Kocaeli, sieved, and packaged. The soil was identified as a clay loam with a high lime and low organic matter concentration. It had a low concentration of extractable K, Mg, and P. Extractable S was found to be sufficient in the soil, whereas extractable Ca was high. The soil has a high Fe concentration where Zn and Mn were available in sufficient quantities.

The soil employed in the broccoli trials was obtained from Gebze, Kocaeli. It was clay-based, exhibiting slight alkalinity and elevated lime characteristics. The organic matter content was moderate. Extractable P and K were present at medium levels, while Ca was present at high levels. Magnesium concentrations were low. Other micronutrients were present in sufficient quantities.

The soil used in the pepper trials was taken from Gebze, Kocaeli, exhibited a clay-loam characteristic and a high lime content. The organic matter content was moderate. The concentration of K and Ca was found to be high, while that of phosphorus and magnesium was relatively low. The concentrations of Fe and Zn were found to be high, while that of Mn was sufficient.

Table 3.1: Soil characteristics of the trials

Parameters	Wheat Trials	Tomato Trials	Broccoli Trials	Pepper Trials
Texture class	Loam	Clay-loam	Clay	Clay-loam
pH	8.1	8.0	7.8	7.5
Lime (%)	2.4	20	37	51
Organic matter (%)	0.6	1.0	2.1	2.0
Extractable Phosphorus (P) (mg/kg)	33	1.6	17	2.5
Extractable Potassium (K) (mg/kg)	18	90	91	172
Extractable Iron (Fe) (mg/kg)	11	8	3.3	7.0
Extractable Zinc (Zn) (mg/kg)	22	0.8	1.4	3.4
Extractable Manganese (Mn) (mg/kg)	14	3.0	2.5	6.1
Extractable Calcium (Ca) (mg/kg)	2109	7841	8428	7974
Extractable Magnesium (Mg) (mg/kg)	128	111	130	144

Soil analyses were made in the GUBRETAS Soil Analysis Laboratory.

3.2. Seaweed Extracts

3.2.1. Collection, Preservation of *C. barbata*

The brown seaweed species *C. barbata* was harvested in February 2020 in Istanbul, Türkiye, from the maximum depth of one meter along the Tuzla coast of the Marmara Sea (40° 49' 30" N, 29° 16' 44" E). The seaweed attached to the stones was harvested by cutting off the floating segments without damaging the attachment points.

Seaweed biomass was rinsed with dH₂O in order to remove any contaminants and seawater. Subsequently, the samples were dried in drying oven at 60°C for a period of three days, after which they were pulverized into a fine powder using a coffee grinder. Some samples were stored at -80°C as frozen samples. Regional observations indicate that *C. barbata* is consistently the most prevalent algal species throughout the winter months at the specified coordinates (**Figure 1**).

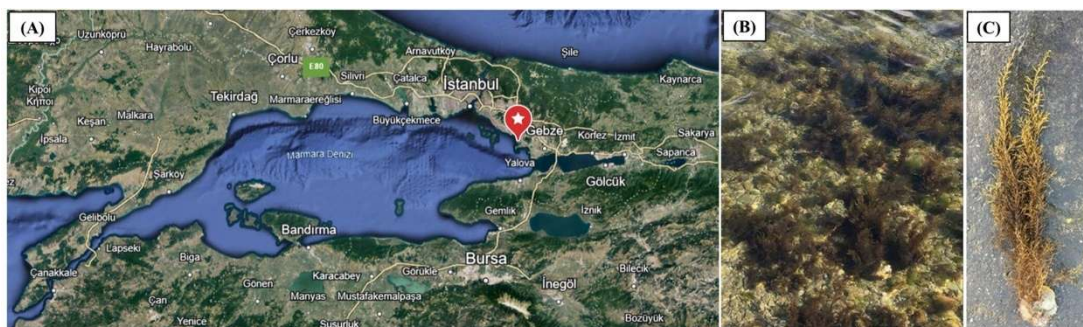


Figure 3.1: The coordinates of the harvested region ($40^{\circ} 49' 30''$ N, $29^{\circ} 16' 44''$ E) (A), the Marmara Sea with seaweed biomass (B), and a detailed image of *C. barbata* (C) [Mutlu-Durak et al., 2024].

3.2.2. Preparation of Extracts of *C. barbata*

Water extract was prepared in accordance with the methodology outlined by **Sharma et al. (2014)** for dried seaweed samples. One hundred grams of finely grounded and dried *C. barbata* was mixed with dH₂O to a total volume of one liter and kept constant at 70°C for three hours of stirring. The sample was then centrifuged for 15 minutes (min) at 8000 g and filtered. The extracts were stored at -80°C until required for use in biostimulant applications.

Acid extract was conducted in accordance with the methodology set out by **Ertani et al. (2018)** and **Shukla et al. (2019)**. The initial stage of the process was grinding 300 grams of frozen samples with the addition of dH₂O. The mixture was acidified with sulfuric acid (H₂SO₄) until the pH reached 2-3. Total volume was then increased to 600 ml with dH₂O. The mixture was incubated in a hot water bath set at 45°C for half an hour, after which it was centrifuged for 15 min. Subsequently, the pH of the extract was adjusted to level of 7-7.5 using potassium hydroxide (KOH). Subsequently, the extracts were stored at -80°C before application.

Alkali extract is regarded as both an extraction and hydrolysis process, carried out in accordance with the methods described by **Craigie (2011)** and **Shukla et al. (2019)**. The process was initiated with H₂SO₄ in the frozen biomass, followed by a subsequent treatment with KOH. Accordingly, the process was applied in the same way until the centrifugation step after the acid extraction incubation at 45°C.

By contrast, the initial hot water step was followed by the basification with KOH of the mixture until a pH of up to 11-12 was reached. The extracts were then incubated again in a hot water bath at 80°C for a period of three hours. Once the centrifugation and filtration steps had been completed as same as other extractions, the extract was neutralized using H₂SO₄. The extract was stored at -80°C for future biostimulant applications.

In addition, a commercial SWE (Acadian Sea Plants Ltd, Dartmouth, NS, Canada) was used in pepper trials to compare the *C. barbata* extracts. The Acadian algae-based biostimulant, derived from *Ascophyllum nodosum* through proprietary biochemical extraction procedures to retain bioactive components.

3.2.3. Biochemical Characterization of *C. barbata* Extracts

A comprehensive biochemical characterization of different *C. barbata* extracts of was performed, involving mineral element concentration, content of total phenolic content (TPC), concentration of total free amino acids, and auxin-like activity assessments.

3.2.3.1. Total Free Amino Acids

The method described by **Sadavisam and Manickam (1996)** was used to determine the contents of free amino acid of different extracts. The procedure consisted of preparing citrate buffer (0.2 M) containing citric acid and adjusting the pH to 5 with NaOH (10 M). A solution of ninhydrin (0.5% (w/v)) was prepared with ethanol (EtOH). 100 ml of ninhydrin solution and 100 ml citrate buffer were mixed together, and SnCl₂.2H₂O (0.05% (w/v)) was added to the mixture. Glutamic acid used as a standard was dissolved in (potassium phosphate) K-P buffer. The K-P buffer (pH:7.6) was prepared by combining 0.1 M monopotassium phosphate (KH₂PO₄) with 0.1 M potassium phosphate dibasic (K₂HPO₄), and it included 0.1 mM etilendiamintetrasetik asit disodyum (Na-EDTA) (Titriplex III). Ninhydrin reagent (1 ml) was added to the sample or standard (0.1 ml), and the mixture was incubated in a water bath at 90 °C for 10 min. It was measured at 570 nm in Ultraviolet–visible (UV-Vis) Spectroscopy (Peak Instruments C-7100S, Houston, USA). Results were expressed as glutamic acid equivalent (mg l⁻¹).

3.2.3.2. Total Phenolic Content (TPC)

Folin-Ciocalteu method was used to determine the TPC according to **Singleton and Rossi (1965)** at UV-Vis Spectroscopy. This approach relies on a redox reaction wherein phenolic are oxidized and the Folin reagent is reduced in a basic environment and forms a blue color. 400 μl of Folin reagent (0.5 N) and 400 μl of sodium carbonate (Na_2CO_3) (10%) solution have been mixed with sample or standard (20 μl). Total mixture volume was adjusted to 1.5 ml with distilled water (dH_2O) to facilitate the reaction. Gallic acid standards of different concentrations (0.125-1 mg ml^{-1}) were prepared. Following 30 min of incubation in darkness, the absorbance (Abs) of the standards and samples was assessed at 760 nm and quantified in accordance with the standard curve. The TPC of extracts was expressed based on mg gallic acid equivalent (mg GAE l^{-1}).

3.2.3.3. Mineral Analysis

Mineral analysis was performed using the method from **Ceylan et al. (2016)**. 0.4 ml of different *C. barbata* extract was transferred to reaction tubes for wet digestion. 2 ml of hydrogen peroxide (H_2O_2) (30%) and 5.0 ml of nitric acid (HNO_3) (65%) were added for acid digestion in a microwave system (MarsExpress; CEM Corp., Matthews, NC, USA). After cooling process, the total mixture volume was completed to 20 ml and then filtered with analytical blue band filter paper. ICP-OES (Agilent 5800 VDV, CA, USA) was used to determine the macro- and micronutrient concentrations found in different extracts.

The essential elements of the three extracts are shown in **Table 3.2** and firstly reported by **Mutlu-Durak et al., 2024**. The concentrations of K and S were highest in the alkali extract compared to the others. The acid extract had a comparatively higher concentration of Ca, while the water extract had a richer concentration of P compared to the others. In all types of extracts, Fe was found to be the micronutrient with the highest concentration. This was particularly evident in the alkali and water extracts. The concentrations of B and Mn were also found to be elevated, while Cu and Zn were found to be lower than other micronutrients.

Table 3.2: The essential (A) macro- (Ca, K, Mg, P and S) and (B) micronutrients (B, Cu, Fe, Mn and Zn) concentrations of the water, alkali and acid extracts [Mutlu-Durak et al., 2024].

(A) Macronutrient Concentration (g l⁻¹)					
Extract	Ca	K	Mg	P	S
Water	0.32 ± 0.01	5.42 ± 0.30	0.32 ± 0.01	0.11 ± 0.01	1.31 ± 0.07
Alkali	0.31 ± 0.01	9.37 ± 0.31	0.14 ± 0.00	0.07 ± 0.00	5.05 ± 0.16
Acid	0.89 ± 0.01	5.64 ± 0.06	0.31 ± 0.00	0.07 ± 0.00	3.02 ± 0.01
(B) Micronutrient Concentration (mg l⁻¹)					
Extract	B	Cu	Fe	Mn	Zn
Water	5.75 ± 0.29	0.23 ± 0.15	11.1 ± 0.2	2.11 ± 0.11	0.23 ± 0.17
Alkali	5.05 ± 0.30	0.43 ± 0.02	16.5 ± 1.5	1.83 ± 0.08	0.41 ± 0.19
Acid	3.35 ± 0.06	0.07 ± 0.04	7.5 ± 0.1	3.16 ± 0.02	0.29 ± 0.23

3.2.3.4. Determination of Antioxidant Capacity

Three different methods were used to determine the total antioxidant capacity of the SWEs.

3.2.3.4.1. ABTS Assay

The assay of 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonate) (ABTS) was utilized to assess the antioxidant capacity of samples in terms of the scavenging activity of free radicals [Re et al., 1999]. The ABTS (7 mM) and potassium persulfate (K₂S₂O₈) (2.45 mM) were prepared with dH₂O, combined in equal quantities, and incubated in the dark for 16 hours to create the ABTS stock solution. Subsequently, the stock solution was diluted with EtOH to achieve an Abs of approximately 0.70 at 734 nm. The SWE (20 µl) was added to of the diluted ABTS solution (2 ml). The mixture was incubated for 10 min in the dark conditions. Trolox (6-hydroxy-2,5,7,8-tetramethyl chromene-2-carboxylic acid) solutions (0–1.5 mM) were employed as a standard. After that, the Abs was determined at 734 nm. The antioxidant activity of the samples was calculated using the formula: % Scavenging activity = ((A₀ - A₁) / A₀) x 100, where A₀ is the control Abs and A₁ is the sample Abs. The results are reported as Trolox equivalent (TE) (mmol TE l⁻¹).

3.2.3.4.2. DPPH Assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was performed as an additional antioxidant assessment. The existence of an unpaired electron in the DPPH radical leads to the generation of color and the establishment of a spectrum of absorption at 516 nm. The incorporation of an electron pair into the radical scavenger results in a decrease in Abs, therefore causing the decolorization of the solution. The scavenging effect on the DPPH radical was evaluated using the methodology previously outlined by several authors, with some modifications. DPPH radical was determined with minor adjustments [Miller and Rice-Evans, 1997; Sánchez-Moreno, 1998]. Trolox solutions, prepared at various concentrations (0–1.5 mM), were used as the standard. A solution of 100 μ M DPPH radical in methanol (MeOH) was prepared. The standard or samples (100 μ l) were added to the DPPH radical solution (3.9 ml). Following a 120-min period of incubation at room temperature in the absence of light, the Abs was determined by UV-Vis spectrophotometry at a wavelength of 515 nm. The percentage of DPPH color change inhibition in the extracts was determined using the formula of scavenging activity above. The results are reported as TE (mmol TE l⁻¹).

3.2.3.4.3. FRAP Assay

The Ferric reducing-antioxidant power (FRAP) was conducted with minor adjustments [Benzie and Strain, 1996]. The FRAP reagent was prepared in a ratio of 10:1:1 (v:v:v) by mixing acetate buffer (300 mM) (pH 3.6) with tripyridyl triazine (TPTZ) (10 mM) in HCl (40 mM), and ferric chloride (FeCl₃·6H₂O) (20 mM), respectively. Sample or standard (30 μ L), dH₂O (90 μ L) and FRAP reagent (900 μ L) were prepared and allowed to incubate at 37 °C for half an hour in a hot water bath. Afterward, the Abs of the reduction of the ferric-tripyridyltriazine (Fe III-TPTZ) complex by the radical scavengers was determined at 593 nm by UV-Vis spectrophotometry. Trolox was employed as the standard solution, with concentrations ranging from 0 to 1.0 mM. and the results were expressed in TE (mmol TE l⁻¹).

The differences measured in the concentration of bioactive chemicals are shown in **Table 3.3**. While the phenolic content of the water and acid extracts was in the range of 0.6-0.7 g l⁻¹, the alkali extract remained the highest at 2 g l⁻¹. Similarly, the free amino acid concentrations of water and acid extracts were about 3 times higher than

the alkali extract. The concentration of alginic acid varied significantly between the extracts, the order being water, alkali and acid having the highest concentrations, respectively. On the other hand, fucoidan was in the same range in the water and alkali extracts, while the acid extract remained lower compared to them. The concentration of laminarin in the alkali and acid extracts was higher than in the water extract, around 50%. All antioxidant results showed a similar trend, with water and acid extracts being relatively lower than alkali extracts. Alkali extracts had been found to have about 2-3 times more antioxidant activity than others.

Table 3.3: The concentrations of TPC, total free amino acid, mannitol; alginic acid, fucoidan, laminarin (g l^{-1}); ABTS, DPPH, and FRAP (mmol l^{-1}) concentrations of water, alkali and acid extracts [Mutlu-Durak et al., 2024]

Extract	TPC	Total free amino acid (g l^{-1})	Mannitol
Water	0.7 $\pm$ 0.1	2.3 $\pm$ 0.1	3.0 $\pm$ 0.3
Alkali	2.0 $\pm$ 0.0	1.6 $\pm$ 0.1	1.1 $\pm$ 0.1
Acid	0.6 $\pm$ 0.0	2.2 $\pm$ 0.1	2.9 $\pm$ 0.4
Extract	Alginic acid	Fucoidan (g l^{-1})	Laminarin
Water	11.6 $\pm$ 0.2	46 $\pm$ 0	4.2 $\pm$ 0.1
Alkali	5.0 $\pm$ 0.1	42 $\pm$ 0	6.5 $\pm$ 0.0
Acid	1.0 $\pm$ 0.0	36 $\pm$ 0	5.9 $\pm$ 0.0
Extract	ABTS	DPPH (mmol l^{-1})	FRAP
Water	0.22 $\pm$ 0.00	6.4 $\pm$ 0.5	7.5 $\pm$ 0.1
Alkali	0.74 $\pm$ 0.00	16.7 $\pm$ 0.9	15.7 $\pm$ 0.2
Acid	0.24 $\pm$ 0.00	6.0 $\pm$ 0.1	5.1 $\pm$ 0.8

Analysis of polysaccharides (alginic acid, fucoidan and laminarin) and mannitol was conducted at Boğaziçi University.

3.2.3.5. Auxin-like Activity Analysis

A corn coleoptile elongation rate assay, as outlined by Colla et al. (2014), was employed as a test for hormone-like activity. The initial stage involved germinating seeds in the absence of light for a period of seven days at an ambient temperature. Twelve coleoptile segments, each measuring 10 cm in length, were positioned on petri dishes containing 20 ml of the solution. Solutions of indole-3-acetic acid (IAA) at a

concentration of 1.75 mg l^{-1} , dH_2O , and several extracts (water extract at 0.04%; alkali extract at 0.1%; acid extract at 0.1%) have been prepared. Each group consisted of four replicates, and the trial duration was 48 hours. Coleoptiles were scanned and later measured using the Image J program to assess auxin-like activity.

A method using maize coleoptile elongation was used to measure auxin-like activity in SWEs compared with the control (water) and IAA (**Figure 3.2**). All three SWEs showed the same trend as those the IAA used. Both IAA and SWE were reported to elongate maize coleoptiles by 25% more than the control.

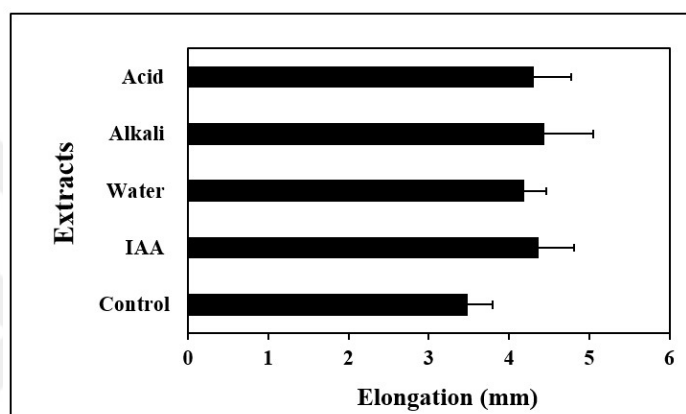


Figure 3.2: Auxin-like activity test conducted by water, alkali, acid extracts, IAA (1.75 mg l^{-1}) and control [Mutlu-Durak et al., 2024]

3.3. Experimental Designs and Seaweed Treatments

3.3.1. Wheat Experiments

This section focuses on the biostimulant effects of different *C. barbata* extracts on wheat, selecting the most effective concentrations for two different application methods: drench and foliar. The drench trials continued with the determination of the effective drought stress application and the investigation of the effects of selected SWEs on drought stress resistance and physiological parameters. Foliar trials continued to investigate the biostimulant effects of SWEs on grain yield and mineral status.

3.3.1.1. Investigation of Biostimulant Effects of *C. barbata* Extracts on Wheat by Drench Application

The biostimulant effects of different *C. barbata* extracts in three different rates were investigated and the most effective SWE rates were selected for drench application to wheat.

Plastic pots with trays containing 2.6 kg of dried soil, specified as the soil used in the wheat trials, were used in the trials. Fertilizer applications were prepared from concentrated solutions of the specified chemicals (100 mg N per kg in the form of commercial CaNO_3 and 25 mg P per kg in the form of KH_2PO_4) prepared in dH_2O and added to the soil. The pots were filled after the soil had been thoroughly mixed and homogenized with the addition of the fertilizers. At the beginning of the experiment, 15 wheat seeds were sown. After germination, the number of seedlings was reduced to 8 to grow uniformly. They were watered regularly with dH_2O according to their water requirements.

The experimental design consisted of a randomized complete block design with 4 replicates in a total of 40 pots. The experiment contained three seaweed levels (low, medium, high) obtained from each of the three SWEs (water extract, alkali extract, and acid extract). The control plants received no SWE.

Each SWE was applied in three different rates (low, medium, and high) with dH_2O as a drench application. The water extract was applied at 10 ml, 20 ml, and 40 ml for the low, medium, and high levels of application, respectively, for each pot. The acid and alkali extract were administered in 2.5 ml, 5 ml, and 10 ml of SWE for the low, medium, and high levels of application, respectively, for each pot. These applications initiated 14 days after sowing (DAS) and conducted three times in total, every week.

Following a 34-day growing period, the shoots were harvested. Subsequently, the plant samples were subjected to a three-day drying process at 70°C , after which their dry weights (DWs) were determined. In this preliminary experiment, the most effective application dose that exhibited a significant positive effect on the growth parameters were determined for each SWE and selected for subsequent experiments.

When multiple doses of SWEs showed similar significant effects, the lowest level of application was selected for use in the next trial. The medium level of water extract,

the low level of alkali extract and the high level of acid extract were selected for further experiment.

3.3.1.2. Determination of the Effective Drought Stress Application Level on Wheat

A second trial was designed to assess the effective level of drought stress on wheat and to select two different levels of drought stress for examination of the stress response.

This trial was conducted utilizing plastic pots with integrated drainage plates capable of holding 2.6 kg of dried soil. The specified fertilizers were prepared in dH₂O and subsequently applied to the soil. The pots were filled after the soil had been thoroughly mixed and homogenized with the addition of the fertilizers. The procedures for the application of fertilizers, the preparation of pots, and the seed sowing, as well as the duration of seedling growth until the two-week period, were identical to those described in section 3.3.1.1.

The experimental design consisted of a randomized complete block design with 4 replicates in a total of 20 pots. Plants were cultivated under four distinct irrigation regimes. The application of irrigation in the context of drought is based on the weighing of pots at regular intervals and the introduction of the required volume of water to achieve the desired level [Craufurd and Wheeler, 1999]. This is conducted manually in greenhouse conditions for each pot. The soil received 90%, 75%, 60% and 45% of the WHC (none, mild, moderate, severe drought) of the soil respectively.

The application of the drought treatments was initiated 14 DAS and continued until the plants reached 34 DAS, at which point they were harvested. Subsequently, the shoots were dried at 70°C for 72 hours to determine the DWs. Drought levels that reduced plant growth parameters by 15-25% and 30-40% were used for the next trials, which were 45% and 60% of the WHC, respectively.

3.3.1.3. Investigation of the Impacts of Selected *C. barbata* Extract Applications on Drought Stress Resistance and Physiological Parameters

Third experiment investigated the effect of biostimulant applications on the drought stress tolerance of wheat under greenhouse conditions. The most effective levels of

three SWEs were selected for use in the trial, in addition to the control application under three different irrigation regimes. The levels of biostimulant and drought were chosen based on the results of the preliminary trials (3.3.1.1 and 3.3.1.2).

The experimental design consisted of a randomized complete block design with 4 replicates in a total of 48 pots. The procedures for the application of fertilizers, the preparation of pots, and the seed sowing, as well as the duration of seedling growth until the two-week period, were identical to those described in section 1.3.1.1. Each SWE was applied with dH₂O as a drench application. The water extract was applied at 20 ml; the alkali extract was applied at 2.5 ml and acid extract were administered at 10 ml for each pot. In addition, three levels of irrigation/drought were applied: 45% WHC, 60% WHC and 90% WHC. The biostimulant and drought applications were initiated a period of two weeks after the sowing. Biostimulant applications were repeated on a weekly basis until they were harvested three times. The experiment was concluded at the 34 DAS.

The first and second samples (0.5 g) were obtained from the first fully expanded young leaf of seven plants, pooled, and employed for protein and antioxidant enzyme analysis. The third and fourth samples (0.5 g) were taken from the second fully expanded young leaf of seven plants, pooled, and were used for MDA and proline measurements. The final sample (0.5 g) was retained as a backup. Remaining plants were harvested, and the FWs were determined. After that, plants were dried at 70 °C for 72 h to measure DWs. Dry matter (%) were calculated. The dried samples were used for mineral analysis and tissue glycine betaine (GB) content.

Roots were pooled and cleaned of soil particles before sampling and collected for proline analysis as 0.5 g from each pot. The samples were stored at -80°C for the determination of free proline.

A study using EcoPlate (Biolog) microplates investigated the changes in the microbial communities in the soil to see how the biostimulant treatments affected the groups of microorganisms living there, described in detail below. After harvest, thoroughly mixing the soil in each pot and removing coarse parts such as stones and roots, 100 g homogeneous soil sample was taken to represent the pot to mediate microbial assay.

3.3.1.4. Investigation of Biostimulant Effects of *C. barbata* Extracts in Wheat by Foliar Application

The biostimulant effects of different *C. barbata* extracts in three different rates were investigated and the most effective SWE rates were defined for foliar application to wheat.

The experimental design consisted of a randomized complete block design with 4 replicates in a total of 40 pots. The experiment contained three levels (low, medium, high) obtained from each of the three SWEs (water extract, alkali extract and acid extract) as previously described. The control pots did not receive any SWE.

The procedures for the application of fertilizers, the preparation of pots, and the seed sowing, as well as the duration of seedling growth until the two-week period, were identical to those described in section 3.3.1.1. Each SWE was applied as foliar at three different rates. Water extract was sprayed at 2 ml, 4 ml and 8 ml of SWE in 150 ml dH₂O for the low, medium and high application rates per pot, respectively. Acid and alkali extracts were sprayed at 0.5 ml, 1 ml and 2 ml of SWE in 150 ml dH₂O for the low, medium and high application levels for each pot, respectively. Control plants were sprayed with dH₂O. Tween 20 was employed as a surfactant in all foliar spray formulations at a concentration of 200 mg l⁻¹. These applications were initiated at 14 DAS and carried out a total of three times each week. They were watered regularly with dH₂O according to their water requirements.

After a 34-day growing period, the shoots were harvested. The plants were then dried for three days at 70°C, and DWs were calculated. This preliminary experiment identified the most effective application level of each SWE that demonstrated a statistically significant positive effect on the growth parameters, which were chosen for further investigation. When effective levels of SWE were comparable, the minimum level of SWE exhibiting this effect was chosen for the long-term foliar trial. For this reason, the high level of water extract, the low level of alkali extract and the high level of acid extract were selected.

3.3.1.5. The Impact of Selected *C. barbata* Extracts Treatment on yield of wheat by foliar application

The study investigated the most effective levels of three different *C. barbata* extracts applied as foliar sprays on wheat in the long-term duration.

Plastic pots with trays containing 4.5 kg of dried soil were used in this trial. The concentrations of fertilizers and the preparation of pots were identical to those described in section 3.3.1.1. At the beginning of the experiment, 12 wheat seeds were sown. After 7 DAS, the number of seedlings was reduced to 5 to grow uniformly. They were watered regularly with dH₂O according to their water requirements.

The experimental design was a randomized complete block design. There were 5 replications in each application group and a total of 20 pots. Each SWE was applied as foliar at selected rates. Water extract was applied at 8 ml in 150 ml dH₂O, acid extract at 2 ml in 150 ml dH₂O and alkali extract were applied at 0.5 ml of SWE in 150 ml dH₂O for each pot. Control plants were sprayed with dH₂O. Tween 20 was employed at a concentration of 200 mg l⁻¹. Biostimulant applications were conducted with foliar sprays when the plants were 3, 6, 9 and 12 weeks old.

The plants were grown for 5 months to the end of the ripening period. When the plants had fully senesced and the grains had reached full maturity, the spikes and straws were harvested separately, and they were dried at 70°C to determine DWs. To determine grain yield, the grains were separated from the husks with a thresher. The growth parameters assessed included grain yield (g plant⁻¹), average grain weight (mg), harvest index, straw weight (g plant⁻¹), spike weight (g plant⁻¹), and spike number. Mineral analysis was conducted on the seeds.

$$\text{Harvest index} = \text{Grain yield} / \text{Total plant weight} \times 100. \quad (3.1)$$

3.3.2. Tomato Experiments

This section focuses on the biostimulant effects of different *C. barbata* extracts on tomato plants with selected levels of different application methods: drench and foliar. These trials investigated the biostimulant effects of the extracts on tomato yield and quality parameters.

3.3.2.1. Investigation of Biostimulant Effects of *C. barbata* Extracts on Tomato by Drench Application

The biostimulant effects of three different *C. barbata* extracts at selected concentrations were studied by long-term drench application on tomato.

Plastic pots with trays containing 3.5 kg of dried soil, specified as the soil used in the tomato trials, were used in the experiments. The required chemicals ($100 \text{ mg kg}^{-1} \text{ CaNO}_3$ and $50 \text{ mg kg}^{-1} \text{ KH}_2\text{PO}_4$) were prepared and, after mixing and homogenizing the soil with the addition of the nutrients, the pots were filled. The tomato seedlings were transplanted into pots and allowed to grow for a period of 100 days. Soil fertilization ($100 \text{ mg kg}^{-1} \text{ CaNO}_3$ and $50 \text{ mg kg}^{-1} \text{ KH}_2\text{PO}_4$) and foliar fertilization (1% (w/v) KH_2PO_4) were applied in the intermediate periods to prevent nutrient deficiency in the long term. Regular irrigation with dH_2O was carried out according to their water requirements.

The experimental design was a randomized complete block design with 5 replications of 20 pots. The SWEs were applied as soil drenches at rates of 100 ml for water extract, 30 ml for alkali extract and 30 ml for acid extract per pot with the control group receiving dH_2O only. SWEs were administered six times at 15-21-day intervals.

The first assessment was made during the early developmental stage 35 days after planting (DAP). The height of the axis of the main plant and the height of the branches were measured and expressed as stem length. The number of flowers is the number of inflorescences in the axis on the branch of the plant. The number of flowers was defined as the total number of flowers at the stage of anthesis, defined as fully opened yellow petals. Ripe fruits were collected over the course of 100 days until sufficient material was available for quality analysis. The experiment was then concluded, and leaves, stems, and fruits were harvested individually, with FWs recorded.

Fresh tomato fruit harvested from each pot was uniformly separated with a knife, mixed, processed through a mixer and aliquoted into falcon tubes at 5 g each. Fresh fruits were stored in the refrigerator at -80°C for quality assessment, which included measurements of electrical conductivity (EC), pH, brix, TPC, ABTS, DPPH, FRAP, lycopene, β -carotene, total and reduced vitamin C, and glutathione (GSH). After separating a portion of the harvested fruits from each pot for fresh analyses, the remaining fruits were oven-dried for mineral analysis. Stems and leaves were also dried for the DW measurements.

3.3.2.2. Investigation of Biostimulant Effects of *C. barbata* Extracts on Tomato by Foliar Application

The biostimulant effects of three different *C. barbata* extracts at selected concentrations were studied by long-term drench application on tomato.

The procedures for the application of fertilizers, the preparation of pots, and the plant transplantation, as well as the duration of seedling growth until the first biostimulant applications were identical to those described in section 3.3.2.1. The tomato seedlings were allowed to grow for a period of 70 days. Regular irrigation with dH₂O was mediated according to their water requirements.

The experimental design was a randomized complete block design with 5 replications of 20 pots. The SWEs were sprayed foliarly at rates of 5 ml for water extract, 1.5 ml for alkali and acid extract in 100 ml dH₂O per pot with the control group receiving dH₂O. SWEs were administered five times at 15-21-day intervals.

The first assessment for early developmental stage was mediated described in section 2.3.2.1. Ripe fruits were collected over the course of 70 days until sufficient material was available for analysis. The experiment was then concluded, and leaves, stems, and fruits were harvested individually. Fruits representing each pot were dried to be used in mineral analysis. Stems and leaves were also dried for the DW measurements.

3.3.3. Broccoli Experiment

3.3.3.1. Investigation of Biostimulant Effects of *C. barbata* Extracts on Plant Growth and Quality of Broccoli by Drench Application

The biostimulant effects of three different *C. barbata* extracts were studied on broccoli growth and quality.

Plastic pots with trays containing 3 kg of dried soil, specified as the soil used in the broccoli trials, were used in the experiments. The required chemicals (200 mg kg⁻¹ CaNO₃, 50 mg kg⁻¹ KH₂PO₄, 25 mg kg⁻¹ K₂SO₄, and 2 mg kg⁻¹ H₃BO₃) were prepared and, after mixing and homogenizing the soil with the addition of the nutrients, the pots were filled. The broccoli seedlings were transplanted into pots and allowed to grow

for a period of 100 days. Regular irrigation with dH₂O was carried out according to their water requirements.

The experimental design was a randomized complete block design with 5 replications of 20 pots. The experiment contained selected three SWEs (water extract, alkali extract, and acid extract) application with the control plants which received no SWE. The water extract was applied as 50 ml; the acid and alkali extract were applied as 10 ml for each pot. SWEs were administered five times at 15–21-day intervals.

The experiment was terminated after 100 DAP. The leaves, stems and heads were harvested separately in order to determine head FWs. Flower buds found in florets were separated from stalks, packed as 0.5 g and stored at -80°C for subsequent quality analysis. The measurements of TPC, ABTS, DPPH, FRAP, total and reduced AsA, GSH were performed on the broccoli heads. After separating a portion of the head from each pot for fresh analyses, the remaining heads were oven-dried for mineral analysis and soluble carbohydrate analysis. Stems and leaves were also dried for the DW measurements. It was also conducted mineral analysis for leaves of broccoli.

3.3.4. Pepper Experiment

3.3.4.1. Investigation of Biostimulant Effects of *C. barbata* Extracts and a Commercial Product on Plant Growth and Quality of Pepper by Drench Application

The biostimulant effects of *C. barbata* extracts and a commercial product which is called Acadian were studied and compared on pepper plants. Pepper seeds were sown in cocopeat medium supplemented with 2 mM Ca in the form of Ca (NO₃)₂·4H₂O and allowed to germinate and grow for 45 d before being transferred to pots. The macro- and micronutrient solutions were prepared as two different solutions and used as stock solutions.

The pots received 10 mM Ca (NO₃)₂·4H₂O, 3 mM KNO₃, 50 µM FeEDTA, 0.5 mM KH₂PO₄, 0.3 mM KCl, 1.5 mM MgSO₄·7H₂O, 5 µM ZnSO₄·7H₂O, 5 µM MnSO₄·H₂O, 1 µM CuSO₄·5H₂O, 10 µM H₃BO₃, 0.5 µM (Na₂)MoO₄·2H₂O. Fertilization was applied twice a week, and one-quarter of the nutrient solution was drained from the bottom of the pots to prevent accumulation of mineral nutrients in the soil.

The experimental design was a randomized, complete block design with four replications of 20 pots. The SWEs were sprayed foliar at rates of 10 ml for the Acadian product, 20 ml for the water extract, 10 ml for the alkali extract, and 10 ml for the acid extract per 1 dH₂O. The control group only received dH₂O. SWEs were applied five times at 15-21-day intervals.

The experiment was terminated after 80 DAP. Ripe pepper fruits were collected over the course of 80 days until sufficient material was available for quality analysis. The experiment was then terminated.

Fresh pepper fruit harvested from each pot was uniformly separated with a knife from their stem, calyx, seed and placenta. The pericarp of pepper was used, and each pot was brought together for further analysis. They were aliquoted at 2 g for each analysis. Fresh fruits were kept in the refrigerator at -80°C for TPC, ABTS, DPPH, FRAP, total and reduced AsA, GSH, Chl a, Chl b, carotenoid assays. After separating a portion of the fruits from each pot for fresh analyses, the remaining fruits were dried for mineral analysis and soluble carbohydrate analysis.

3.4. Applied Methods and Plant Analyses

3.4.1. Dry Matter (%) Calculation

The dry matter content, also known as the dry matter fraction in the International System of Units (SI), is the ratio of DW to FW expressed as a percentage. Dry matter content refers to the percentage of all components in a plant sample, excluding water, such as protein, fiber, fat and other substances.

3.4.2. Mineral Analysis

Dried ground plant samples weighing 0.2 g were transferred to digestion vessels. Microwave digestion was performed as indicated in section 3.2.3.3, followed by mineral analysis by ICP-OES.

3.4.3. Glycine Betaine (GB)

The GB was measured according to the method developed by **Grieve and Grattan (1983)**. 0.5 g of dried ground plant sample was added to 20 ml of dH₂O. The mixture was shaken for 24 hours at 25°C and then filtered. 1 ml of 2N H₂SO₄ was added to 1 ml of the filtered sample. 0.2 ml of pre-chilled potassium iodide-iodine (KI-I₂) reagent was added after cooling 0.5 ml of the sample in an ice bath for 1 hour. The reagent KI-I₂ was prepared by dissolving 15.7 g I₂ and 20 g potassium KI in 100 ml dH₂O. After a waiting period of 16 hours at 0-4°C, the mixture was centrifuged at 0°C for 15 min at 8000 g. The remaining iodide crystals were redissolved by vortexing in 9 ml of 1,2-dichloroethane after careful removal of the supernatant. Different concentrations of GB were prepared as standards in 1N H₂SO₄. The Abs of the samples were measured at 360 nm in UV-Vis spectrophotometry. The GB concentrations were expressed in mg g⁻¹.

3.4.4. Bradford Protein Assay

Fresh plant samples (0.5 g) were extracted using K-P buffer (5 ml). The mixture was extracted utilizing liquid nitrogen with using Mixer Mill (MM 400, Retsch, Haan, Germany). Following this, the homogenate underwent centrifugation for 15 min at 8000 g. Thereafter, the collected upper phase was utilized for Bradford protein analysis and antioxidant enzyme examination, as detailed below.

The linearized Bradford technique was utilized to ascertain the total soluble protein concentration [**Zor and Selinger, 1996**]. The following reagents were mixed: 50 ml of ethanol, 100 ml of orthophosphoric acid (85%), 450 ml of dH₂O, 100 mg of Coomassie Brilliant Blue, and 100 ml of glycerin. The final volume was adjusted to 1 L with dH₂O, and then the solution was filtered. The prepared Bradford's reagent was stored at 4°C overnight before use.

The standards of different concentrations in the range of 0-800 mg ml⁻¹ of bovine serum albumin (fraction V) were prepared in K-P buffer. 100 µl of the plant extract or standards and 5 ml of the reagent were mixed. The mixture was then incubated in the dark for 5 min. The Abs of samples was measured at 450 nm and 590 nm using UV-Vis spectrophotometry. The protein concentrations calculated from the ratio of Abs₅₉₀ / Abs₄₅₀ and expressed as mg g⁻¹.

3.4.5. Antioxidative Enzyme Activities

The total antioxidant enzyme activities of such as SOD, CAT, APX, and GR were analyzed. The specific antioxidant enzyme activities were calculated as the division of total activities to total protein concentration.

3.4.5.1. Superoxide dismutase (SOD)

The methodology employed in this analysis was adapted from the procedures established by **Giannopolitis and Ries (1977)** and **Mengutay et al. (2013)**, incorporating minor modifications for enhanced efficacy. 100 μl of plant extract was mixed with 500 μl of Na_2CO_3 (50 mM), 500 μl of L-methionine (12 mM), and 500 μl of p-nitro blue tetrazolium chloride (NBT) (75 μM). The solution was completed with K-P buffer to a total volume of 4.5 ml. 500 μl of riboflavin (2 μM) was added to initiate the reaction, after which the mixture was exposed to intense light for 8 min. The inhibition of NBT photochemically by SOD was measured at 560 nm. The definition of one unit of activity is as follows: it is the reduction of NBT activity by 50%. The results were expressed as U mg^{-1} protein.

3.4.5.2. Catalase (CAT)

The K-P buffer and 100 mM H_2O_2 were prepared. The reaction was initiated by the addition of 100 μl of H_2O_2 solution to 100 μl of plant extract and 800 μl of K-P buffer. The kinetic reaction was measured by observing the reduction of Abs in H_2O_2 for 2 min at 240 nm using UV-Vis spectrophotometry. The CAT activity was reported as $\mu\text{mol H}_2\text{O}_2 \text{ mg}^{-1} \text{ protein (prt.) min}^{-1}$ [**Mengutay et al., 2013**].

3.4.5.3. Ascorbate peroxide (APX)

First, 700 μl of K-P buffer was mixed with 100 μl of ascorbic acid ($\text{C}_6\text{H}_8\text{O}_6$) (0.25 mM) and 100 μl of a plant extract solution. To initiate the reaction, 100 μl of H_2O_2 (12 mM) was added to the mixture. The average rate of depletion of L-ascorbic acid, the APX activity, was determined by UV-Vis spectrophotometry at 290 nm for 2 min. APX activity was expressed as $\text{nmol H}_2\text{O}_2 \text{ mg}^{-1} \text{ prt. min}^{-1}$ [**Nakano and Asada, 1981**].

3.4.5.4. Glutathione reductase (GR)

The 100 µl of plant extract was mixed with 100 µl of 0.5 mM oxidized glutathione (GSSG) and 700 µl of K-P buffer containing 0.1 mM Na₃-EDTA. To initiate the reaction, 100 µl of nicotinamide dihydronicotinamide adenine dinucleotide phosphate tetrasodium salt (NADPH-Na₄) (0.12 mM) was added to the mixture [Foyer and Halliwell, 1976; Mengutay et al., 2013]. Nicotinamide adenine dinucleotide phosphate (NADPH) oxidation was determined by monitoring the activity at 340 nm for 2 min using UV-Vis spectrophotometry. The results were expressed as nmol NADPH mg⁻¹ prt. min⁻¹.

3.4.6. Malondialdehyde (MDA)

0.5 g of fresh plant sample was homogenized with 25 ml of 80% ethanol for plant extraction using liquid nitrogen in mixer mill for the extraction. The mixture was then centrifuged at 8000 g for 10 min, and the supernatant was collected [Heath and Packer, 1968; Hodges et al., 1999].

Trichloroacetic acid (TCA) (20%) containing butylated hydroxytoluene (BHT) 0.01% was prepared as (-) TBA solution. TCA (20%) containing Thio barbituric acid (TBA) (0.65%) and 0.01% BHT was prepared as (+) TBA solution. 1 ml of each solution was added to 1 ml of plant extract to initiate the reaction. The mixture was incubated in a hot water bath at 95°C for 25 min. The samples were then centrifuged at 8000 g for 10 min at 4°C. The Abs values were measured at 440, 532 and 600 nm using UV-Vis spectrophotometry. The results have been calculated and reported according to the formulas shown below:

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

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

$$MDA_{(nmol\ ml^{-1})} = \frac{A-B}{157\ 000} \times 106 \quad (3.2)$$

3.4.7. Microbial Community Analysis

10 g soil sample was taken from 100 g of soil mixture, in 90 ml 0.85% NaCl buffer vortexed at 150 rpm for 30 min at room temperature. The sample was then centrifuged

at 500 rpm. The liquid phase was diluted with 0.85% NaCl buffer to a volume of 1:10, and then 125 µl of samples were added to Eco Plate (Biolog) microplates that had 31 different carbon sources already on them. These were then incubated in the dark at 25°C. Carbon source consumption rates were determined by measuring absorbance at 590 nm after 120 hours [Gomez et al., 2006]. Following this measurement, the average well color development (AWCD), substrate average well color development (SAWCD), Shannon index (H'), substrate richness (SR) and Shannon evenness (E) were calculated according to the provided instructions to provide insight into the microbial community analysis.

3.4.8. Free Proline

For extraction, 0.5 g of fresh plant samples were homogenized with 10 ml of sulphosalicylic acid (3% (w/v)) in a mixer mill using liquid nitrogen. The mixture was then centrifuged at 8000 g for 15 min at 4°C. The supernatant was used for free proline analysis as described by Bates et al. (1973). An acid ninhydrin solution was prepared by dissolving 6.25 g ninhydrin in 150 ml glacial acetic acid and 100 ml phosphoric acid (6 M). The plant extracts (2 ml) were mixed with glacial acetic acid (2 ml) and acid ninhydrin solution (2 ml). The resulting mixture was then incubated in a hot water bath at 100°C for 60 min, then cooled. Then 4 ml of toluene was added and vortexed. The chromophore phase present in both plant samples and standards was measured at 520 nm by UV-Vis spectrophotometry. Different proline concentrations dissolved in sulfosalicylic acid (3% w/v) were evaluated as a standard curve. The results were expressed as mg kg⁻¹ FW.

3.4.9. Brix, EC and pH

The brix value which defines the amount of total soluble solid of tomato and pepper was conducted with a digital refractometer (MASTER-53M, Atago Co., Tokyo, Japan). After centrifugation for 15 min at 8000 g, the EC and pH values of tomato samples was conducted from the supernatant with electrical conductivity (Mettler-Toledo Meter, Switzerland) and pH meter (Mettler Toledo InLab® Routine Pro-ISM).

3.4.10. Total Phenolic Content (TPC)

The Folin-Ciocalteu procedure was employed for the purpose of ascertaining the TPC of the plant samples [Singleton et al., 1999]. Initially, plant extracts were prepared with 70% MetOH at a ratio of 1:1 (w/v) for tomato, 1:10 (w/v) for broccoli, and 1:10 (w/v) for pepper [Paradić et al., 2011; Kalużewicz et al., 2017; Mannino et al., 2020]. They were extracted using a mixing mill with liquid nitrogen, then centrifuged at 4 °C for 15 min at 8000 g and aliquoted for TPC and total antioxidant analysis. As described under 1.2.3.2 section, TPC analysis was conducted with UV-Vis spectrophotometry. The results were expressed as mg GAE 100 g⁻¹ FW or mg GAE g⁻¹ FW.

3.4.11. Total Antioxidant Capacity

Three different methods were used to determine the total antioxidant capacity of the plants. Aliquots of the 70% MetOH extract of the plants were used for analysis.

3.4.11.1. ABTS

As described under 3.2.3.5.1 section, ABTS analysis was conducted with UV-Vis spectrophotometry. The results were expressed as µmol TE 100 g⁻¹ or mmol TE kg⁻¹.

3.4.11.2. DPPH

As described under 3.2.3.5.2 section, DPPH analysis was mediated with UV-Vis spectrophotometry. The results were reported as µmol TE 100 g⁻¹ or mmol TE kg⁻¹.

3.4.11.3. FRAP

As described in section 3.2.3.5.3, the FRAP analysis was carried out using UV-Vis spectrophotometry. The results were reported as µmol TE 100 g⁻¹ or mmol TE kg⁻¹.

3.4.12. Ascorbic acid

Total ascorbic acid (AsA) and reduced AsA were measured in three different plants [Kampfenkel et al., 1995; Ilahy et al., 2011]. Initially, plant extracts were prepared

with 5% metaphosphoric acid at a ratio of 1:3 (w/v) for tomato, 1:10 (w/v) for broccoli, and 1:10 (w/v) for pepper. They were extracted using a mixing mill with liquid nitrogen, then centrifuged at 4 °C for 15 min at 8000 g and aliquoted for GSH analysis. The analysis based on the reduction of ferric iron (Fe⁺³) to ferious iron (Fe⁺²) by ASA, then the spectrophotometric identification of Fe⁺² complexes with 2,2-dipyridyl. Preincubation of the sample with dithiothreitol (DTT) reduces the oxidized form of dehydroascorbic acid (DHA) to ascorbic acid (AsA). Excess DTT is subsequently eliminated using N-ethylmaleimide (NEM), and the total ascorbic acid (AsA) is quantified. Absorbance of the samples or standards had been measured at 525 nm via UV-Vis spectrophotometry. The findings were presented as mg of total AsA and reduced AsA 100 g⁻¹ or kg⁻¹ FW.

3.4.13. Glutathione (GSH)

Aliquots of the 5% metaphosphoric acid extract of the plants that were used for the AsA analysis were used for the analysis of reduced GSH [Kampfenkel et al., 1995]. K-P buffer (150 mM) containing EDTA (5 mM) at pH 7.4 was added to the extracts [Zhou et al., 2022]. The resulting mixture was centrifuged at 4 °C at 8000 g for 15 min. The 5,5'-dithio-bis (2-nitrobenzoic acid) (DTNB) (6 mM) was then added to the reaction vessel. The Abs were read at 412 nm after 20 min incubation. Results were expressed in mg GSH 100 g⁻¹ or mg GSH kg⁻¹ FW according to a standard curve constructed from various concentrations of GSH.

3.4.14. Lycopene and β-carotene

Tomato samples were extracted in a ratio of 1:10 (w/v) with acetone:hexane (2:3). They were then sonicated for half an hour and incubated overnight at room temperature. The extracts were centrifuged at 8000 g at 4 °C for 15 min. The Abs of the samples was measured at multiple wavelengths: 453, 505, 645, and 663 nm, utilizing a UV-Vis spectrophotometry [Nagata and Yamashita., 1992; Fernández-Garcí., 2004]. The calculation method for the results is below.

$$\text{Lycopene (mg 100 ml}^{-1}\text{)} = - 0.0806 A_{453} + 0.372 A_{505} + 0.204 A_{645} - 0.0458 A_{663}$$

$$\beta\text{-Carotene (mg 100 ml}^{-1}\text{)} = + 0.452 A_{453} - 0.304 A_{505} - 1.220 A_{645} + 0.2160 A_{663} \quad (3.3)$$

3.4.15. Soluble Carbohydrate

Dried tomato and pepper samples were extracted in a ratio of 1:100 (w/v) with EtOH (80%). The extracts were centrifuged at 8000 g at 4°C for 20 min. Soluble carbohydrates were measured by UV-Vis spectroscopy, based on the method of **Yemm and Wills (1954)** and **Mengutay et al. (2013)** with minor adjustments. First, 600 mg of anthrone was dissolved in 100 ml of EtOH (20%) and 300 ml of H₂SO₄ (98%). Then 125 µl of plant extract and 2 ml of anthrone reagent were added. The mixture was incubated for 20 min at 90°C in a hot-water bath. The mixture was then cooled on ice, and the Abs were measured at 612 nm. The results were expressed as mg g⁻¹ DW and were based on a standard curve generated from different concentrations of D-glucose.

3.4.16. Chlorophyll and Total Carotenoids

Broccoli and pepper samples were extracted with 80% acetone at a ratio of 1:10 (w/v) using a homogenizer. After a period, the homogenate was centrifuged at 4°C at 8000 g for 15 min. To mediate measurement, it has been diluted with the plant sample (100 µl) with 80% acetone (1900 µl) [**Wellburn, 1994**]. The Abs of the samples were measured at 470 nm, 646.8 nm and 663.2 nm. The following equations were used for calculations:

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

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

$$C_{x+c} = (1000\text{Abs}_{470} - 1.82C_a - 85.02C_b) / 198. \quad (3.4)$$

3.5. Calculations and Statistical Analysis

The statistical software of JMP (version 14.0.0) was applied to the data to analyze the results. Analysis of variance (ANOVA) was used to show that the different effects of applications and interactions were significant. The least significant differences between the means of the values were determined by Fisher's protected least significant difference (LSD) test (significance of 5%) for one-factorial experiment. For multiple-factorial experiments, Tukey's honestly significant difference (HSD) test (significance of 5%) was applied.

4. RESULTS

4.1. Wheat Experiments

4.1.1. Investigation of Biostimulant Effects of *C. barbata* Extracts on Wheat by Drench Application

The experiment was designed to determine the effective level of *C. barbata* extracts on wheat growth. All plants treated with different *C. barbata* extracts showed increased growth performance (**Figure 4.1**) and significant enhancement in shoot DW (**Figure 4.2**) when compared to the control plants, as confirmed by ANOVA results (**Table 4.1**). The application of medium level of water extract, low level of alkali extract, and the high level of acid extract had the most pronounced effects with increments of 42%, 18%, and 26%, respectively. These rates of SWEs were chosen for testing in a further experiment.



Figure 4.1: Effect of biostimulant applications (control (water); water extracts (low, medium, high); alkali extracts (low, medium, high); acid extracts (low, medium, high) on wheat growth

Table 4.1: The effect of biostimulant applications on wheat shoot DW according to one-way ANOVA ($\alpha = 0.05$)

Source of Variation	DF ¹	Shoot DW
Biostimulant	9	**

¹DF; Degree of freedom; * $0.01 \leq F \text{ Pr.} < 0.05$; ** $0.001 \leq F \text{ Pr.} < 0.01$; *** $F \text{ Pr.} < 0.001$

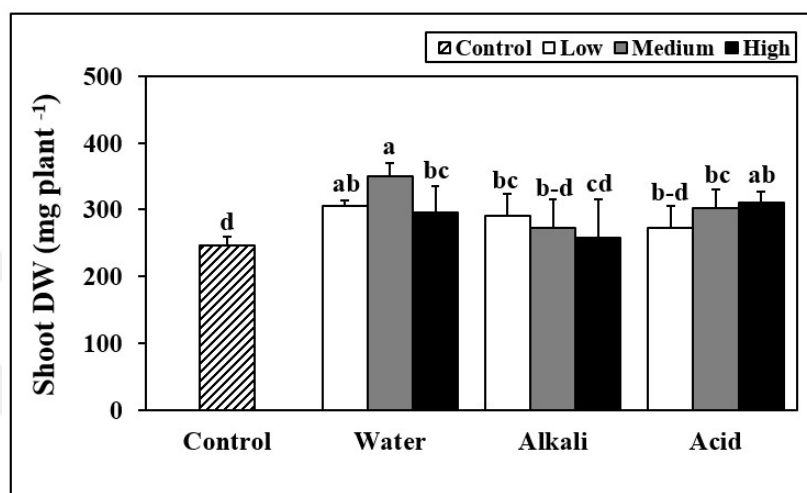


Figure 4.2: Effect of biostimulant applications (control (water); water extracts (low, medium, high); alkali extracts (low, medium, high); acid extracts (low, medium, high) on shoot DWs of wheat. Values are means and standard deviations of four replicates. Different letters indicate significant differences between means according to Fisher's protected LSD test ($p < 0.05$).

4.1.2. Determination of the Effective Drought Stress Application Level on Wheat

The experiment was conducted to identify the most effective level of drought stress according to the results of plant growth. It has been shown that rising levels of drought stress negatively affected wheat growth and development (**Figure 4.3**), and ANOVA revealed a significant effect in shoot DW (**Table 4.2**). According to **Figure 4.4**, an increase in drought severity resulted in reductions of 17%, 26% and 40%. Based on the findings, 60% and 45% WHC were applied for the subsequent trial to represent two different drought stress level.

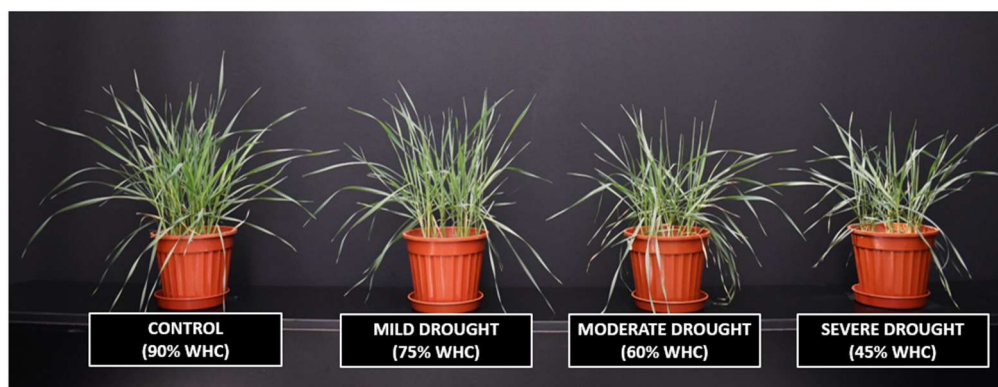


Figure 4.3: Effect of drought stress levels (control (%90 WHC); mild drought (75% WHC); moderate drought (60% WHC); severe drought (45% WHC) on wheat growth

Table 4.2: Significance of the effect of drought stress applications on wheat shoot DW according to one-way ANOVA ($\alpha = 0.05$)

Source of Variation	DF ¹	Shoot DW
Drought Stress	3	***

¹DF; Degree of freedom; * $0.01 \leq F \text{ Pr.} < 0.05$; ** $0.001 \leq F \text{ Pr.} < 0.01$; *** $F \text{ Pr.} < 0.001$

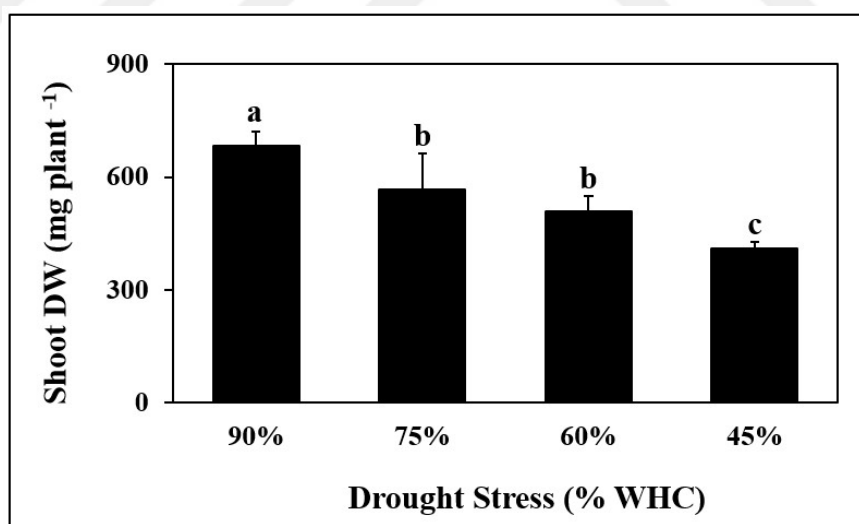


Figure 4.4: Effect of drought stress levels (control (%90 WHC); mild drought (75% WHC); moderate drought (60% WHC); severe drought (45% WHC) on shoot DW (mg plant⁻¹) of wheat. Values are means and standard deviations of four replicates. Different letters indicate significant differences between means according to Fisher's protected LSD test ($p < 0.05$).

4.1.3. Investigation of the Impacts of Selected *C. barbata* Extract Applications on Drought Stress Resistance and Physiological Parameters

In an extensive experiment, the selected SWEs from the first trial were used together with moderate and severe drought stress levels to assess their interactive effects on plant growth. ANOVA results revealed that both SWE and drought stress applications exerted significant effects on shoot DW individually, and their interaction was also found to be significant (**Table 4.5**). As drought severity increased, a substantial decline in growth performance was observed in plants (**Figure 4.5**), together with shoot DW decreasing by approximately 27% under moderate and 40% under severe drought conditions irrespective of biostimulant applications (**Figure 4.6**). The water extract application improved shoot DW by 31%, while the acid extract resulted in a 21% increase compared to the control regardless of drought stress. In addition, under moderate drought conditions, water extract application resulted in a 41% increase whereas acid extract application led to a 30% increase. Under severe drought stress, shoot DW increments reached up to 58% with water extract and 50% with acid extract, compared to the stress-applied controls.

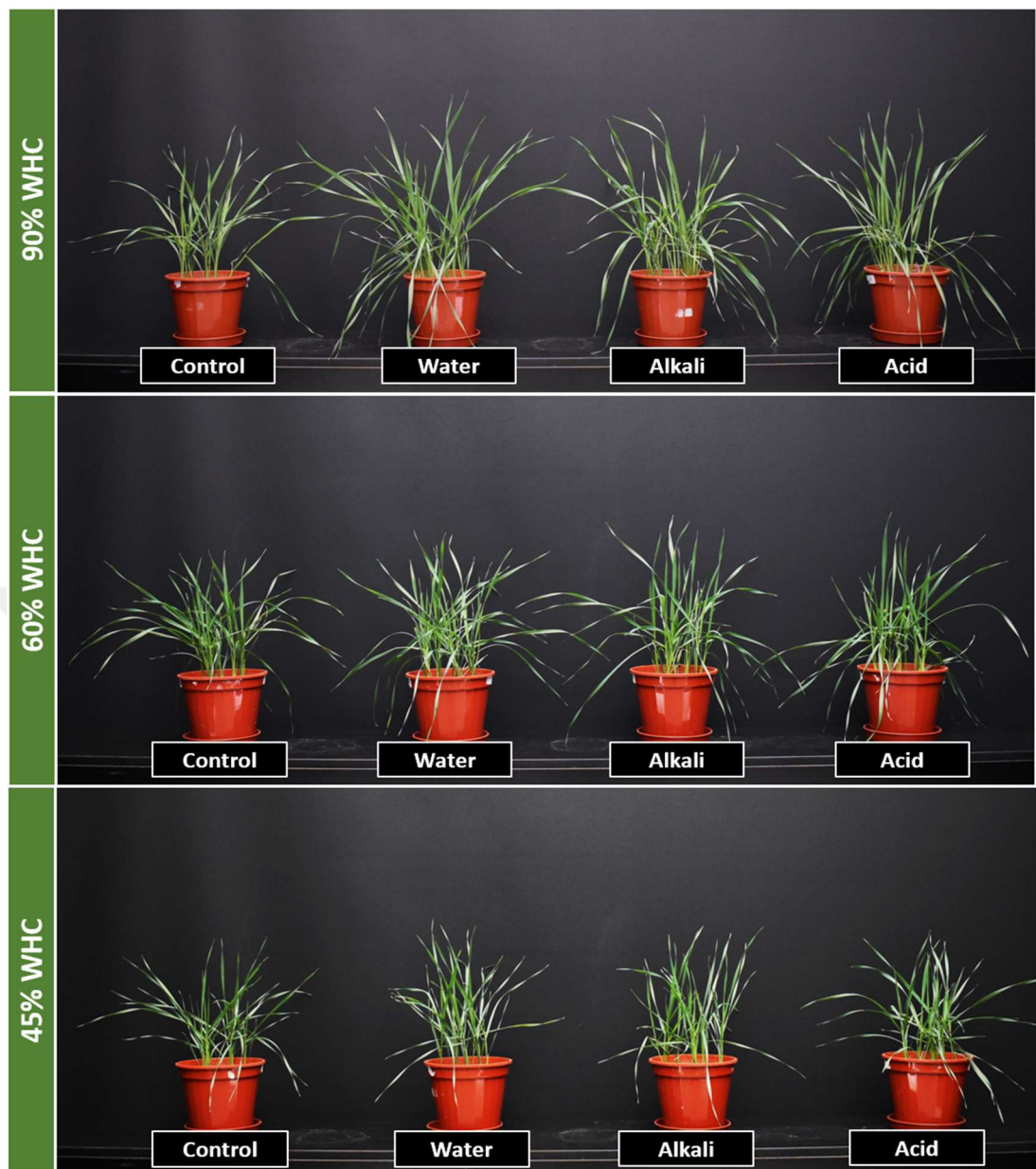


Figure 4.5: Effect of biostimulant applications (control; water extracts; alkali extracts; acid extracts) and drought stress levels (%90 WHC; 60% WHC; 45% WHC) on the growth of wheat growth

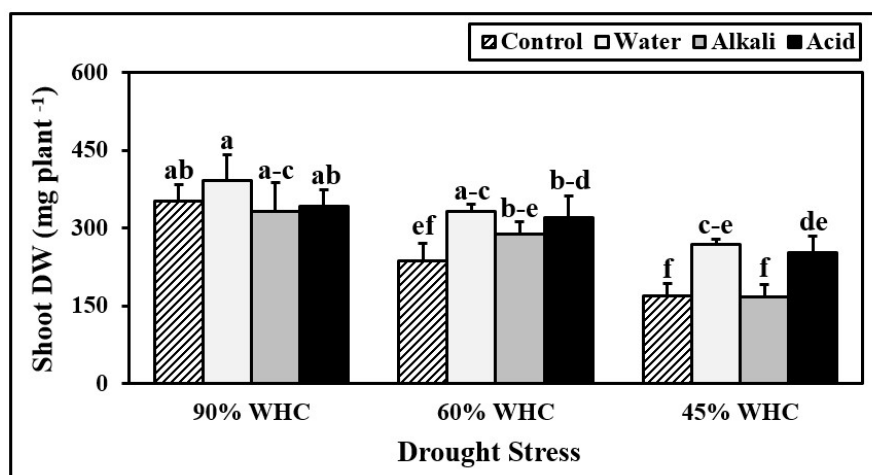


Figure 4.6: Effect of biostimulant applications (control; water extract; alkali extract; acid extract) and drought stress levels (90% WHC; 60% WHC; 45% WHC) on shoot DW (mg plant⁻¹) of wheat. Values are means and standard deviations of four replicates. Different letters indicate significant differences between means according to protected HSD test ($p < 0.05$)

According to ANOVA results, both the main effects and the interaction of biostimulant and drought stress on dry matter ratio were found to be significant (**Table 4.5**). Water extract improved dry matter ratio by 18% and 21% relative to drought stressed controls under moderate and severe drought conditions, respectively. On the other hand, acid extract resulted in modest enhancement of approximately 7-8% under both drought levels (**Figure 4.7**).

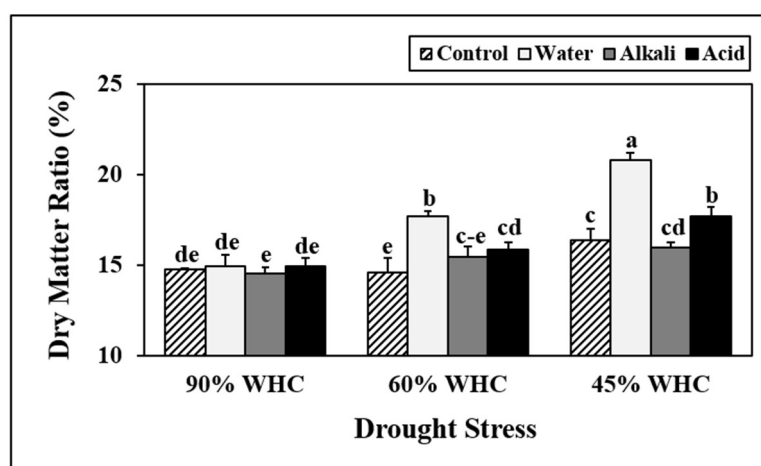


Figure 4.7: Effect of biostimulant applications (control; water extract; alkali extract; acid extract) and drought stress levels (90% WHC; 60% WHC; 45% WHC) on dry matter ratio (%) of wheat. Values are means and standard deviations of four replicates. Different letters indicate significant differences between means according to protected HSD test ($p < 0.05$)

Shoot P concentration increased with increasing drought severity regardless of *C. barbata* applications. Although K and Ca were significantly affected by interactions of drought stress and SWEs treatment (**Table 4.5**), their responses exhibited unsteady variations lacking a discernible pattern (**Table 4.3**). Moreover, increasing drought severity led to a modest decline in Mg concentrations. Water and alkali extract applications significantly increased the S concentration of plants compared to the control under non-stressed conditions.

Table 4.3: Effect of biostimulant applications (control; water extract; alkali extract; acid extract) and drought stress levels (90% WHC; 60% WHC; 45% WHC) on macronutrient concentrations (P, K, Ca, Mg, and S) of wheat

Biostimulant	Drought Stress (% WHC)	P (mg g ⁻¹)	K (mg g ⁻¹)	Ca (mg g ⁻¹)	Mg (mg g ⁻¹)	S (mg g ⁻¹)
Control	90	5.1 ± 0.3 a	41 ± 1 b	8.6 ± 0.6 ab	1.8 ± 0.1a	3.6 ± 0.2 bc
	60	7.2 ± 1.1 a	44 ± 3 ab	9.8 ± 0.6 a	1.7 ± 0.1ab	3.9 ± 0.6 a-c
	45	7.1 ± 0.4 a	45 ± 1 ab	9.6 ± 0.6 ab	1.7 ± 0.0ab	3.8 ± 0.3 a-c
Water	90	6.2 ± 0.6 a	46 ± 2 a	9.4 ± 0.5 ab	1.8 ± 0.0a	4.5 ± 0.3 a
	60	7.2 ± 0.3 a	44 ± 1 ab	9.0 ± 0.5 ab	1.5 ± 0.1bc	3.7 ± 0.2 a-c
	45	6.4 ± 1.3 a	43 ± 4ab	7.8 ± 1.4 b	1.3 ± 0.2c	3.1 ± 0.4 c
Alkali	90	5.8 ± 0.4 a	44 ± 1 ab	9.5 ± 0.6 a	1.8 ± 0.1a	4.5 ± 0.3 a
	60	7.5 ± 0.1 a	45 ± 1 ab	9.1 ± 0.1 ab	1.6 ± 0.0ab	3.9 ± 0.0 a-c
	45	8.2 ± 0.7 a	46 ± 1 a	8.7 ± 0.3 ab	1.5 ± 0.2bc	3.6 ± 0.6 bc
Acid	90	6.1 ± 1.5 a	44 ± 2 ab	9.0 ± 0.5 a	1.7 ± 0.1ab	4.1 ± 0.1 ab
	60	7.6 ± 0.3 a	45 ± 1 ab	9.3 ± 0.6 ab	1.6 ± 0.0ab	4.0 ± 0.1 ab
	45	6.8 ± 0.4 a	44 ± 2 ab	9.8 ± 0.8 a	1.6 ± 0.0ab	3.9 ± 0.2 a-c

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

The concentration of Fe and Zn was significantly affected by both the individual effects of SWEs and drought stress applications and their interaction, as indicated by ANOVA (**Table 4.5**). Regardless of the application of SWEs, there is a declining trend in Fe and Zn concentrations as drought severity increased (**Table 4.4**). Other than that, there were no consistent effects of treatments on Cu, Mn, and B concentrations of plants.

Table 4.4: Effect of biostimulant applications (control; water extract; alkali extract; acid extract) and drought stress levels (90% WHC; 60% WHC; 45% WHC) on micronutrient concentrations (Fe, Zn, Mn, Cu, and B) of wheat

Biostimulant	Drought Stress (% WHC)	Fe (mg kg ⁻¹)	Zn (mg kg ⁻¹)	Mn (mg kg ⁻¹)	Cu (mg kg ⁻¹)	B (mg kg ⁻¹)
Control	90	136±11 ab	106 ± 2 a	45 ± 6 a	11 ± 1 ab	12 ± 1 a-c
	60	128±6 bc	78 ± 2 c	49 ± 2 a	10 ± 2 bc	9 ± 1 bc
	45	105±7 b-d	76 ± 8 c	48 ± 4 a	11 ± 1 a-c	10 ± 2 a-c
Water	90	167±36 a	104 ± 6 a	42 ± 2 a	12 ± 1 a	13 ± 1 a
	60	117±10 b-d	67 ± 6 cd	45 ± 1 a	10 ± 1 bc	11 ± 0 a-c
	45	92±3 cd	57 ± 1 d	38 ± 4 a	10 ± 1 c	9 ± 1 bc
Alkali	90	124±19 bc	105 ± 7 a	40 ± 6 a	11 ± 0 a-c	11 ± 0 a-c
	60	116±4 b-d	83 ± 6 bc	47 ± 4 a	10 ± 0 bc	9 ± 1 a-c
	45	83±13 d	76 ± 13 c	43 ± 4 a	11 ± 0 a-c	8 ± 2 c
Acid	90	114±10 b-d	100 ± 10 ab	46 ± 7 a	11 ± 0 a-c	10 ± 1 a-c
	60	120±15 b-d	80 ± 7 c	43 ± 5 a	10 ± 0 a-c	10 ± 1 a-c
	45	118±17 b-d	85 ± 5 bc	44 ± 2 a	11 ± 0 a-c	12 ± 3 ab

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

Table 4.5: Significance of the effects of biostimulants (A), drought (B) and their interactions on reported traits of soil-grown wheat plants according to two-way ANOVA ($\alpha = 0.05$)

Source of Variation	DF	Shoot DW	Dry Matter Ratio	P	K	Ca
Biostimulant (A)	3	***	***	<i>n.s.</i> ²	<i>n.s.</i>	<i>n.s.</i>
Drought (B)	2	***	***	***	<i>n.s.</i>	<i>n.s.</i>
A x B	6	**	***	<i>n.s.</i>	*	**
Source of Variation	DF	Mg	S	Fe	Zn	Mn
Biostimulant (A)	3	***	<i>n.s.</i>	*	***	*
Drought (B)	2	***	***	***	***	<i>n.s.</i>
A x B	6	**	***	***	**	<i>n.s.</i>
Source of Variation	DF	Cu	B	Glycine betaine	Proline _(shoot)	Proline _(root)
Biostimulant (A)	3	<i>n.s.</i>	<i>n.s.</i>	***	*	***
Drought (B)	2	***	**	***	***	***
A x B	6	*	**	<i>n.s.</i>	***	***
Source of Variation	DF	MDA	Protein	APX _(total)	CAT _(total)	GR _(total)
Biostimulant (A)	3	***	**	***	***	***
Drought (B)	2	***	**	***	***	*
A x B	6	***	***	***	*	***
Source of Variation	DF	SOD _(total)	APX _(sp.)	CAT _(sp.)	GR _(sp.)	SOD _(sp.)
Biostimulant (A)	3	<i>n.s.</i>	***	**	***	*
Drought (B)	2	<i>n.s.</i>	***	***	<i>n.s.</i>	<i>n.s.</i>
A x B	6	<i>n.s.</i>	***	**	***	***
Source of Variation	DF	AWCD	H'	SR	E	
Biostimulant (A)	3	***	<i>n.s.</i>	***	<i>n.s.</i>	
Drought (B)	2	***	***	***	***	
A x B	6	**	<i>n.s.</i>	**	<i>n.s.</i>	

¹ DF: degree of freedom

² *n.s.*: not significant; * $0.01 < p < 0.05$; ** $0.001 < p < 0.01$; *** $p < 0.001$

The ANOVA results revealed that both drought stress and biostimulant applications individually had significant effects on GB concentrations (**Table 4.5**). Meanwhile, their interaction did not produce a significant effect. While water extract led to a significant increase of 25% relative to the control, irrespective of drought levels, acid extract resulted in a 12% increase (**Figure 4.8A**). On the other hand, GB concentrations increased with the severity of the drought by 48% and 87% under moderate and severe stress conditions, respectively (**Figure 4.8B**).

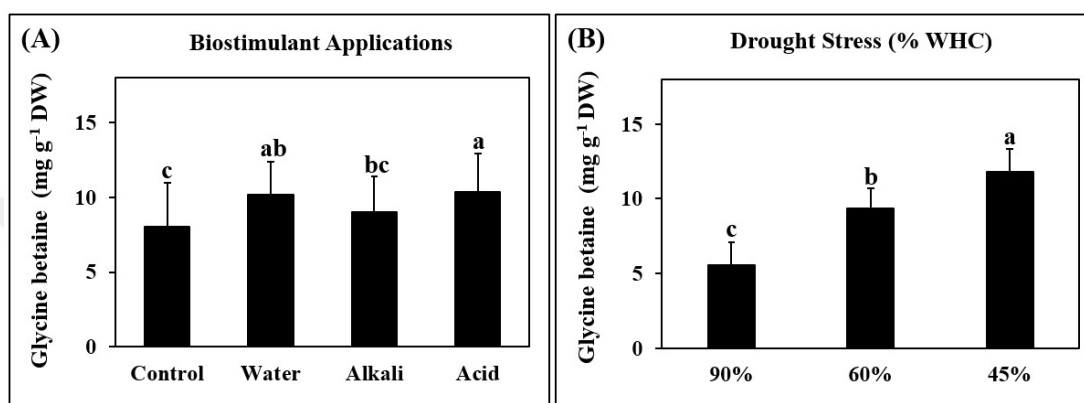


Figure 4.8: Effect of (A) biostimulant applications (control; water extract; alkali extract; acid extract) and (B) drought stress levels (90% WHC; 60% WHC; 45% WHC) on GB concentration (mg g⁻¹ DW) of wheat. Values are means and standard deviations of four replicates. Different letters indicate significant differences between means according to protected HSD test ($p < 0.05$).

The ANOVA results indicated that the effects of both SWEs and drought applications, both individually and in combination, on both shoot and root proline concentration were significant (**Table 4.5**). The most pronounced effect on shoot proline concentration was observed under severe drought stress with the application of water and acid extracts (**Figure 4.9A**). Proline concentrations nearly doubled with water extract application, whereas acid extract application represented an approximately 45% increase compared to the drought-stressed control. The trend continues, with both water and acid extracts led to a significant increase in root proline concentration at 60% WHC (**Figure 4.9B**). However, only the acid extract demonstrated an increase at 45% WHC.

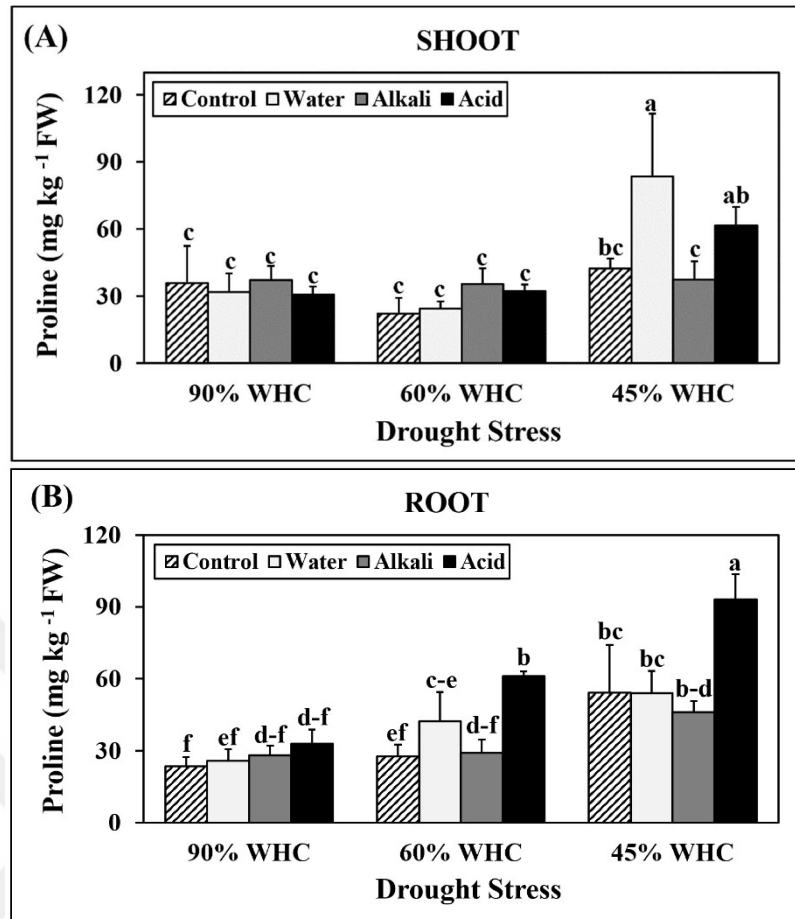


Figure 4.9: Effect of biostimulant applications (control; water extract; alkali extract; acid extract) and drought stress levels (90% WHC; 60% WHC; 45% WHC) on (A) shoot and (B) root proline concentration (mg kg⁻¹FW) of wheat. Values are means and standard deviations of four replicates. Different letters indicate significant differences between means according to protected HSD test ($p < 0.05$)

The significant correlation between drought stress and SWEs treatments on MDA concentration was demonstrated according to ANOVA (**Table 4.5**). As the severity of drought stress increased, the effect of the water extract became more pronounced (**Figure 4.10**). It has been demonstrated that an approximate 2.8-fold increase relative to the control at moderate stress and a six-fold increase at severe stress conditions.

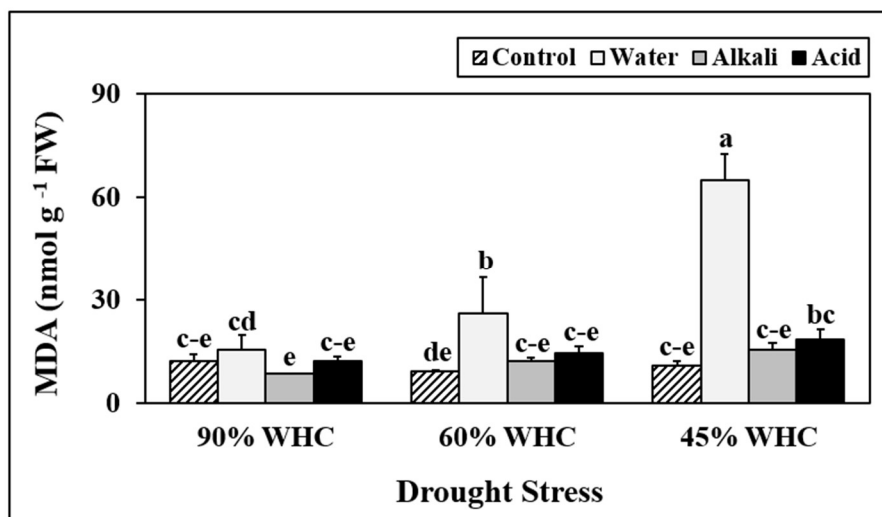


Figure 4.10: Effect of biostimulant applications (control; water extract; alkali extract; acid extract) and drought stress levels (90% WHC; 60% WHC; 45% WHC) on MDA concentration (nmol g⁻¹ FW) of wheat. Values are means and standard deviations of four replicates. Different letters indicate significant differences between means according to protected HSD test ($p < 0.05$).

The protein concentrations of plants were significantly influenced by both the main effects of drought and SWEs applications and their interaction as shown in ANOVA (Table 4.5). The marked increase was reported at moderate drought stress, where acid extract application resulted highest increase among all treatment groups (Figure 4.11).

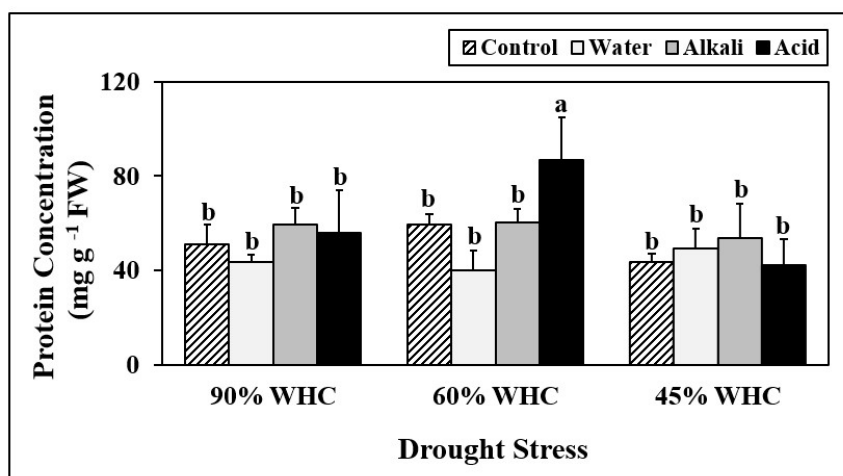


Figure 4.11: Effect of biostimulant applications (control; water extract; alkali extract; acid extract) and drought stress levels (90% WHC; 60% WHC; 45% WHC) on protein concentration (mg g⁻¹) of wheat. Values are means and standard deviations of four replicates. Different letters indicate significant differences between means according to protected HSD test ($p < 0.05$).

The ANOVA results indicated a significant interaction of biostimulant application and drought stress on total and specific enzyme activities (APX, CAT, and GR) (**Table 4.5**). Total APX activity declined as drought severity increased, independent of SWEs applications (**Table 4.6**). Water and alkali extracts resulted in reductions of 40% and 60% compared to control in total APX level, respectively, irrespective of drought level. Specific APX activity results exhibited a similar trend (**Table 4.7**). Specifically, acid extract applications at severe stress level resulted in a two-fold increase in both total and specific APX activity relative to the stressed control. Other than that, severe drought stress markedly elevated total CAT activity, with all biostimulants enhancing it by approximately 86%. The specific CAT activity exhibited a consistent trend. In addition, acid extract significantly increased specific activity of GR regardless of the drought level. Moreover, total SOD activity remained constant, while specific SOD activity exhibited small increases with SWEs applications.

Table 4.6: Effect of biostimulant applications (control; water extract; alkali extract; acid extract) and drought stress levels (90% WHC; 60% WHC; 45% WHC) on the total antioxidant enzyme activities of shoot of wheat

Biostimulant	Drought Stress (% WHC)	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	90	17.2 ± 5.0 a	2.9 ± 0.2 b	2.2 ± 0.3 c-e	105 ± 9 a
	60	8.1 ± 1.3 bc	3.1 ± 0.2 b	1.6 ± 0.1 e	109 ± 10 a
	45	8.5 ± 2.7 bc	3.8 ± 0.3 b	2.1 ± 0.4 c-e	106 ± 11 a
Water	90	7.2 ± 2.7 cd	3.6 ± 0.2 b	2.4 ± 0.4 c-e	112 ± 10 a
	60	3.6 ± 1.3 cd	3.6 ± 0.4 b	2.3 ± 0.3 c-e	108 ± 10 a
	45	3.5 ± 1.0 cd	5.4 ± 0.2 a	1.8 ± 0.2 de	115 ± 13 a
Alkali	90	13.9 ± 7.3 ab	3.3 ± 0.2 b	2.6 ± 0.1 b-d	113 ± 6 a
	60	4.1 ± 1.2 cd	3.5 ± 0.4 b	2.6 ± 0.2 b-d	107 ± 2 a
	45	2.0 ± 0.4 d	5.4 ± 0.5 a	2.8 ± 0.6 bc	106 ± 9 a
Acid	90	7.5 ± 1.1 cd	3.3 ± 0.3 b	3.5 ± 0.6 ab	110 ± 4 a
	60	2.9 ± 0.9 cd	3.4 ± 0.1 b	4.1 ± 0.3 a	109 ± 9 a
	45	17.2 ± 0.4 a	5.5 ± 1.4 a	2.7 ± 0.5 b-d	111 ± 6 a

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

Table 4.7: Effect of biostimulant applications (control; water extract; alkali extract; acid extract) and drought stress levels (90% WHC; 60% WHC; 45% WHC) on the specific antioxidant enzyme activities of shoot of wheat

Biostimulant	Drought Stress (% WHC)	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	90	302 ± 96 b	45 ± 8 de	38 ± 9 b-d	1.6 ± 0.4 bc
	60	137 ± 29 d-g	52 ± 3 c-e	28 ± 3 d	1.8 ± 0.3 a-c
	45	192 ± 52 b-d	87 ± 13 b-d	49 ± 9 a-d	2.4 ± 0.2 ab
Water	90	159 ± 50 b-e	82 ± 8 b-e	54 ± 5 a-c	2.6 ± 0.2 ab
	60	72 ± 25 de	80 ± 24 b-e	61 ± 20 ab	2.8 ± 0.6 a-c
	45	83 ± 7 de	114 ± 29 ab	34 ± 9 cd	1.8 ± 0.5 a-c
Alkali	90	242 ± 153 bc	57 ± 9 c-e	45 ± 6 a-d	1.9 ± 0.4 a-c
	60	69 ± 24 de	58 ± 9 c-e	44 ± 6 a-d	1.8 ± 0.2 a-c
	45	33 ± 2 e	91 ± 14 bc	54 ± 14 a-c	1.8 ± 0.5 a
Acid	90	156 ± 73 b-e	63 ± 17 c-e	65 ± 14 a	2.1 ± 0.7 a-c
	60	55 ± 41 de	41 ± 13 e	51 ± 15 a-d	1.4 ± 0.5 c
	45	470 ± 128 a	151 ± 52 a	66 ± 13 a	2.8 ± 0.8 a

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

A significant effect of interaction of biostimulant and drought treatments was recorded for average well color development (AWCD) and substrate richness (SR) values as shown in ANOVA results (**Table 4.5**). AWCD demonstrated a significant decline, irrespective of SWEs applications, with increasing drought severity (**Table 4.8**). In contrast, the water extract application demonstrated a 37% rise in AWCD at 90% WHC and a 30% increase at 60% WHC. In addition, drought stress application caused by 25% and 36% decrease in SR, regardless of biostimulant applications, at moderate and severe stress conditions, respectively.

Table 4.8: Effect of biostimulant applications (control; water extract; alkali extract; acid extract) and drought stress levels (90% WHC; 60% WHC; 45% WHC) on average well color development (AWCD), Shannon index (H'), substrate richness (SR), and evenness index (E) of soil microbiota

Biostimulant	Drought Stress (% WHC)	AWCD	Shannon Index (H')	Substrate Richness (SR)	Evenness Index (E)
Control	90	0.87 ± 0.12 bc	3.1 ± 0.2 a	26 ± 2 a	0.98 ± 0.01 a
	60	0.67 ± 0.12 cd	2.7 ± 0.2 a	16 ± 3 c	1.03 ± 0.05 a
	45	0.30 ± 0.10 e	2.5 ± 0.2 a	11 ± 3 de	1.14 ± 0.11 a
Water	90	1.19 ± 0.18 a	3.1 ± 0.1 a	25 ± 3 a	0.98 ± 0.01 a
	60	0.87 ± 0.20 bc	2.8 ± 0.2 a	19 ± 4 bc	1.02 ± 0.05 a
	45	0.37 ± 0.06 e	2.5 ± 0.2 a	7 ± 2 e	1.19 ± 0.17 a
Alkali	90	0.94 ± 0.14 b	3.1 ± 0.0 a	22 ± 2 ab	1.00 ± 0.01 a
	60	0.43 ± 0.15 de	2.7 ± 0.3 a	11 ± 2 de	1.04 ± 0.02 a
	45	0.28 ± 0.04 e	2.3 ± 0.1 a	8 ± 1 e	1.13 ± 0.10 a
Acid	90	0.99 ± 0.09 ab	3.0 ± 0.1 a	22 ± 4 ab	1.00 ± 0.04 a
	60	0.44 ± 0.04 de	2.7 ± 0.1 a	15 ± 2 cd	1.05 ± 0.07 a
	45	0.35 ± 0.10 e	2.5 ± 0.1 a	11 ± 1 de	1.06 ± 0.03 a

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

A comparison of the percentage distribution of substrate average well color development (SAWCD) reveals that the utilization of different substrates varies according to drought stress applications (**Figure 4.12**). The utilization of carbohydrates, carboxylic acids, and polymers increased simultaneously with the severity of the drought. Amino acids demonstrated only minor fluctuations in utilization across varying drought levels. When stress levels were high, phenolic acids were used less except acid extract application under 60% WHC. Moreover, the amins were only used in the control. Although biostimulant applications did not exert a consistent influence on the percentage distribution of SAWCD across the substrate groups, the water extract notably enhanced carbohydrate utilization.

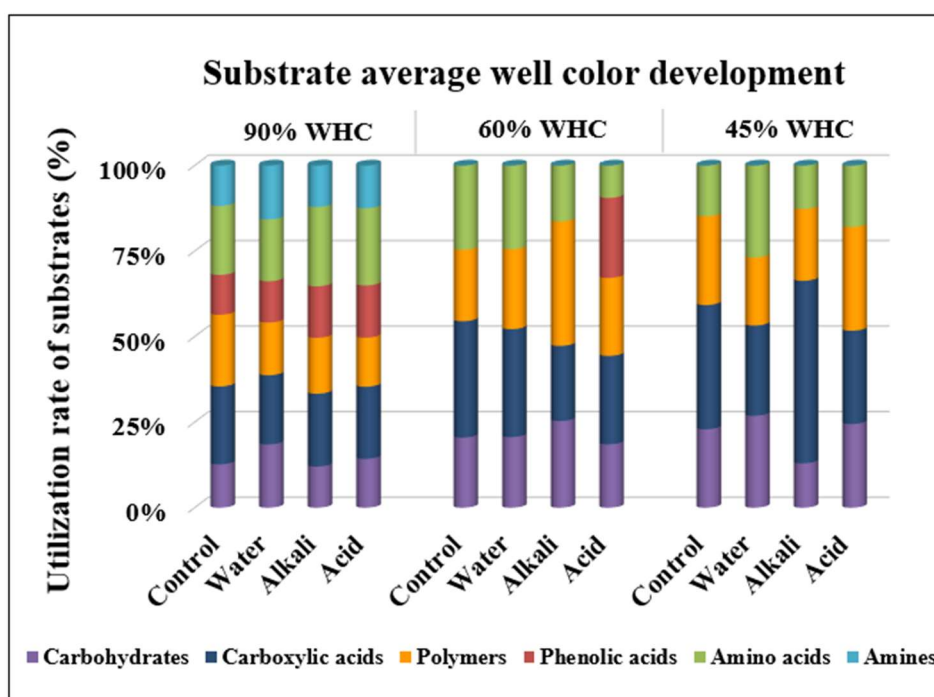


Figure 4.12: Effect of biostimulant applications (control; water extract; alkali extract; acid extract) and drought stress levels (90% WHC; 60% WHC; 45% WHC) on Substrate Average Well Color Development (SAWCD) of soil microbiota

4.1.4. Investigation of Biostimulant Effects of *C. barbata* Extracts in Wheat by Foliar Application

The experiment was carried out to establish effective concentration on wheat growth of *C. barbata* extracts when applied as a foliar treatment. As shown in **Figure 4.13**, plants treated with SWEs exhibited improved growth performance compared to the control. Seaweed extracts demonstrated a significant effect on the growth of the plants (**Table 4.9**), exhibiting significant increases in the range of 13-22% in shoot DW (**Figure 4.14**). The high rate of water, the low rate of alkali extract, the high rate of acid extract was selected based on growth responses.



Figure 4.13: Effect of foliar biostimulant applications (control (water); water extracts (low, medium, high); alkali extracts (low, medium, high); acid extracts (low, medium, high) on wheat growth

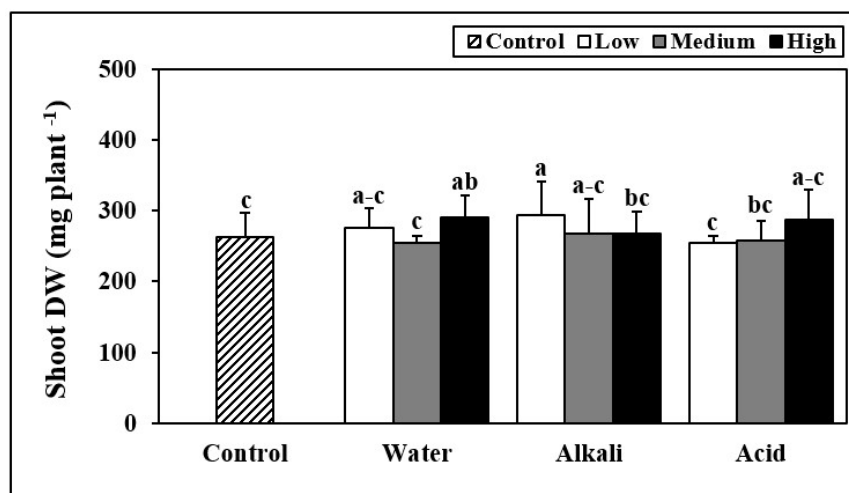


Figure 4.14: Effect of foliar biostimulant applications (control (water); water extracts (low, medium, high); alkali extracts (low, medium, high); acid extracts (low, medium, high) on wheat shoot DWs. Values are means and standard deviations of four replicates. Different letters indicate significant differences between means according to Fisher's protected LSD test ($p < 0.05$).

Table 4.9: The effects of foliar application of biostimulants on reported traits of soil grown wheat according to one-way ANOVA ($\alpha = 0.05$)

Source of Variation	DF ¹	Shoot DW	P	K	Ca
Biostimulant	9	*	<i>n.s.</i> ²	**	<i>n.s.</i>
Source of Variation	DF	Mg	S	Fe	Zn
Biostimulant	9	***	***	***	<i>n.s.</i>
Source of Variation	DF	Mn	Cu	B	
Biostimulant	9	<i>n.s.</i>	**	*	

¹DF; Degree of freedom

²*n.s.* Not significant; * $0.01 \leq F \text{ Pr.} < 0.05$; ** $0.001 \leq F \text{ Pr.} < 0.01$; *** $F \text{ Pr.} < 0.001$

Significant effects in macroelement concentrations (K, Mg, and S) according to SWE applications has been shown in **Table 4.9**. While P and Ca concentrations did not change among SWEs applications, K and Mg concentrations decreased slightly with the application of water extract irrespective of the dose (**Table 4.10**). On the other hand, the alkali and acid extracts treatments led to an average reduction of approximately 15% in S level regardless of drought level.

Table 4.10: Effect of foliar biostimulant applications (control (water); water extracts (low, medium, high); alkali extracts (low, medium, high); acid extracts (low, medium, high) on macroelement concentration (P, K, Ca, Mg, S) (mg g^{-1}) of wheat

Biostimulant	P (mg g^{-1})	K (mg g^{-1})	Ca (mg g^{-1})	Mg (mg g^{-1})	S (mg g^{-1})
Control	9.8 ± 0.5 a	52 ± 1 ab	10.7 ± 0.6 a	1.96 ± 0.10 bc	4.7 ± 0.3 a
Water-L	9.8 ± 0.6 a	49 ± 1 cd	10.9 ± 0.3 a	1.86 ± 0.03 e	4.5 ± 0.4 ab
Water-M	9.4 ± 0.6 a	49 ± 1 b-d	10.6 ± 0.4 a	1.87 ± 0.01 de	4.3 ± 0.2 bc
Water-H	9.4 ± 0.2 a	49 ± 3 d	10.8 ± 0.9 a	1.91 ± 0.07 c-e	4.5 ± 0.1 ab
Alkali-L	10.4 ± 0.8 a	54 ± 2 a	11.1 ± 0.6 a	2.05 ± 0.02 a	4.0 ± 0.1 d
Alkali-M	9.9 ± 0.5 a	52 ± 2 a	11.1 ± 0.2 a	2.03 ± 0.01 ab	3.9 ± 0.0 d
Alkali-H	9.9 ± 0.5 a	53 ± 2 a	10.4 ± 0.3 a	1.93 ± 0.03 c-e	3.8 ± 0.1 d
Acid-L	10.0 ± 0.1 a	53 ± 2 a	10.8 ± 0.6 a	1.93 ± 0.04 c-e	3.9 ± 0.2 d
Acid-M	10.3 ± 0.4 a	52 ± 3 ab	11.1 ± 0.6 a	1.94 ± 0.06 cd	4.2 ± 0.0 c
Acid-H	10.0 ± 0.3 a	52 ± 2 a-c	11.0 ± 0.2 a	1.95 ± 0.03 bc	4.1 ± 0.1 cd

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

The concentration of Fe exhibited a significant increase, with high rates of water and acid extractions with 47% and 25%, respectively (**Table 4.11**). Biostimulant applications did not lead to noteworthy effects on Zn and Mn concentrations. Similarly, Cu and B concentrations exhibited fluctuations, though they were not consistent.

Table 4.11: Effect of foliar biostimulant applications (control (water); water extracts (low, medium, high); alkali extracts (low, medium, high); acid extracts (low, medium, high) on microelement concentration (Fe, Zn, Mn, Cu, B) (mg kg⁻¹) of wheat

Biostimulant	Fe (mg kg ⁻¹)	Zn (mg kg ⁻¹)	Mn (mg kg ⁻¹)	Cu (mg kg ⁻¹)	B (mg kg ⁻¹)
Control	144 ± 34 cd	98 ± 6 a	44 ± 4 a	10.9 ± 0.5 b-d	9.5 ± 1.6 bc
Water-L	131 ± 13 d	94 ± 6 a	38 ± 3 a	10.4 ± 0.8 cd	9.5 ± 0.6 a-c
Water-M	168 ± 27 bc	100 ± 4 a	43 ± 3 a	10.2 ± 0.3 d	10.1 ± 1.1 ab
Water-H	212 ± 63 a	101 ± 2 a	44 ± 4 a	10.8 ± 0.3 b-d	11.0 ± 1.4 a
Alkali-L	128 ± 5 d	104 ± 4 a	46 ± 8 a	12.0 ± 0.7 a	8.3 ± 1.4 c
Alkali-M	154 ± 22 b-d	106 ± 1 a	47 ± 3 a	11.5 ± 0.5 ab	10.3 ± 0.5 ab
Alkali-H	135 ± 29 cd	106 ± 8 a	42 ± 2 a	11.2 ± 0.5 a-c	10.2 ± 1.2 ab
Acid-L	147 ± 15 b-d	103 ± 3 a	40 ± 2 a	10.9 ± 0.6 b-d	11.0 ± 0.6 ab
Acid-M	140 ± 24 cd	111 ± 10 a	43 ± 5 a	11.2 ± 0.4 a-c	10.4 ± 0.9 ab
Acid-H	180 ± 10 ab	106 ± 14 a	43 ± 2 a	10.5 ± 0.8 cd	10.5 ± 0.5 ab

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

4.1.5. The Impact of Selected *C. barbata* Extracts Treatment on Yield of Wheat by Foliar Application

The figure illustrates the differences in the long-term growth of wheat plants in response to foliar applications of selected rates of SWEs (**Figure 4.15**). When compared other SWEs, the use of water extract exhibited a greater growth response. This observation was further supported by the ANOVA results (**Table 4.13**). Significant increases in grain yield have also been reported as a result of water and acid extracts (**Table 4.12**). In addition, other growth parameters were not positively or negatively affected by the biostimulant applications.

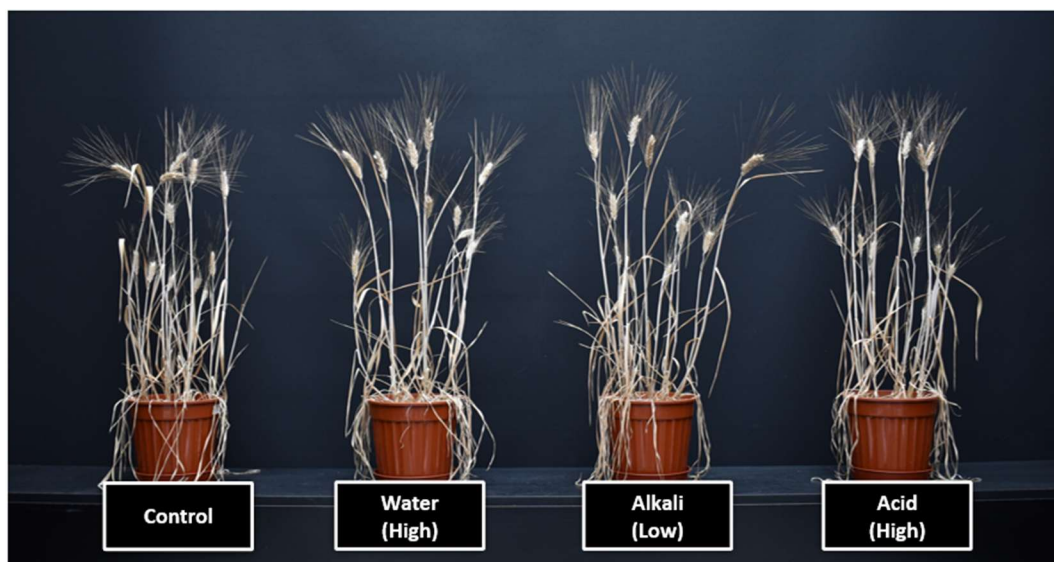


Figure 4.15: Effect of foliar biostimulant applications (control (water); water extract (high); alkali extract (low); acid extract (high) on long-term wheat growth

Table 4.12: Effect of foliar biostimulant applications (control (water); water extract (high); alkali extract (low); acid extract (high) on grain yield (g plant^{-1}), average grain weight (mg), harvest index, straw weight (g plant^{-1}), spike weight (g plant^{-1}), and spike number (plant^{-1}) of wheat

Biostimulant	Grain yield (g plant^{-1})	Average grain weight (mg)	Harvest index
Control	2.5 ± 0.2 b	49 ± 2 a	43 ± 3 a
Water	3.1 ± 0.2 a	47 ± 3 a	47 ± 1 a
Alkali	2.8 ± 0.3 ab	51 ± 2 a	44 ± 4 a
Acid	2.9 ± 0.1 a	51 ± 3 a	47 ± 4 a
Biostimulant	Straw weight (g plant^{-1})	Spike weight (g plant^{-1})	Spike number (plant^{-1})
Control	2.6 ± 0.5 a	3.9 ± 0.6 a	2.6 ± 0.5 a
Water	2.3 ± 0.1 a	4.1 ± 0.3 a	2.5 ± 0.3 a
Alkali	2.3 ± 0.1 a	4.1 ± 0.4 a	2.4 ± 0.3 a
Acid	2.3 ± 0.2 a	3.9 ± 0.2 a	2.3 ± 0.2 a

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

Table 4.13: The effects of foliar application of selected biostimulants on reported traits of soil grown wheat according to one-way ANOVA ($\alpha = 0.05$)

Source of Variation	DF ¹	Grain yield	Average grain weight	Harvest index	Straw weight
Biostimulant	3	*	<i>n.s</i>	<i>n.s</i>	<i>n.s</i>
Source of Variation	DF	Spike weight	Spike number	P	K
Biostimulant	3	<i>n.s</i>	<i>n.s</i>	<i>n.s</i>	<i>n.s</i>
Source of Variation	DF	Ca	Mg	S	Fe
Biostimulant	3	<i>n.s</i>	<i>n.s</i>	<i>n.s</i>	<i>n.s</i>
Source of Variation	DF	Zn	Mn	Cu	Mo
Biostimulant	3	<i>n.s</i>	<i>n.s</i>	<i>n.s</i>	<i>n.s</i>

¹DF; Degree of freedom

²*n.s.* Not significant; * $0.01 \leq F \text{ Pr.} < 0.05$; ** $0.001 \leq F \text{ Pr.} < 0.01$; *** $F \text{ Pr.} < 0.001$

The alterations in macro- and microelement concentrations in the grains in response to SWEs applications have been reported in **Table 4.14** and **Table 4.15**. Notably, all treatment groups were found to be within the same concentration range with no significant differences in response to biostimulant applications.

Table 4.14: Effect of foliar biostimulant applications (control (water); water extract (high); alkali extract (low); acid extract (high) on macroelement concentration (P, K, Ca, Mg, S) (mg g^{-1}) of wheat

Biostimulant	P (mg g^{-1})	K (mg g^{-1})	Ca (mg g^{-1})	Mg (mg g^{-1})	S (mg g^{-1})
Control	4.6±0.1 a	5.6±0.4 a	0.6±0.0 a	1.3±0.1 a	2.1±0.1 a
Water	4.5±0.2 a	5.6±0.3 a	0.6±0.1 a	1.3±0.1 a	2.1±0.1 a
Alkali	4.3±0.2 a	5.7±0.1 a	0.6±0.1 a	1.3±0.1 a	2.0±0.1 a
Acid	4.5±0.2 a	5.7±0.3 a	0.6±0.1 a	1.3±0.1 a	2.1±0.1 a

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

Table 4.15: Effect of foliar biostimulant applications (control (water); water extracts (high); alkali extracts (low); acid extracts (high) on microelement concentration (Fe, Zn, Mn, Cu, Mo) (mg kg⁻¹) of wheat

Biostimulant	Fe (mg kg ⁻¹)	Zn (mg kg ⁻¹)	Mn (mg kg ⁻¹)	Cu (mg kg ⁻¹)	Mo (mg kg ⁻¹)
Control	42 ± 3 a	79 ± 6 a	32 ± 4 a	7.6 ± 0.3 a	2.3 ± 0.1 a
Water	41 ± 5 a	76 ± 12 a	32 ± 6 a	7.7 ± 0.7 a	2.4 ± 0.5 a
Alkali	39 ± 2 a	73 ± 6 a	28 ± 3 a	7.4 ± 0.2 a	2.4 ± 0.4 a
Acid	40 ± 2 a	77 ± 14 a	29 ± 4 a	7.5 ± 0.7 a	2.3 ± 0.3 a

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

4.2. Tomato Experiments

4.2.1. Investigation of Biostimulant Effects of *C. barbata* Extracts on Tomato by Drench Application

This experiment was carried out to establish biostimulant effects of SWEs on tomato yield and quality in the long term. The results of ANOVA demonstrated a significant effect of SWEs applications on the early growth parameters of tomato (**Table 4.20**). While alkali and acid extracts led to a significant increase in stem length of approximately 40–60%, acid extract had the highest number of inflorescences among treatments (**Table 4.16**). On the other hand, the application of acid and water extracts increased the number of flowers by an average of 56%.

Table 4.16: Effect of biostimulant applications (control (water); water extract; alkali extract; acid extract) on stem length (cm), number of inflorescence (plant⁻¹), and number of flowers (plant⁻¹) of tomato at 35 DAP

Biostimulant	Stem Length (cm)	Number of Inflorescence (plant ⁻¹)	Number of Flowers (plant ⁻¹)
Control	60 ± 10 b	3.8 ± 0.4 b	9 ± 2 b
Water	65 ± 12 b	4.8 ± 1.5 b	14 ± 3 a
Alkali	84 ± 7 a	5.5 ± 1.3 ab	10 ± 2 b
Acid	97 ± 12 a	6.8 ± 1.7 a	14 ± 2 a

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

The significant impact of SWE applications on the tomato yield was revealed by ANOVA (**Table 4.20**). The tomato yield was increased according to the application of alkali and acid extracts, by average increase of 64%. In addition, water extract led to modest increase in fruit yield (**Figure 4.16**).

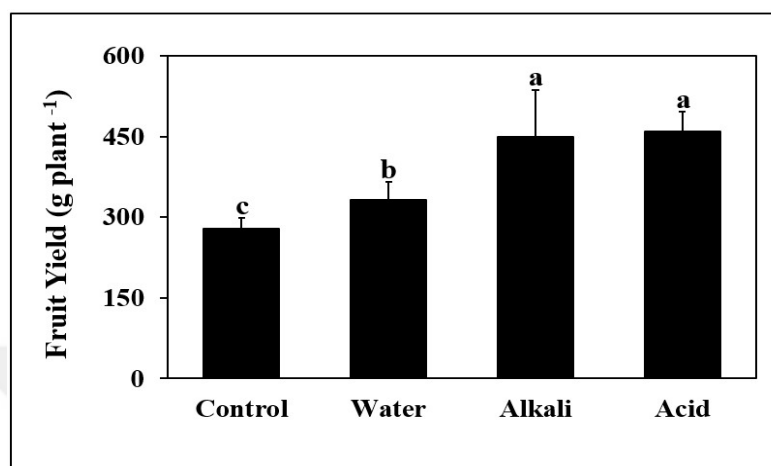


Figure 4.16: Effect of biostimulant applications (control (water); water extract; alkali extract; acid extract) on fruit yield (g plant⁻¹) of tomato. Values are means and standard deviations of five replicates. Different letters indicate significant differences between means according to Fisher's protected LSD test ($p < 0.05$).

The application of *C. barbata* extracts facilitated significant effects in vegetative growth characteristics of tomato plants according to ANOVA (**Table 4.20**). All three SWEs resulted notable improvements with increases reaching 55% in stem DW, 61% in leaf DW, and 58% in total shoot DW (**Figure 4.17**).

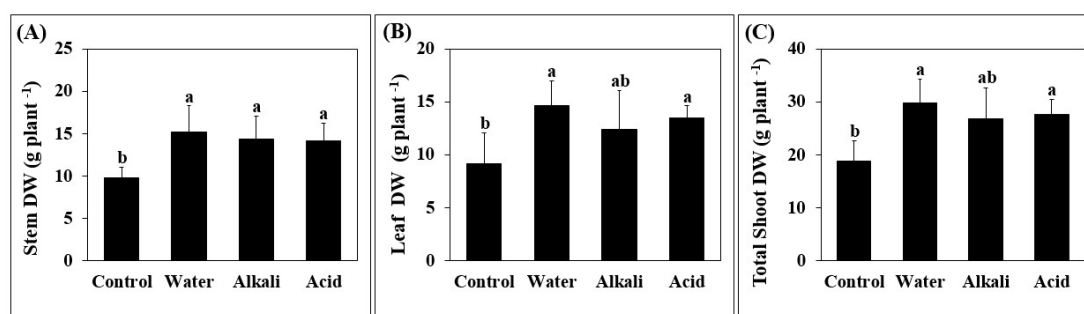


Figure 4.17: Effect of biostimulant applications (control (water); water extract; alkali extract; acid extract) on (A) stem, (B) leaf, and (C) total shoot DW (g plant⁻¹). Values are means and standard deviations of five replicates. Different letters indicate significant differences between means according to Fisher's protected LSD test ($p < 0.05$).

The SWEs treatments resulted in a significant reduction in fruit pH (**Table 4.20**). In contrast, The EC values increased by between 30% and 70% in SWEs-treated plants compared to control (**Table 4.17**). The Brix values of the fruits elevated by 15 to 39% in response to SWE treatments.

Table 4.17: Effect of biostimulant applications (control (water); water extract; alkali extract; acid extract) on pH, EC ($\mu\text{S cm}^{-1}$), Brix ($^{\circ}\text{Bx}$) of tomato fruit

Biostimulant	pH	EC ($\mu\text{S cm}^{-1}$)	Brix ($^{\circ}\text{Bx}$)
Control	4.8 $\pm$ 0.1 a	3409 $\pm$ 110 d	5.4 $\pm$ 0.5 c
Water	4.2 $\pm$ 0.1 b	5739 $\pm$ 140 a	7.5 $\pm$ 0.5 a
Alkali	4.3 $\pm$ 0.0 b	4913 $\pm$ 219 b	6.9 $\pm$ 0.7 ab
Acid	4.3 $\pm$ 0.1 b	4381 $\pm$ 155 c	6.2 $\pm$ 0.3 b

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

Significant effects of SWEs on K, Ca, and S concentrations demonstrated by ANOVA (**Table 4.20**). The utilization of water and alkali extracts resulted slight increments in K level of fruits, whereas Ca level decreased in all SWE-treated plants relative to control (**Table 4.18**). Application of *C. barbata* extracts resulted in fruit S levels that were 1.5 to 1.75 times higher than those of control plants.

Table 4.18: Concentrations of tomato fruits macronutrient (P, K, Ca, Mg, S) (mg g^{-1}) concentrations of tomato fruits in response to biostimulant applications (control (water); water extract; alkali extract; acid extract)

Biostimulant	P (mg g^{-1})	K (mg g^{-1})	Ca (mg g^{-1})	Mg (mg g^{-1})	S (mg g^{-1})
Control	3.0 $\pm$ 0.3 a	25 $\pm$ 1 b	3.1 $\pm$ 0.2 a	1.2 $\pm$ 0.1 a	0.8 $\pm$ 0.1 c
Water	2.6 $\pm$ 0.1 a	27 $\pm$ 0 a	2.1 $\pm$ 0.5 b	1.2 $\pm$ 0.1 a	1.2 $\pm$ 0.1 b
Alkali	3.0 $\pm$ 0.2 a	27 $\pm$ 1 a	2.3 $\pm$ 0.1 b	1.3 $\pm$ 0.1 a	1.4 $\pm$ 0.1 a
Acid	2.9 $\pm$ 0.3 a	25 $\pm$ 1 b	2.4 $\pm$ 0.1 b	1.2 $\pm$ 0.1 a	1.2 $\pm$ 0.1 b

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

The concentrations of micronutrients of tomato fruits in response to the biostimulants are shown in **Table 4.19**. Seaweed extracts did not have a significant effect on the fruit concentrations of Fe, Zn, and Cu. The Mn concentration was elevated by 25% in alkali-

and acid-extract-treated plants, while the B concentration was increased by 26% with water extract treatment.

Table 4.19: Concentrations of tomato fruits micronutrient (Fe, Zn, Mn, Cu, B) (mg kg⁻¹) concentrations of fruits in response to biostimulant applications (control (water); water extract; alkali extract; acid extract)

Biostimulant	Fe (mg kg ⁻¹)	Zn (mg kg ⁻¹)	Mn (mg kg ⁻¹)	Cu (mg kg ⁻¹)	B (mg kg ⁻¹)
Control	32 ± 2 a	5.6 ± 0.5 a	8.6 ± 0.7 b	3.2 ± 0.2 a	8.8 ± 0.6 b
Water	31 ± 4 a	5.5 ± 0.6 a	8.8 ± 0.9 b	3.1 ± 0.4 a	11.1 ± 1.9 a
Alkali	35 ± 3 a	5.7 ± 0.7 a	11.0 ± 0.5 a	3.5 ± 0.6 a	9.0 ± 0.9 b
Acid	33 ± 3 a	5.6 ± 0.5 a	10.4 ± 0.6 a	3.2 ± 0.3 a	9.5 ± 0.4 b

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

All biostimulant applications significantly increased the TPC of tomato fruits by about 20% compared to control (**Figure 4.18A**). The antioxidant activity, as measured by ABTS, DPPH, and FRAP, was approximately 20-40% higher in plants treated with SWEs (**Figures 4.18B-D**).

Table 4.20: The effects of drench application of biostimulants on reported traits of soil grown tomato according to one-way ANOVA ($\alpha = 0.05$)

Source of Variation	DF ¹	Stem Length	Number of Inflorescence	Number of Flowers	Fruit Yield
Biostimulant	3	**	*	**	***
Source of Variation	DF	Stem DW	Leaf DW	Total Shoot DW	pH
Biostimulant	3	*	*	***	***
Source of Variation	DF	EC	Brix	P	K
Biostimulant	3	***	***	<i>n.s.</i>	**
Source of Variation	DF	Ca	Mg	S	Fe
Biostimulant	3	***	<i>n.s.</i>	***	<i>n.s.</i>
Source of Variation	DF	Zn	Mn	Cu	B
Biostimulant	3	<i>n.s.</i>	***	<i>n.s.</i>	*
Source of Variation	DF	TPC	ABTS	DPPH	FRAP
Biostimulant	3	*	**	***	***
Source of Variation	DF	Lycopene	β -Carotene	Total AsA	Reduced AsA
Biostimulant	3	*	***	*	***
Source of Variation	DF	GSH	Soluble Carbohydrate		
Biostimulant	3	*	**		

¹DF; Degree of freedom

²*n.s.* Not significant; * $0.01 \leq F \text{ Pr.} < 0.05$; ** $0.001 \leq F \text{ Pr.} < 0.01$; *** $F \text{ Pr.} < 0.001$

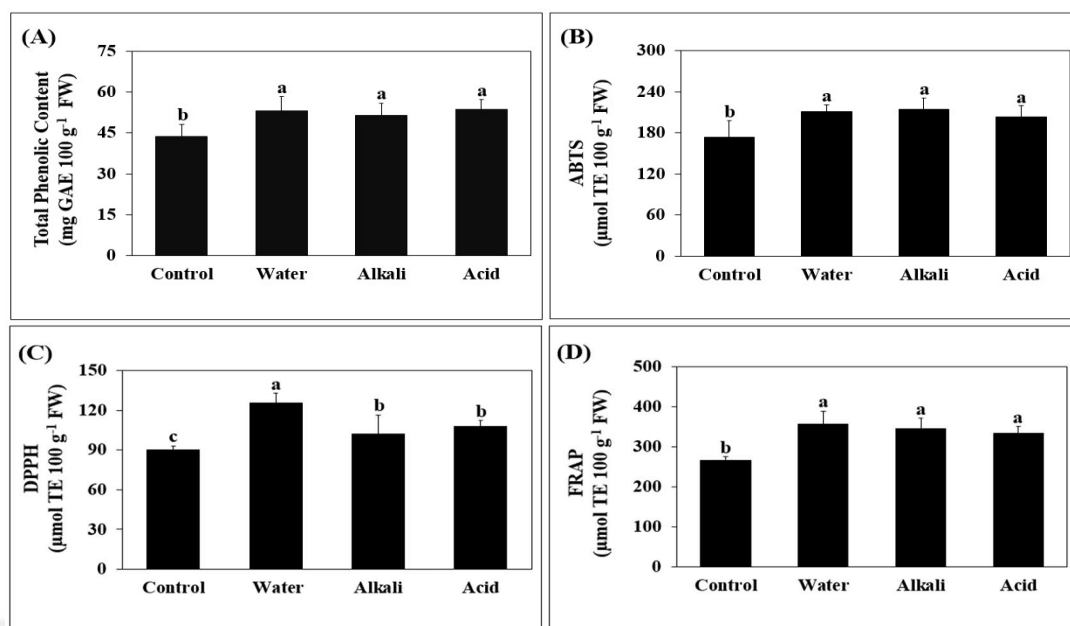


Figure 4.18: Effect of biostimulant applications (control (water); water extract; alkali extract; acid extract) on the (A) TPC as Gallic acid equivalent (mg GAE 100 g⁻¹FW), (B) ABTS, (C) DPPH, and (D) FRAP antioxidant activity as Trolox equivalent (μmol TE 100 g⁻¹FW) of tomato fruits. Values are means and standard deviations of five replicates. Different letters indicate significant differences between means according to Fisher's protected LSD test ($p < 0.05$).

Biostimulant applications demonstrated significant effects on both lycopene and β -carotene concentration of tomato fruits according to ANOVA (**Table 4.20**). Applying water extract resulted in a 43% improvement in lycopene concentration (**Figure 4.19A**). Furthermore, β -carotene concentrations were almost twofold in plants subjected to water and alkali extracts (**Figure 4.19B**).

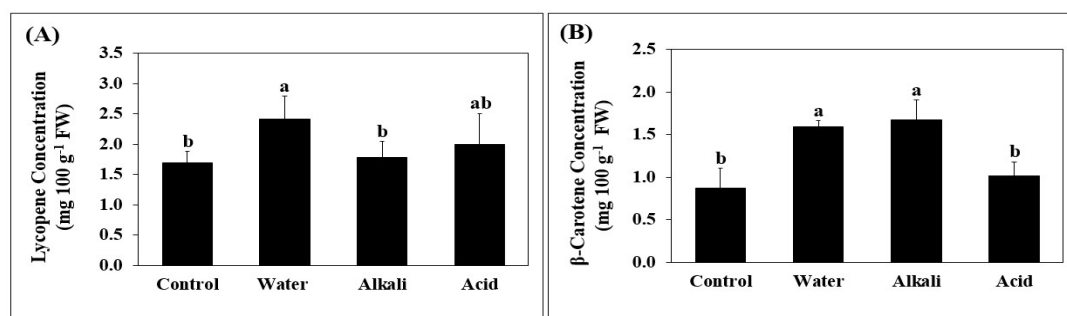


Figure 4.19: Effect of biostimulant applications (control (water); water extract; alkali extract; acid extract) on the (A) lycopene concentration and (B) β -carotene concentration (mg 100 g⁻¹ FW) of tomato fruits. Values are means and standard deviations of five replicates. Different letters indicate significant differences between means according to Fisher's protected LSD test ($p < 0.05$).

The variations in both total and reduced AsA concentrations of tomato fruits in response to SWEs treatments were presented in **Table 4.20**. A notable increase of up to 1.5-fold in total AsA was reported in both water and acid extracts applications relative to control. The improvement in reduced AsA varied between 80% and 130% in response to SWE applications.

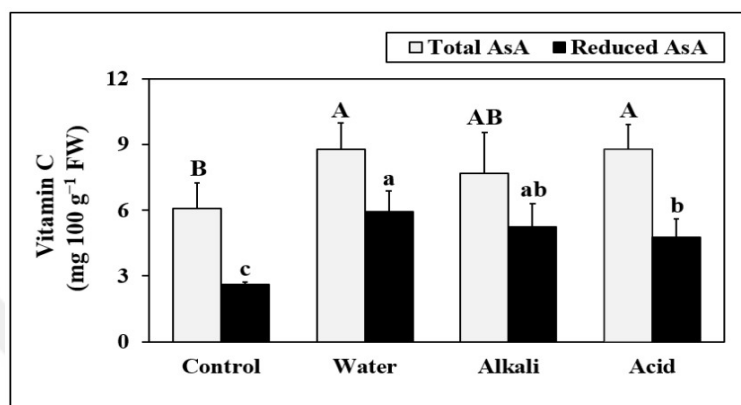


Figure 4.20: Effect of biostimulant applications (control (water); water extract; alkali extract; acid extract) on the total AsA and reduced AsA concentration (mg 100 g⁻¹FW) of tomato fruits. Values are means and standard deviations of five replicates. Uppercase letters and lowercase letters indicate significant differences between means according to Fisher's protected LSD test ($p < 0.05$) in the total and reduced AsA, respectively.

According to ANOVA (**Table 4.20**), the GSH concentration was significantly affected by the *C. barbata* applications, increasing by 24% relative to the control (**Figure 4.21**).

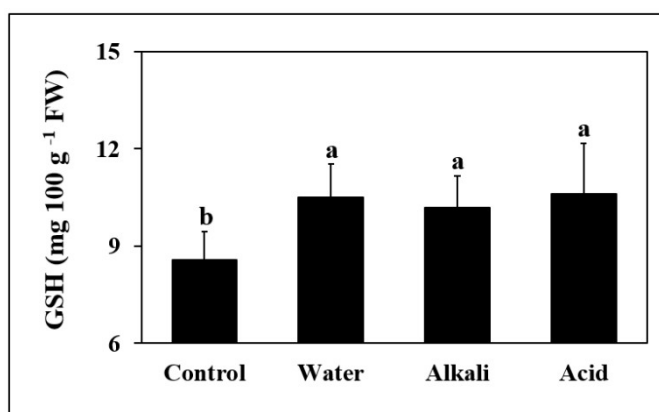


Figure 4.21: Effect of biostimulant applications (control (water); water extract; alkali extract; acid extract) on the GSH concentration (mg 100 g⁻¹ FW) of tomato. Values are means and standard deviations of five replicates. Different letters indicate significant differences between means according to Fisher's protected LSD test ($p < 0.05$).

Water or alkali extract applications exhibited a significant increase in soluble carbohydrates according to ANOVA (**Table 4.20**) by a 39% increase relative to control (**Figure 4.22**).

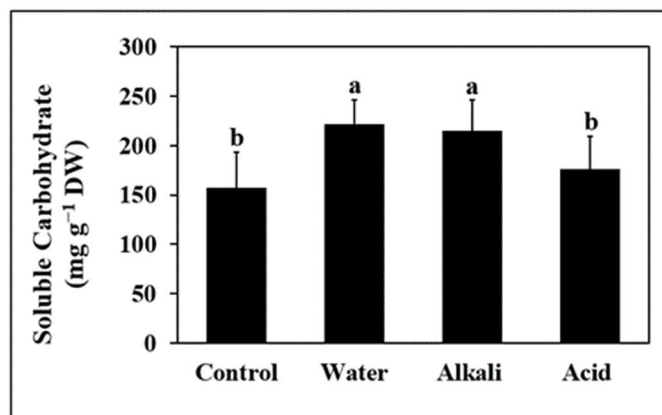


Figure 4.22: Effect of biostimulant applications (control (water); water extract; alkali extract; acid extract) on the soluble carbohydrate concentration (mg g⁻¹ FW) of tomato. Values are means and standard deviations of five replicates. Different letters indicate significant differences between means according to Fisher's protected LSD test ($p < 0.05$).

4.2.2. Investigation of Biostimulant Effects of *C. barbata* Extracts on Tomato by Foliar Application

This research was assessed to define biostimulant effects of SWEs which applied as foliar treatments on tomato growth and yield in the long term. There was no significant effect of SWEs on stem length and number of inflorescences, whereas number of flowers were decreased significantly in response to acid and alkali extracts applications in the early growth (**Table 4.21**).

Table 4.21: Effect of foliar biostimulant applications (control (water); water extract; alkali extract; acid extract) on stem length (cm plant⁻¹), number of inflorescence (plant⁻¹), and number of flowers (plant⁻¹) of tomato

Biostimulant	Stem Length (cm plant ⁻¹)	Number of Inflorescence (plant ⁻¹)	Number of Flowers (plant ⁻¹)
Control	65 ± 8 a	4.8 ± 0.8 a	14 ± 1 a
Water	69 ± 2 a	4.4 ± 1.1 a	13 ± 2 ab
Alkali	77 ± 11 a	4.8 ± 0.4 a	10 ± 3 bc
Acid	75 ± 7 a	4.6 ± 0.9 a	10 ± 1 c

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

ANOVA revealed a significant effect of SWEs on fruit yield, but not on plant DW results (**Table 4.22**). The alkali and acid extracts led to an increase of approximately 40% in tomato yield (**Figure 4.23**) whereas the application of SWEs did not produce any noticeable impact on vegetative growth (**Figure 4.24**).

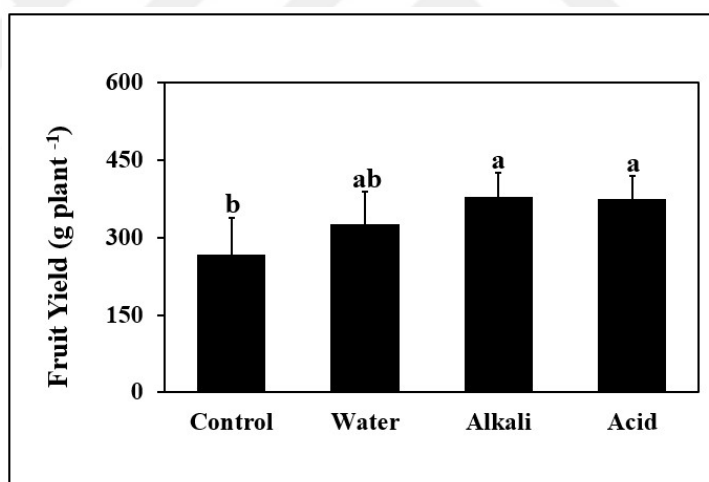


Figure 4.23: Effect of foliar biostimulant applications (control (water); water extract; alkali extract; acid extract) on fruit yield (g plant⁻¹) of tomato. Values are means and standard deviations of five replicates. Different letters indicate significant differences between means according to Fisher's protected LSD test ($p < 0.05$).

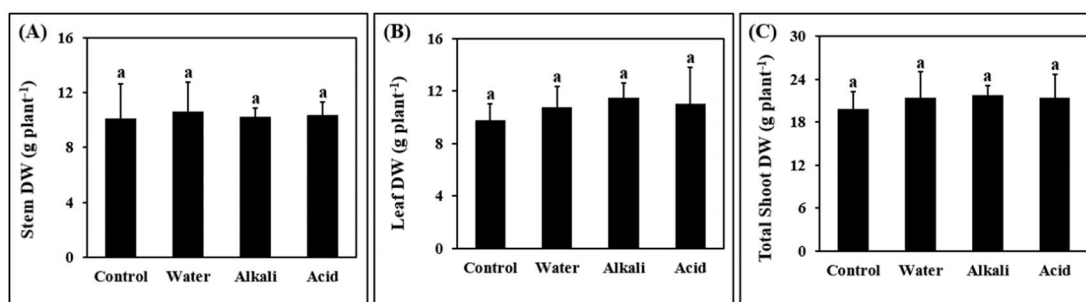


Figure 4.24: Effect of foliar biostimulant applications (control (water); water extract; alkali extract; acid extract) on (A) stem, (B) leaf, and (C) total shoot DW (g plant⁻¹). Values are means and standard deviations of five replicates. Different letters indicate significant differences between means according to Fisher's protected LSD test ($p < 0.05$).

Table 4.22: The effects of foliar application of biostimulants on reported traits of soil grown tomato according to one-way ANOVA ($\alpha = 0.05$)

Source of Variation	DF ¹	Stem Length	Number of Inflorescence	Number of Flowers	Fruit Yield
Biostimulant	3	<i>n.s.</i> ²	<i>n.s.</i>	**	*
Source of Variation	DF	Stem DW	Leaf DW	Total Shoot DW	pH
Biostimulant	3	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
Source of Variation	DF	EC	Brix	P	K
Biostimulant	3	<i>n.s.</i>	***	<i>n.s.</i>	<i>n.s.</i>
Source of Variation	DF	Ca	Mg	S	Fe
Biostimulant	3	*	<i>n.s.</i>	**	<i>n.s.</i>
Source of Variation	DF	Zn	Mn	Cu	B
Biostimulant	3	***	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>

¹DF; Degree of freedom

²*n.s.* Not significant; * $0.01 \leq F \text{ Pr.} < 0.05$; ** $0.001 \leq F \text{ Pr.} < 0.01$; *** $F \text{ Pr.} < 0.001$

The pH and EC values of the tomatoes are presented in **Table 4.23**, showing no consistent increase or decrease in response to SWE applications. The Brix value increased significantly in response to SWEs.

Table 4.23: Effect of foliar biostimulant applications (control (water); water extract; alkali extract; acid extract) on pH, EC ($\mu\text{S cm}^{-1}$), Brix ($^{\circ}\text{Bx}$) of tomato fruit

Biostimulant	pH	EC ($\mu\text{S cm}^{-1}$)	Brix ($^{\circ}\text{Bx}$)
Control	4.6 $\pm$ 0.2 a	4256 $\pm$ 347 a	3.5 $\pm$ 0.3 b
Water	4.6 $\pm$ 0.1 a	4503 $\pm$ 623 a	4.6 $\pm$ 0.4 a
Alkali	4.8 $\pm$ 0.2 a	4286 $\pm$ 345 a	4.5 $\pm$ 0.3 a
Acid	4.7 $\pm$ 0.1 a	4222 $\pm$ 310 a	4.3 $\pm$ 0.4 a

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

The level of macronutrients (P, K and Mg) was not significantly changed among treatments except Ca concentration according to ANOVA (**Table 4.22**), which decreased following water and alkali extracts applications in the range between 30-45% (**Table 4.24**). Furthermore, the S level of fruits was found to be slightly higher in plants treated with acid extract.

Table 4.24: Changes in concentrations of tomato fruits macronutrient (P, K, Ca, Mg, S) (mg g^{-1}) concentrations in response to foliar biostimulant applications (control (water); water extract; alkali extract; acid extract)

Biostimulant	P (mg g^{-1})	K (mg g^{-1})	Ca (mg g^{-1})	Mg (mg g^{-1})	S (mg g^{-1})
Control	4.2 $\pm$ 0.4 a	30 $\pm$ 2 a	3.1 $\pm$ 1.0 a	1.4 $\pm$ 0.2 a	0.6 $\pm$ 0.1 b
Water	4.1 $\pm$ 0.8 a	29 $\pm$ 2 a	2.1 $\pm$ 0.7 b	1.4 $\pm$ 0.2 a	0.6 $\pm$ 0.1 b
Alkali	3.6 $\pm$ 0.4 a	29 $\pm$ 1 a	1.7 $\pm$ 0.4 b	1.3 $\pm$ 0.1 a	0.7 $\pm$ 0.0 b
Acid	3.7 $\pm$ 0.6 a	30 $\pm$ 2 a	2.7 $\pm$ 0.8 ab	1.4 $\pm$ 0.1 a	0.8 $\pm$ 0.0 a

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

Likewise, the concentrations of Fe, Mn, Cu, and B were not significantly affected from SWEs applications (**Table 4.25**). The Zn concentration showed a marked decrease with *C. barbata* extracts applications by up to 25% compared to control.

Table 4.25: Changes in concentrations of tomato fruits micronutrient (Fe, Zn, Mn, Cu, B) (mg kg^{-1}) concentrations of fruits in response to foliar biostimulant applications (control (water); water extract; alkali extract; acid extract)

Biostimulant	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	Cu (mg kg^{-1})	B (mg kg^{-1})
Control	23 $\pm$ 2 a	7.0 $\pm$ 0.5 a	7.5 $\pm$ 0.8 a	3.9 $\pm$ 0.4 a	11 $\pm$ 3 a
Water	24 $\pm$ 3 a	6.0 $\pm$ 0.3 b	7.5 $\pm$ 0.5 a	4.2 $\pm$ 0.6 a	11 $\pm$ 4 a
Alkali	23 $\pm$ 2 a	6.2 $\pm$ 0.5 b	7.5 $\pm$ 0.7 a	3.9 $\pm$ 0.5 a	9 $\pm$ 1 a
Acid	23 $\pm$ 2 a	5.2 $\pm$ 0.5 c	7.7 $\pm$ 0.9 a	4.0 $\pm$ 0.4 a	10 $\pm$ 2 a

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

4.3. Broccoli Experiment

4.3.1. Investigation of Biostimulant Effects of *C. barbata* Extracts on Plant Growth and Quality of Broccoli by Drench Application

The present experiment was conducted to assess the biostimulant effects of SWEs on broccoli yield and quality. It has been established that SWEs applications promote broccoli growth compared to control plant in the early-stage growth (**Figure 4.25**).

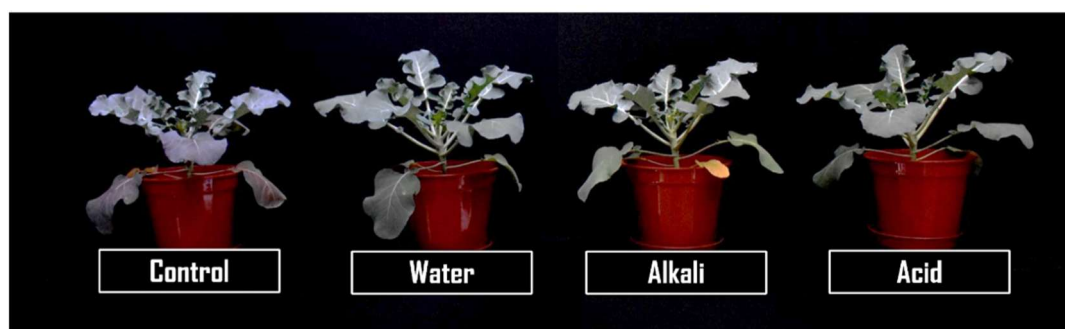


Figure 4.25: Effect of biostimulant applications (control (water); water extract; alkali extract; acid extract) on broccoli growth at 50 DAP

According to ANOVA, biostimulant applications had a significant effect on head FW (**Table 4.31**), which is depicted in **Figure 4.26** with a notable increase of 46% and 38% respectively in response to alkali and acid extracts applications. This is also visually evident in **Figure 4.27**.

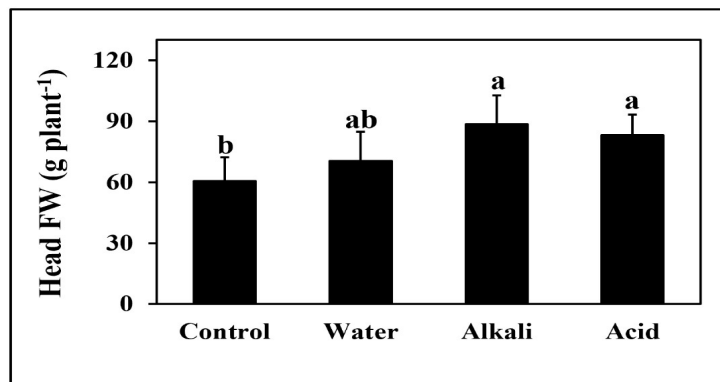


Figure 4.26: Effect of biostimulant applications (control (water); water extract; alkali extract; acid extract) on head FW (g plant⁻¹) of broccoli. Values are means and standard deviations of five replicates. Different letters indicate significant differences between means according to Fisher's protected LSD test ($p < 0.05$).

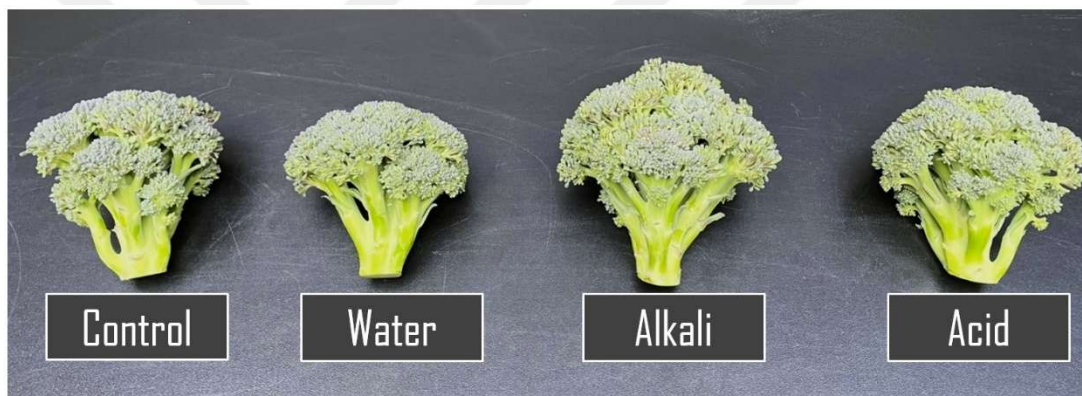


Figure 4.27: Effect of biostimulant applications (control (water); water extract; alkali extract; acid extract) on growth of broccoli head

The impact of biostimulant applications on vegetative growth of broccoli has been shown in **Table 4.26**. Leaf DW increased with SWEs applications in the range of 33-53% whereas shoot DW exhibited an average rise of 30-45 %.

Table 4.26: Effect of biostimulant applications (control (water); water extract; alkali extract; acid extract on stem, leaf and shoot DW (g plant⁻¹) of broccoli

Biostimulant	Stem DW	Leaf DW (g plant ⁻¹)	Shoot DW
Control	9.7 ± 0.9 a	30 ± 4 c	40 ± 3 b
Water	12.0 ± 1.0 a	46 ± 5 a	58 ± 6 a
Alkali	11.5 ± 2.2 a	40 ± 5 b	51 ± 7 a
Acid	10.1 ± 0.3 a	43 ± 1 ab	53 ± 1 a

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

Table 4.31 demonstrated the significant effect of biostimulant applications on the concentrations of mineral elements in the leaves and heads of broccoli plants according to ANOVA. On average, the application of *C. barbata* extracts applications decreased the P concentration of broccoli leaves by 12%. The K level increased by 2.3 times in water-extract-treated plants compared to control, while alkali and acid extracts led to a 33% increase (**Table 4.27**). Water extract alone significantly decreased Ca concentration compared to other treatments. On the other hand, Mg and S concentrations decreased by up to 25-35% with SWE applications, respectively.

Table 4.27: Effect of biostimulant applications (control (water); water extract; alkali extract; acid extract on macroelement concentration (P, K, Ca, Mg, S) (mg g⁻¹) of broccoli leaves

Biostimulant	P (mg g ⁻¹)	K (mg g ⁻¹)	Ca (mg g ⁻¹)	Mg (mg g ⁻¹)	S (mg g ⁻¹)
Control	1.06 ± 0.10 a	9 ± 1 c	36 ± 5 a	1.36 ± 0.17 a	3.5 ± 0.3 a
Water	0.94 ± 0.04 b	20 ± 1 a	26 ± 1 b	1.00 ± 0.04 c	2.3 ± 0.3 c
Alkali	0.92 ± 0.05 b	12 ± 1 b	34 ± 2 a	1.13 ± 0.06 bc	3.0 ± 0.1 b
Acid	0.94 ± 0.07 b	12 ± 2 b	37 ± 6 a	1.28 ± 0.15 ab	2.8 ± 0.3 b

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

The alterations in micronutrient levels in broccoli leaves with application of SWEs are presented in **Table 4.28**. The Fe, Zn, and Cu level were not changed among treatments whereas Mn and B concentrations decreased by 35% and 42%, respectively following water extract application compared to control.

Table 4.28: Effect of biostimulant applications (control (water); water extract; alkali extract; acid extract on microelement concentration (Fe, Zn, Mn, Cu, B) (mg kg⁻¹) of broccoli leaves

Biostimulant	Fe (mg kg ⁻¹)	Zn (mg kg ⁻¹)	Mn (mg kg ⁻¹)	Cu (mg kg ⁻¹)	B (mg kg ⁻¹)
Control	59 ± 9 a	8.8 ± 1.3 a	64 ± 5 ab	2.4 ± 0.5 a	154 ± 13 a
Water	67 ± 6 a	8.7 ± 0.8 a	42 ± 3 c	2.1 ± 0.1 a	89 ± 6 c
Alkali	63 ± 3 a	8.9 ± 0.7 a	60 ± 6 b	2.2 ± 0.1 a	113 ± 4 b
Acid	68 ± 6 a	8.8 ± 1.2 a	69 ± 8 a	2.1 ± 0.1 a	127 ± 15 b

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

ANOVA results revealed significant effects of K, Ca, and S (**Table 4.31**). While K concentrations increased slightly with SWE treatments, Ca concentrations decreased by up to 30% with water extract application (**Table 4.29**). Moreover, water and acid extract applications improved S level by approximately 60% whereas alkali extract nearly doubled S concentration.

Table 4.29: Effect of biostimulant applications (control (water); water extract; alkali extract; acid extract on macroelement concentration (P, K, Ca, Mg, S) (mg g⁻¹) of broccoli heads

Biostimulant	P (mg g ⁻¹)	K (mg g ⁻¹)	Ca (mg g ⁻¹)	Mg (mg g ⁻¹)	S (mg g ⁻¹)
Control	3.0 ± 0.4 a	20 ± 2 c	7.5 ± 0.9 a	1.4 ± 0.2 a	3.8 ± 0.6 c
Water	3.0 ± 0.2 a	27 ± 1 a	5.3 ± 0.2 c	1.2 ± 0.0 a	5.9 ± 0.4 b
Alkali	3.1 ± 0.4 a	25 ± 2 ab	6.7 ± 0.5 b	1.5 ± 0.1 a	7.4 ± 0.6 a
Acid	2.9 ± 0.5 a	23 ± 2 bc	6.4 ± 0.3 b	1.5 ± 0.3 a	6.1 ± 0.8 b

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

Iron level of heads exhibited a notable increase of 38% compared to control with alkali and acid extract applications (**Table 4.30**). In addition, Cu concentrations increased significantly following alkali extract application. While Mn levels had a declining trend with water and alkali extract applications, all SWE applications led to a marked decrease in B concentrations.

Table 4.30: Effect of biostimulant applications (control; water extracts; alkali extracts; acid extracts on microelement concentration (Fe, Zn, Mn, Cu, B) (mg kg⁻¹) of broccoli heads

Biostimulant	Fe (mg kg ⁻¹)	Zn (mg kg ⁻¹)	Mn (mg kg ⁻¹)	Cu (mg kg ⁻¹)	B (mg kg ⁻¹)
Control	39 ± 3 b	21 ± 3 a	24 ± 3 a	3.9 ± 0.4 b	45 ± 4 a
Water	39 ± 3 b	21 ± 2 a	17 ± 1 c	3.8 ± 0.4 b	29 ± 1 b
Alkali	54 ± 5 a	25 ± 3 a	21 ± 2 b	4.7 ± 0.5 a	33 ± 1 b
Acid	54 ± 5 a	25 ± 2 a	24 ± 2 ab	4.3 ± 0.5 ab	32 ± 2 b

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

Table 4.31: The effects of drench application of biostimulants on reported traits of soil grown broccoli according to one-way ANOVA ($\alpha = 0.05$)

Source of Variation	DF ¹	Head FW	Stem DW	Leaf DW	Shoot DW
Biostimulant	3	*	<i>n.s.</i> ²	***	***
Source of Variation	DF	P _(leaf)	K _(leaf)	Ca _(leaf)	Mg _(leaf)
Biostimulant	3	*	***	**	**
Source of Variation	DF	S _(leaf)	Fe _(leaf)	Zn _(leaf)	Mn _(leaf)
Biostimulant	3	***	<i>n.s.</i>	<i>n.s.</i>	***
Source of Variation	DF	Cu _(leaf)	B _(leaf)	P _(head)	K _(head)
Biostimulant	3	<i>n.s.</i>	***	<i>n.s.</i>	***
Source of Variation	DF	Ca _(head)	Mg _(head)	S _(head)	Fe _(head)
Biostimulant	3	***	<i>n.s.</i>	***	***
Source of Variation	DF	Zn _(head)	Mn _(head)	Cu _(head)	B _(head)
Biostimulant	3	<i>n.s.</i>	**	*	***
Source of Variation	DF	Chl a	Chl b	Chl a/b	Caretonoid
Biostimulant	3	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	***
Source of Variation	DF	TPC	ABTS	DPPH	FRAP
Biostimulant	3	<i>n.s.</i>	*	***	**
Source of Variation	DF	Total AsA	Reduced AsA	GSH	
Biostimulant	3	<i>n.s.</i>	***	***	

¹DF; Degree of freedom

²*n.s.* Not significant; * $0.01 \leq F \text{ Pr.} < 0.05$; ** $0.001 \leq F \text{ Pr.} < 0.01$; *** $F \text{ Pr.} < 0.001$

The significant effect of biostimulant applications on carotenoid concentrations was demonstrated by ANOVA (**Table 4.31**). Although Chl a, Chl b and their ratio remained unchanged between treatments, carotenoid concentration increased by 26% with alkali extract application (**Table 4.32**).

Table 4.32: Effect of biostimulant applications (control (water); water extract; alkali extract; acid extract on Chl a, and Chl b (mg kg^{-1}), Chl a/b ratio, and carotenoids (mg kg^{-1}) of broccoli

Biostimulant	Chl a (mg kg^{-1})	Chl b (mg kg^{-1})	Chl a/b ratio	Carotenoids (mg kg^{-1})
Control	218 ± 14 a	104 ± 7 a	2.1 ± 0.1 a	70 ± 7 b
Water	222 ± 27 a	105 ± 9 a	2.1 ± 0.2 a	79 ± 6 ab
Alkali	248 ± 14 a	108 ± 12 a	2.2 ± 0.3 a	88 ± 6 a
Acid	237 ± 34 a	109 ± 15 a	2.1 ± 0.5 a	73 ± 8 b

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

Seaweed extract applications did not significantly alter the TPC of plants (**Figure 4.28A**). While alkali extract application led to a 25% increment in ABTS concentration (**Figure 4.28B**), DPPH concentration increased by 32% relative to control (**Figure 4.28C**). In addition, antioxidant activity measured by FRAP assay exhibited an enhancement of 14% following both alkali and acid extract applications compared to control (**Figure 4.28D**).

The level of reduced AsA increased dramatically by 48% and 19% following alkali and acid extracts, respectively (**Table 4.33**). However, total AsA concentration remained unchanged between treatments.

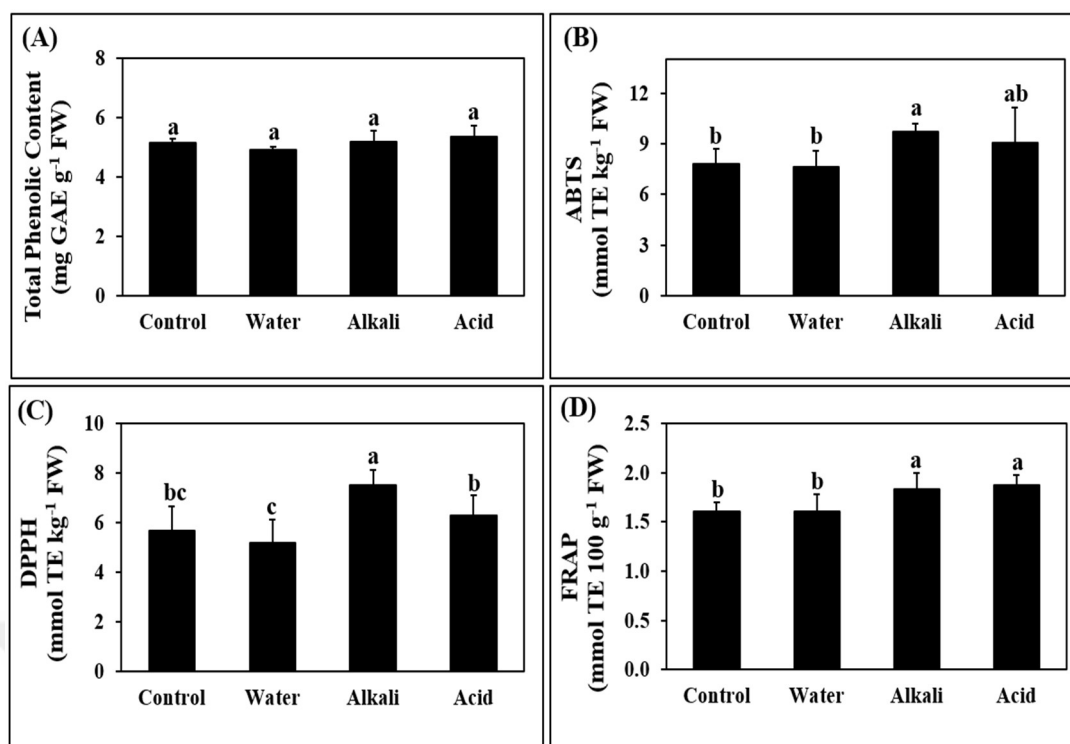


Figure 4.28: Effect of biostimulant applications (control (water); water extracts (high); alkali extracts (low); acid extract (low)) on (A) TPC (mg GAE g⁻¹ FW), (B) ABTS (mmol TE kg⁻¹ FW), (C) DPPH (mmol TE kg⁻¹ FW), and (D) FRAP (mmol TE 100 g⁻¹ FW) of broccoli. Values are means and standard deviations of five replicates. Different letters indicate significant differences between means according to Fisher's protected LSD test ($p < 0.05$).

Table 4.33: Effect of biostimulant applications (control (water); water extracts (high); alkali extracts (low); acid extract (low)) on total and reduced AsA (mg kg⁻¹ FW) of broccoli

Biostimulant	Total AsA (mg kg ⁻¹ FW)	Reduced AsA (mg kg ⁻¹ FW)
Control	562 ± 113 a	98 ± 6 c
Water	656 ± 137 a	103 ± 6 c
Alkali	546 ± 136 a	145 ± 10 a
Acid	569 ± 70 a	117 ± 4 b

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

ANOVA results indicated that *C. barbata* extracts significantly influence the GSH levels in broccoli (**Table 4.31**). Water and alkali extract elevated GSH levels by an average of 45% compared to the control, while acid extract application contributed to a 27% increase (**Figure 4.29**).

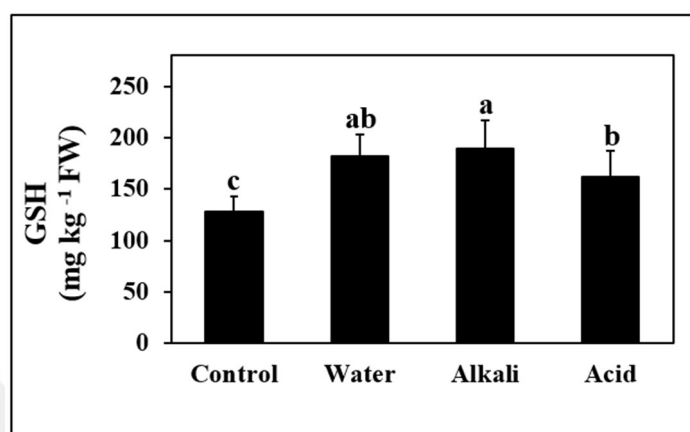


Figure 4.29: Effect of biostimulant applications (control (water); water extract; alkali extract; acid extract) on GSH (mg kg⁻¹ FW) of broccoli. Values are means and standard deviations of five replicates. Different letters indicate significant differences between means according to Fisher's protected LSD test ($p < 0.05$).

4.4. Pepper Experiment

4.4.1. Investigation of Biostimulant Effects of Selected *C. barbata* Extracts and a Commercial Product on Plant Growth and Quality of Pepper by Drench Application

The study assessed the efficacy of various SWEs, contrasting their performance with that of a commercial seaweed product as well as a control. ANOVA results indicated that tested SWEs applications and Acadian as a commercial product had significant effects on fruit yield and number (**Table 4.34**). Acid and water extracts increased in the range of 35-40% increase in pepper yield relative to the control (**Figure 4.30A**). The improvements were comparable to those observed effects with Acadian. A comparable trend was also noted for fruit number; water and acid extract treatments significantly increased compared to control, producing results that are like those obtained with the commercial product (**Figure 4.30B**).

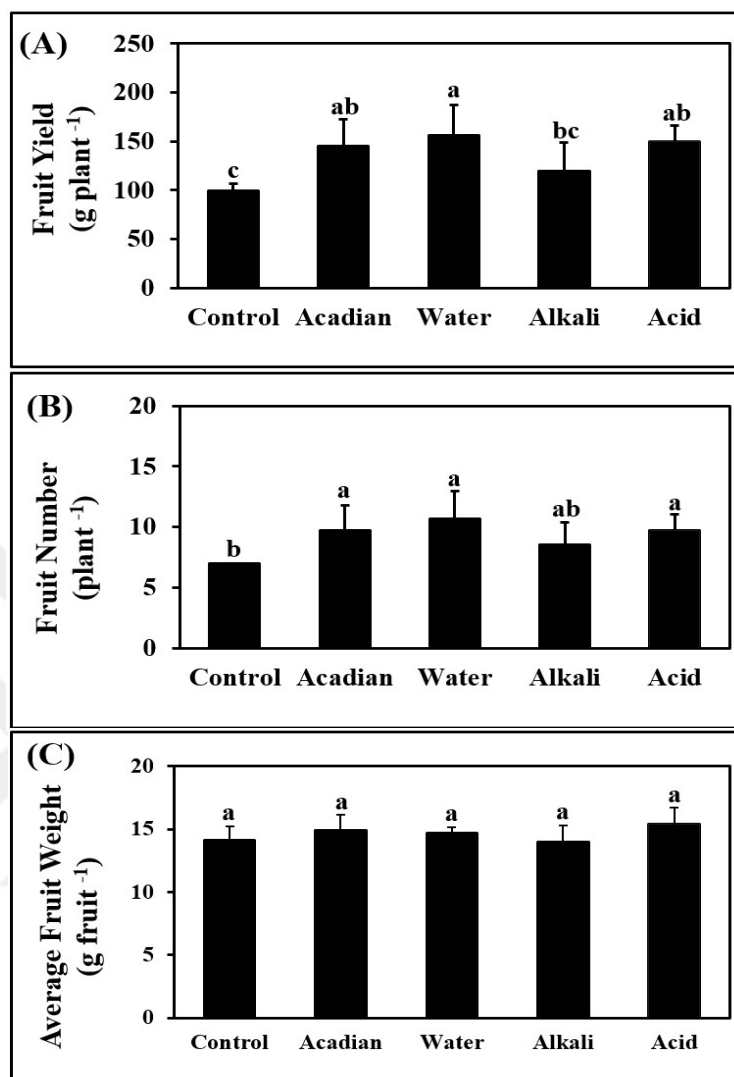


Figure 4.30: Effect of biostimulant applications (control (water); Acadian; water extract; alkali extract; acid extract) on (A) fruit yield (g plant⁻¹), (B) fruit number (plant⁻¹), (C) average fruit weight (g fruit⁻¹) of pepper fruit. Values are means and standard deviations of four replicates. Different letters indicate significant differences between means according to Fisher's protected LSD test ($p < 0.05$).

Table 4.34: The effects of drench application of biostimulants on reported traits of soil grown pepper according to one-way ANOVA ($\alpha = 0.05$)

Source of Variation	DF ¹	Fruit Yield	Fruit Number	Average Fruit Weight	TPC
Biostimulant	3	**	*	<i>n.s.</i> ²	<i>n.s.</i>
Source of Variation	DF	ABTS	DPPH	FRAP	Total AsA
Biostimulant	3	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	*
Source of Variation	DF	Reduced AsA	GSH	Soluble carbohydrate	Chl a
Biostimulant	3	<i>n.s.</i>	<i>n.s.</i>	*	<i>n.s.</i>
Source of Variation	DF	Chl b	Carotenoid	P	K
Biostimulant	3	<i>n.s.</i>	<i>n.s.</i>	*	<i>n.s.</i>
Source of Variation	DF	Ca	Mg	S	Fe
Biostimulant	3	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
Source of Variation	DF	Zn	Mn	Cu	B
Biostimulant	3	<i>n.s.</i>	*	*	<i>n.s.</i>

DF; Degree of freedom

²*n.s.* Not significant; * $0.01 \leq F \text{ Pr.} < 0.05$; ** $0.001 \leq F \text{ Pr.} < 0.01$; *** $F \text{ Pr.} < 0.001$

The TPC and antioxidant activity results did not exhibit any significant differences among the SWE treatments (**Figure 4.31**). The effects of the tested SWEs were similar with Acadian.

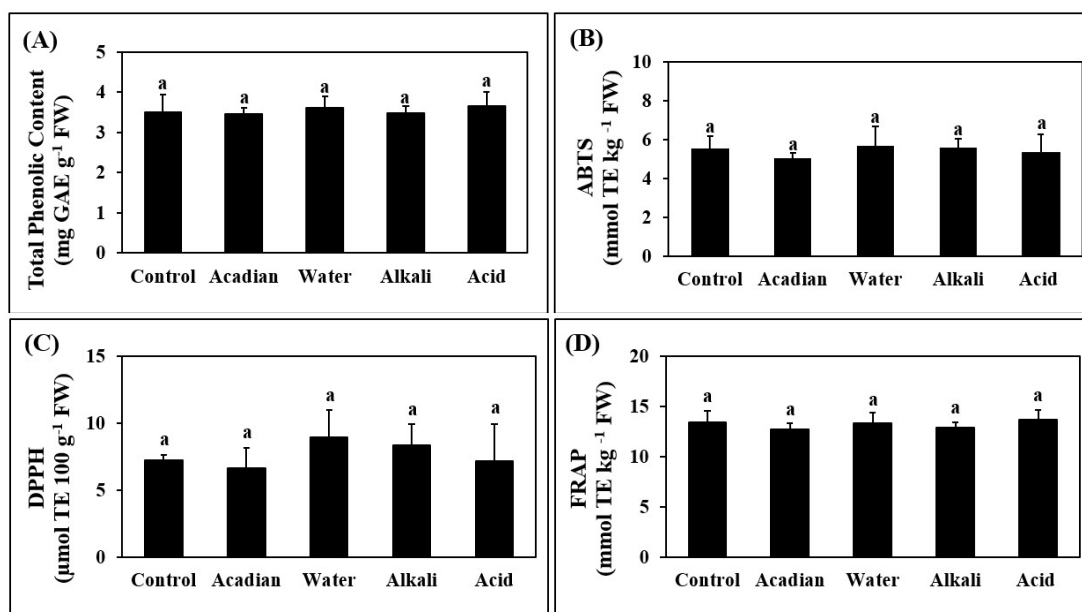


Figure 4.31: Effect of biostimulant applications (control (water); water extracts; Acadian; alkali extract; acid extract) on (A) TPC (mg GAE g⁻¹ FW), (B) ABTS (mmol TE kg⁻¹ FW), (C) DPPH (μmol TE 100 g⁻¹ FW), and (D) FRAP (mmol TE kg⁻¹ FW) of pepper fruit. Values are means and standard deviations of four replicates. Different letters indicate significant differences between means according to Fisher's protected LSD test ($p < 0.05$).

According to ANOVA, total AsA was significantly affected by biostimulant applications, whereas no significant effect was reported on reduced AsA (**Table 4.34**). All tested SWE-treated groups, along with the commercial product, exhibited an increase in total AsA levels compared to the control, with an average of 35-50%. (**Figure 4.32**). Tested SWEs exhibited similar effects with the commercial product.

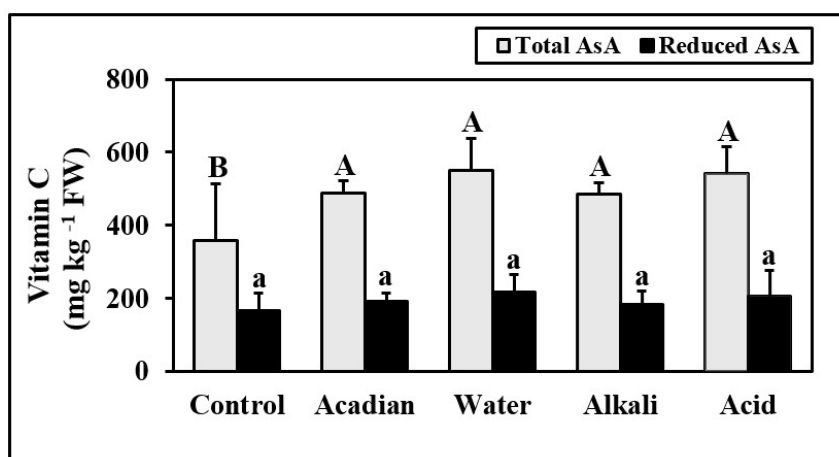


Figure 4.32: Effect of biostimulant applications (control (water); Acadian; water extract; alkali extract; acid extract) on the total AsA and reduced AsA concentration (mg kg⁻¹FW) of pepper fruit. Values are means and standard deviations of four replicates. Uppercase letters and lowercase letters indicate significant differences between means according to Fisher's protected LSD test ($p < 0.05$) in the total and reduced AsA, respectively.

Figure 4.33 indicates that GSH concentrations showed no significant differences among different treatments.

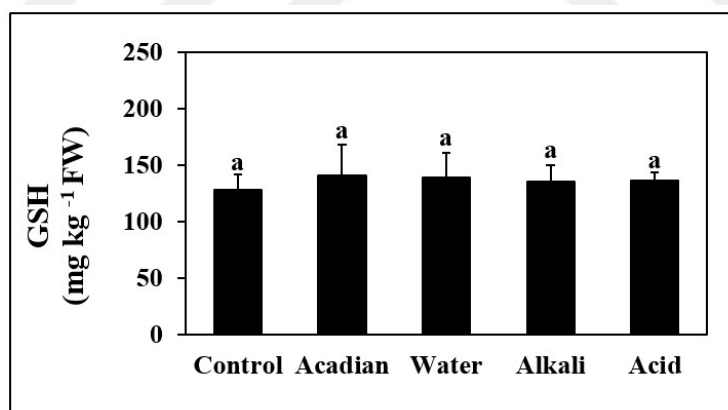


Figure 4.33: Effect of biostimulant applications (control (water); Acadian; water extract; alkali extract; acid extract) on GSH (mg kg⁻¹ FW) of pepper fruit. Values are means and standard deviations of four replicates. Different letters indicate significant differences between means according to Fisher's protected LSD test ($p < 0.05$).

The effects of biostimulant applications on Chl a, Chl b, and carotenoid levels of pepper fruits have been shown in **Figure 4.34**. The levels of Chl a, Chl b, and carotenoids did not changed following biostimulant treatments.

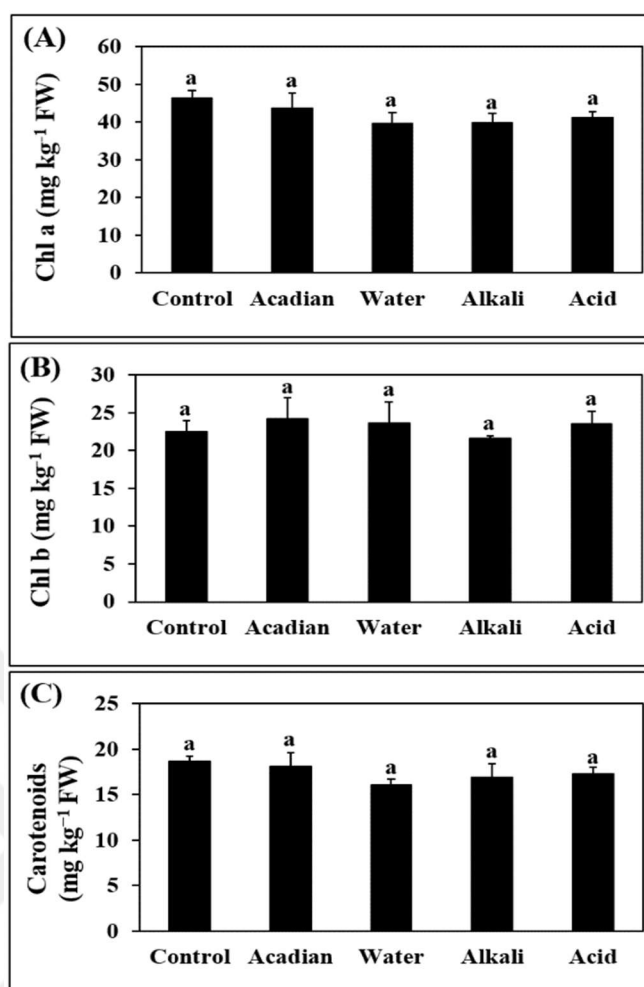


Figure 4.34: Effect of biostimulant applications (control (water); Acadian; water extract; alkali extract; acid extract) on (A) chl a, (B) chl b and (C) caretonoids ($\text{mg kg}^{-1} \text{FW}$) of pepper fruit. Values are means and standard deviations of four replicates. Different letters indicate significant differences between means according to Fisher's protected LSD test ($p < 0.05$).

ANOVA indicated a significant effect of the biostimulant applications on the soluble carbohydrate concentrations (**Table 4.34**). The alkali extract application led to a significant increase in soluble carbohydrate concentration compared to the control, whereas water and acid as well as Acadian applications were not significantly different from control (**Figure 4.35**).

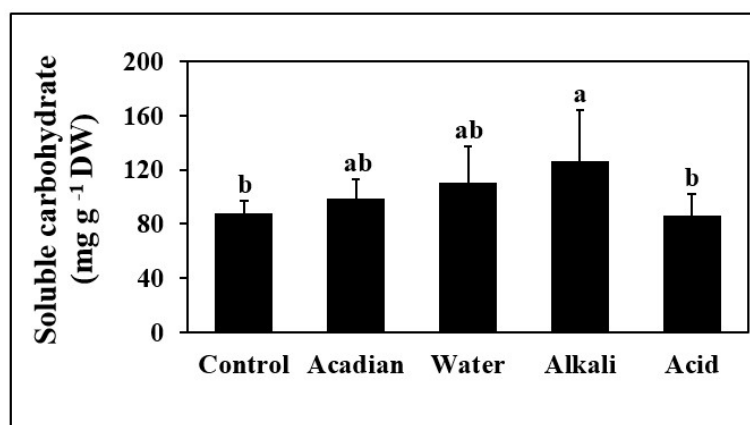


Figure 4.35: Effect of biostimulant applications (control (water); Acadian; water extract; alkali extract; acid extract) on soluble carbohydrate (mg g^{-1} DW) of pepper fruit. Values are means and standard deviations of four replicates. Different letters indicate significant differences between means according to Fisher's protected LSD test ($p < 0.05$).

The effects of the different biostimulant treatments on macronutrient concentrations has been shown in **Table 4.35**. While Acadian application led to modest decrease in the P level compared control, the concentration of K, Ca, Mg, and S did not change according to different biostimulant applications.

Table 4.35: Effect of biostimulant applications (control (water); Acadian; water extract; alkali extract; acid extract) on macroelement concentration (P, K, Ca, Mg, S) (mg g^{-1}) of pepper fruit

Biostimulant	P (mg g^{-1})	K (mg g^{-1})	Ca (mg g^{-1})	Mg (mg g^{-1})	S (mg g^{-1})
Control	2.6 ± 0.2 ab	30 ± 1 a	1.2 ± 0.1 a	1.5 ± 0.1 a	2.2 ± 0.1 a
Acadian	2.3 ± 0.1 c	29 ± 1 a	1.3 ± 0.3 a	1.4 ± 0.1 a	2.3 ± 0.2 a
Water	2.4 ± 0.1 bc	30 ± 1 a	1.3 ± 0.2 a	1.5 ± 0.1 a	2.3 ± 0.2 a
Alkali	2.7 ± 0.2 a	30 ± 1 a	1.4 ± 0.2 a	1.5 ± 0.1 a	2.3 ± 0.2 a
Acid	2.6 ± 0.2 a-c	29 ± 0 a	1.3 ± 0.2 a	1.5 ± 0.1 a	2.4 ± 0.1 a

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

Similarly, the level of Fe, Zn, and B did not change significantly (**Table 4.36**). While Mn concentrations increased slightly with all biostimulant applications, alkali extract led to modest increase in Cu concentration compared to control.

Table 4.36: Effect of biostimulant applications (control (water); Acadian; water extract; alkali extract; acid extract) on microelement concentration (Fe, Zn, Mn, Cu, B) (mg kg^{-1}) of pepper fruit

Biostimulant	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	Cu (mg kg^{-1})	B (mg kg^{-1})
Control	45 ± 6 a	19 ± 1 a	16 ± 1 c	8.3 ± 0.6 bc	10.8 ± 0.9 a
Acadian	44 ± 8 a	21 ± 8 a	17 ± 1 bc	7.9 ± 0.5 c	11.0 ± 1.2 a
Water	52 ± 6 a	20 ± 2 a	18 ± 1 ab	8.3 ± 0.7 bc	10.9 ± 0.9 a
Alkali	53 ± 7 a	21 ± 1 a	18 ± 1 a	9.2 ± 0.5 a	10.9 ± 0.8 a
Acid	55 ± 5 a	20 ± 1 a	18 ± 1 ab	8.9 ± 0.3 ab	11.0 ± 0.4 a

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

5. DISCUSSION

5.1. Wheat Experiments

5.1.1. Investigation of Biostimulant Effects of *C. barbata* Extracts on Wheat by Drench Application

Among the evaluated *C. barbata* extracts, the medium, low, and high rate of the water, alkali, and acid extract, respectively produced the most pronounced wheat growth-promoting effects (**Figure 4.2**). These results are consistent with previous studies demonstrating that the application of SWEs enhances wheat growth and yield under control conditions by improving physiological performance and nutrient uptake [**Patel et al., 2018; Hamouda et al., 2022; Phakatkar et al., 2025**].

The SWEs constitute a diverse category, with compositional changes contingent upon the species utilized and the extractions employed to get bioactive substances [**Sharma et al., 2014; Ali et al., 2016**]. The application rate and various extraction processes significantly impacted the growth performance of the SWEs [**Godlewska et al., 2016**]. The application rates for the evaluated SWEs were determined based on the findings of **Mutlu-Durak et al. (2024)** and growth promoting in terms of DWs results leading to the selection of three SWE treatments.

5.1.2. Determination of the Effective Drought Stress Application Level on Wheat

Shoot DW decreased by 17%, 26%, and 40% as drought intensified relative to control (**Figure 4.4**). These results align with previous investigations indicating that drought stress can decrease wheat productivity by between 20% and 40% [**Daryanto et al., 2016; Fahad et al., 2017; Zhang et al., 2017**]. Drought continues to have a major limitation on crop productivity, and efficient water management process are essential for sustaining high crop yields and guaranteeing food security [**Vafa et al., 2024**].

5.1.3. Investigation of the Impacts of Selected *C. barbata* Extract Applications on Drought Stress Resistance and Physiological Parameters

On-going droughts, driven by global warming, pose an increasing threat to crop production, emphasizing the need for sustainable solutions, such as the application of biostimulants to promote plant growth under stressful conditions [Trivedi et al., 2018; Bisht and Chhabra, 2024]. Prior investigations have shown that seaweed based biostimulants promote wheat growth in non-drought growing conditions by enhancing physiological traits and mineral nutrient absorption [Patel et al., 2018; Hamouda et al., 2022; Phakatkar et al., 2025]. In addition, SWE applications have also been indicated to improve the plant growth parameters of soybean [Shukla et al., 2018] and faba bean [El Boukhari et al., 2023] under water-deficit conditions via different biochemical pathways. Utilization of *C. barbata* extracts significantly improves plant growth performance and increases shoot DW under all WHC levels (Figure 4.5 and Figure 4.6). Under well-watered conditions, the water extract increased shoot DW; however, during drought conditions, both the water and acid extracts yielded more significant enhancements. The beneficial effects of seaweed based biostimulants under drought stress conditions can be ascribed to the bioactive compounds present in the SWEs. The presence of laminarin, fucoidan, and alginate decreases adverse effects of water stress by stimulating antioxidant mechanism of plant [Carillo, 2025].

These ingredients alleviate oxidative damage within cells, reinforce cellular structures, and preserve photosynthetic mechanism under stress conditions. Molecular analyses indicate that these extracts can maintain cell cycle activity by upregulating genes involved in cell division, while simultaneously downregulating drought-induced growth-inhibitory genes [Rasul et al., 2021]. The stimulatory effects of water extract of *C. barbata* can be ascribed to its elevated levels of alginic acid and alginates (Table 3.3), which promote both plant growth and improve stress tolerance [González et al., 2013; Hernández-Herrera et al., 2016]. Furthermore, the active ingredients like laminarins and fucoidans in *C. barbata* extract (Table 3.3) can encourage plant development and stress tolerance through their potential biostimulant actions [Ali et al., 2021a]. On the other hand, the osmoprotectant and antioxidant effects of mannitol, present in *C. barbata* extracts, enhance stress tolerance [Patel et al., 2023].

The extracts' phenolic and antioxidant contents also helped the plant's oxidative defense mechanism, which led to improved performance in both stress and non-stress environments [Mughunth et al., 2024]. Furthermore, *C. barbata* extracts utilized in this study were indicated to have auxin-like activity, which could indicate a mechanism that improves developing roots and phytohormonal homeostasis [Mutlu-Durak et al., 2024].

In addition, recent literature has demonstrated that brown SWEs can improve stomatal conductivity, which in turn maintains cellular turgor balance and makes plants more resistant to dehydration [Shukla et al., 2018]. Furthermore, the application of SWEs to plants subjected to drought stress has been demonstrated to enhance crop yield, antioxidant activity and sugar production [Jacomassi et al., 2022]. The results indicate that the bioactive compounds in *C. barbata* extracts work harmoniously to enhance wheat growth, mitigate drought-related growth deficiencies, and improve plant resilience.

The utilization of *C. barbata* extracts has been demonstrated to enhance the dry matter ratio (Figure 4.7), presumably by improving water use efficiency and fostering physiological endurance in drought conditions. The observed effect is due to the accumulation of compatible solutes [Shukla et al., 2018] and the adjustment of phytohormone activity that facilitates regulation of stoma movement [Rouphael and Colla, 2020]. In addition, studies have reported that biostimulants can enhance the architecture of plant root systems, leading to better absorption of nutrients and water [Battacharyya et al., 2015], enhancement of dry matter of plants which are exposed to water-deficit conditions [Xu and Geelen, 2018]. The findings align with previous investigations show that safflowers treated with biostimulants reduce damage from water stress by increasing dry matter accumulation. [Davari et al., 2022].

Macro- and micronutrient concentrations revealed slight differences following SWE application (Table 4.3 and Table 4.4). Changes in the concentration of macro minerals were noted in wheat, which improved through the application of water and alkali extracts under non-stress conditions. Similar elevations in mineral nutrients have also been reported in different plants [Stasio et al., 2017; Chen et al., 2021] following the application of brown SWE. The bioactive components of the extracts, especially the polysaccharides which include S, can both deliver S and trigger changes in metabolism that initiate S assimilation [Bouissil et al., 2020; Nivetha et al., 2024].

While biostimulants contained essential nutrients do not completely meet a plant's nutritional needs [Rathore et al., 2009], it has been established that they are able to improve plant development by altering gene expression that are related with nutrient metabolism [Shukla et al., 2019]. Additionally, it has been proposed that regulatory hormones involved in plant growth and active phytochemicals present in SWEs improve nutrient uptake and internal transport within plants, resulting in increased biomass accumulation [Gupta et al., 2021]. Administering biostimulants enhances the levels of trace elements, like Fe, in wheat plants under well-watered conditions and maintains these levels under water deficit conditions [Basu et al., 2023]. The concentration of Fe in wheat was shown to increase via water extract treatments (Table 4.4). Previous research has shown that micronutrient absorption is reduced by drought stress [d'Oria et al., 2022], and our results reveal that the concentration of Fe and Zn also decreases significantly as the drought level increases. The concentration of Fe and Zn in wheat, maize, and rice is significantly reduced when subjected to water stress, according to literature [Zahra et al., 2023].

Glycine betaine functions as an osmolyte, contributing to stabilization of turgor pressure and osmotic equilibrium, thereby alleviating drought stress in plants [Ashraf and Foolad, 2007]. Research indicates that maize plants subjected to SWE treatment exhibited elevated GB levels, thereby validating their recognized role in enhancing drought resilience and maximizing agricultural output [Trivedi et al., 2018]. The utilization of SWEs also reportedly elevates the GB content in bean plants [Mansori et al., 2015]. In line with the recent literature, both water and acid extracts substantially increased GB concentration, regardless of drought level (Table 4.8 and Figure 4.8). Brown SWEs inherently contain GB [MacKinnon et al., 2010], which may explain the GB accumulation noted in the *C. barbata* treated wheat plants. Furthermore, drought stress itself caused a significant rise in GB concentrations (Figure 4.8B). The buildup of GB in plants is linked to improved resistance against numerous abiotic stressors, such as drought [Niu et al., 2021]. A similar trend was observed in proline concentration, which increased in shoot tissues as WHC decreased. Likewise, water and acid extraction caused further accumulation (Figure 4.8A). At 60% WHC, the application of acid extract also led to an increase in soluble protein concentration. These findings are consistent with previous studies reporting that various wheat cultivars tend to accumulate higher levels of compatible solutes, such as proline and

proteins, in response to SWE treatments [Patel et al., 2018]. Seaweed based extracts have been shown to improve plant growth performance under drought conditions by enhancing stress tolerance through the promotion of osmotic adjustment, preservation of cellular hydration, and stabilization of membrane integrity [Ahmed et al., 2023]. Furthermore, under drought stress, SWEs application to tomato plants had elevated level of proline which supported the role of osmolytes in stress reduction [Ali et al., 2024]. Both the acid and water extracts of *C. barbata* enhanced GB; however, the proline level exhibited a significant rise only at the severe drought level (Figure 4.9). The synthesis of endogenous GB occurs simultaneously with proline accumulation under abiotic stress conditions, underscoring the synergistic effect [Sun et al., 2020].

Root proline level escalated with the degree of drought severity, especially with acid extract at 45% WHC. Both water and acid extracts application significantly increased proline concentrations in roots at 60% WHC, corroborating the assertion that proline accumulation facilitates drought resistance through osmotic adjustment in the roots [Vartanian et al., 1992].

Similarly, elevated proline levels in both shoots and roots of barley have been shown to preserve cell turgor maintenance, promote root growth, and improve the plant's ability to cope with drought stress [Frimpong et al., 2021]. The use of proline improves maize growth during drought by enhancing antioxidant defense and facilitating osmotic and physiological adjustments [Khan et al., 2025]. It is mainly synthesized in leaves and transported to roots during water stress, accompanied by upregulation of biosynthesis genes [Wang et al., 2022a]. The increased shoot and root proline levels suggest a similar adaptation mechanism to that observed with the application of acid extracts, which indicates the possibility of proline transfer from shoots to roots.

The MDA level significantly increased as a result of the water and acid extract applications under severe stress conditions (Figure 4.10). Additionally, there were no apparent indicators of oxidative damage despite the elevated MDA accumulation under severe drought stress, particularly in plants subjected to water extract (Figure 4.5). In contrast to this, MDA stands out as a key indicator of cellular membrane damage [Zhang et al., 2021]. Through the activation of defense-related and developmental genes, rising levels of MDA can indicate an acclimation mechanism rather than a marker of oxidative damage, when regulated properly [Morales and

Munné-Bosch, 2019]. Considering this, it is possible that MDA may also function as a protective signalling molecule. Our findings are consistent with previous research indicating that, when subjected to salt stress, rosemary plants increase ROS scavenging within cells and produce MDA without exhibiting signs of oxidative stress [**Tounekti et al., 2011**].

Soluble proteins that act as osmolytes or osmoprotectants which are frequently synthesized and stored by plants under water-deficit conditions [**Ozturk, 2021**]. In drought conditions, osmotic stress may be alleviated by increasing the number of soluble proteins in response to acid extract application (**Figure 4.11**). On the other hand, the antioxidant enzyme activity was found to show different variations with the drench application of *C. barbata* extract, with both stimulatory and inhibitory effects being noted (**Table 4.6** and **Table 4.7**). In the present investigation, APX activity decreased over time due to drought stress, although CAT activity improved by SWEs applications under severe stress conditions. The outcomes show that CAT and APX, two important antioxidant enzymes, serve distinct functions in stress-related ROS detoxification depending on substrate specificity and subcellular location [**Sofo et al., 2015**]. The steady decline in APX activity could be due to either a lack of ascorbate concentration or an enzyme-regulated optimum level [**Corpas et al., 2024**] or oxidative reduction during extended periods of severe stress [**Pandey et al., 2017**], whereas the observed increase in CAT activity could be an indication of an improved enzymatic response to elevated H₂O₂ levels [**Luna et al., 2005**]. Our findings showed an improved enzymatic antioxidant defence in terms of CAT activity, which is consistent with the literature and suggests increased CAT activity in tomato plants under drought conditions [**Wang et al., 2022b**]. Biostimulants' bioactive ingredients led to enhancements of antioxidative enzyme activities in different plants [**Panfili et al., 2019; Irani et al., 2021**] when experiencing stress stimuli.

While applications of *C. barbata* extracts did not increase APX activity in general, using a specific level of acid extract under higher water-deficit conditions significantly improved APX activity, bringing it almost to the level of well-watered control. This suggests that different regulatory pathways related to antioxidant defense may be engaged by acid extract formulations. Of particular interest, the application of acid extract resulted in an increase in GR activity, independent of drought stress levels. Recent investigations indicates that the GR upregulation with biostimulant treatments

may enhance GSH production, which is linked to improved plant growth under both control and stress conditions [Hasanuzzaman et al., 2017; Naboulsi et al., 2022]. The consistent level of total SOD activity, coupled with the modest increase in specific SOD activity, might demonstrate enhanced enzymatic effectiveness. The enhancement of SOD activity via SWE application correlates with the presence of bioactive compounds, including polyphenolics and proteins, which can activate antioxidant enzyme activity [Latique et al., 2021].

Drought and biostimulant treatments caused changes in the soil microbiome, as seen in **Table 4.8**. Research indicates that brown SWEs can enhance microbial activity, hence improving soil physical properties and increasing the proportion of water-stable macro-aggregates [Chen et al., 2023]. One possible explanation for this action is the presence of bioactive compounds in seaweed, such as polysaccharides and alginic acid, which have significant binding properties to soil. Out of all the extracts examined, the water extract had the highest alginic acid content [Mutlu-Durak et al., 2024] and produced a positive response in the microbe activity found in the soil. Likewise using the water extract has shown elevated AWCD value (**Table 4.8**), which indicates a rise in the microbial community's overall metabolic activity [Németh et al., 2021]. The repeated application of SWEs was observed to elevate overall bacterial number and accessible nitrogen in the soil, suggesting a possible enhancement of microbial activity and an improved role in soil C and N cycle via bacterial mineralization [Hussain et al., 2021]. Key soil enzymes such as urease, phosphatase, and peroxidase may exhibit increased activity in response to the stimulation of soil microflora by SWEs [Trivedi et al., 2022; Chen et al., 2023]. These enzymes are crucial for enhancing nutrient availability for plant absorption, sustaining soil organic matter, supporting soil structure, and establishing microbial interactions [Moscatelli et al., 2018].

A well-known technique for evaluating how stress conditions affect the diversity of soil bacteria and fungi is the Biolog EcoPlate [Wang et al., 2017; Galazka and Grzadziel, 2018]. One important metric in these assessments is the H' index, which is strongly impacted by species diversity within a microbial population [Liu et al., 2023]. Our findings were confirmed by Ferioun et al. (2025), who found that as drought intensity increased, both the H' index and SR significantly decreased. Despite the fact that applying plant growth-promoting rhizobacteria (PGPR) to drought-stressed barley

enhanced both soil H⁺ and SR, our research did not find a significant correlation between drought stress and the use of biostimulants (**Table 4.8**).

A close relationship between soil microbial dynamics and plant root exudation is demonstrated by the differences in SAWCD following various SWE treatments (**Figure 4.12**). Microbial activity in soil is stimulated by plant root exudates [Kawasaki et al., 2016]. According to Iannucci et al. (2017), sugars make up the majority of the chemicals found in wheat root exudates, followed by polyalcohols, organic acids and amino acids.

Microorganisms primarily obtain their carbon sources from sugars, amino acids are known to act as chemoreceptors, facilitating early root colonization [Sasse et al., 2018]. Nevertheless, plants' ability to absorb carbon usually declines during drought, which restricts the amount of carbon that may enter the rhizosphere [Karlowsky et al., 2018]. By promoting microbial interactions in the rhizosphere, the water extract application enhanced the consumption of carbohydrates and amino acids, pointing to a possible drought tolerance mechanism. Additionally, it has been proposed that during drought stress, increasing amino acid exudation may improve the WHC surrounding the roots [Bobille et al., 2019]. By strengthening the cell wall, phenolic acids which increased when alkali and acid extracts were applied can provide interactions between microbial community and plants [Deng and Lu, 2017]. Alkali extract led to increase the consumption of carboxylic acid and amine, suggesting the induction of root exudates rich in organic acids, such as citrate and malate. These are known to improve nutrient solubilization in soil [Canarini et al., 2019]. However, there are some limitations to Biolog EcoPlates because only cultivable microorganisms that can grow in nutrient-rich conditions [Stefanowicz, 2006], despite the carbon sources tested closely resembling compounds commonly found in plant root exudates [Campbell et al., 1997].

5.1.4. Investigation of Biostimulant Effects of *C. barbata* Extracts in Wheat by Foliar Application

Various levels of SWE application have led to positive effects on wheat growth in the range of 13% to 22% (**Figure 4.14**). Consistent with the current literature; the beneficial effects of foliar sprays of SWEs on wheat plant growth and yield parameters have been highlighted [Stirk et al., 2003; Chernane et al., 2015; Shah et al., 2013;

Pačuta et al., 2021; Ali et al., 2022]. Leaf spraying is a useful application technology for biostimulants that have been used in many agricultural products to improve plants growth and physiology [**Mariani and Ferrante, 2017**].

5.1.5. The Impact of Selected *C. barbata* Extracts Treatment on Yield of Wheat by Foliar Application

The grain yield of wheat plants was significantly boosted by the foliar application of water and acid extracts of *C. barbata* (**Table 4.12**). Similarly, a study investigating the application of a biostimulant to wheat grown in the soil showed that grain yield increased by up to 15% but did not significantly affect other growth parameters [**Amaral et al., 2022**]. The impact of biostimulants on winter wheat yield under field conditions revealed a notable increase in 1000-grain weight compared to control [**Popko et al., 2018**]. A foliar application of brown SWEs at the grain-filling stage enhanced grain yield in maize under normal irrigation conditions by improving growth components and reducing oxidative stress [**Trivedi et al., 2018**].

Bioactive substances found in SWEs such phytohormones, vitamins, and amino acids that enhance photosynthesis, regulate source-sink dynamics, and promote cell division are mainly responsible for these yield gains [**Mughunth et al., 2024**]. It has been determined that biostimulants did not cause any notable changes in the mineral nutrients of wheat grains with the exception of Cu levels [**Popko et al., 2018**]. In fact, experiments have shown that the observed effect is not due to the nutrients present in biostimulants [**Ricci et al., 2019**]. It has been reported that the foliar application of brown SWEs (acid extract) activates a complex sensing mechanism involving membrane-bound receptors. This mechanism may activate the MAPK signalling cascade, altering the expression of genes associated with auxin and abscisic acid (ABA) signalling. Ultimately, this affects sugar accumulation and root architecture in sugar beet [**Bertoldo et al., 2023**]. Similarly, water extracts of brown seaweeds have been reported to contain plant growth regulators such as gibberellins, abscisic acid, indole-3-acetic acid (IAA), and salicylic acid [**Benítez García et al., 2020**], which may elicit physiological responses comparable to those observed with acid extracts in the present study. Subsequently it was pointed out that yields were higher even when SWEs were used later in the growth process of plants [**Cai et al., 2014**]. Furthermore, foliar applied brown SWEs enhance nitrate uptake and its incorporation into organic

compounds [Shukla et al., 2023], while also promoting soluble sugar accumulation and upregulating genes associated with nitrogen transporters, potentially aiding in photoassimilate remobilization [de Castro et al., 2024]. In a similar study, wheat yield was efficiently improved by the foliar application of SWE, which is applied at certain growth stages [Stamatiadis et al., 2021]. Together, these results highlight how crucial biostimulant composition and application timing are to maximizing the biostimulant activity of SWEs.

5.2. Tomato Experiments

5.2.1. Investigation of Biostimulant Effects of *C. barbata* Extracts on Tomato by Drench Application

Increasing food productivity and ensuring food safety are major concerns in agricultural efforts which has concentrated on creating sustainable approaches [González-González et al., 2020]. Numerous research indicate that SWEs positively influences tomato yield and quality of [Ali et al., 2016; Weisser et al., 2024]. This chapter examines the application of SWEs on tomato plants to assess their potential biostimulatory effects on yield and quality via soil drenching.

The variety of seaweeds, extraction methods, and additional substances in the SWEs formulations can influence the efficacy of SWEs when applied as soil drenches for tomato plants [Di Stasio et al., 2018; Mzibra et al., 2021; Vagnela et al., 2023]. Although the application levels of SWEs vary according to the literature, optimising application levels and frequencies is important for providing economic benefits. This is particularly important for tomato cultivation [Colla et al., 2017; Yao et al., 2020]. The application level of SWEs optimized according to previous results [Mutlu-Durak et al., 2024; Arikan-Algul et al., 2025].

In addition to fruit yield, the vegetative and generative development of tomato plants was enhanced by *C. barbata* extracts (Table 4.16, Figure 4.16 and Figure 4.17). Among biostimulant treatments, alkali and acid extracts enhance stem length during early growth and result in the maximum tomato output. This study employed a determinate tomato cultivar characterized by a branched growth habit, but lateral growth preserved to avoid any negative effects. Enhancing stem length underlines improved seedling performance in this case. Tomato growth and flowering

performance are essential factors that influence yield performance in determinate varieties [Uzun., 2006]. Water and acid extract applications enhanced flower number on the plants which may lead to yield improvement. Providing evidence for our conclusions, brown SWEs have been shown to improve the inflorescence and flower number of tomato plants [Hussain et al., 2021], as well as the vegetative development [Hernández-Herrera et al., 2022]. Increasing crop yield and upregulating the genes responsible for tomato flowering have been noted [Dookie et al., 2021]. It has demonstrated the efficacy of SWEs in enhancing tomato yield, both in terms of floral production and fruit productivity [Weisser et al., 2023]. Additionally, tomato ripening can be accelerated by SWEs applications, allowing for earlier harvests and greater financial benefits [Mannino et al., 2020; Yao et al., 2020].

The acidity and Brix value of tomato fruits are commercially important for defining the quality and sensory properties of tomato [Zhu et al., 2018; Zhang et al., 2023]. In the process of tomato ripening, there is a decrease in pH corresponding to a reduction in organic acids, particularly citric acid. This metabolica activity led to enhancement in the brix value [Iglesias et al., 2015]. The pH of ripe, excellent in quality tomatoes generally remains below 4.6, which provides a balanced flavor and adequate acidity without the need for heat treatment [Hernandez Suarez et al., 2008; Anthon and Barrett, 2012]. Tomato fruits showed more acidic character, high EC, and Brix values relative to non-SWEs-treated plants in this study (Table 4.17). Moreover, fruit maturity is linked to EC [Lamsal and Jindal, 2014], while sweetness and commercial acceptability are connected to the Brix value [Zhang et al., 2023]. The Brix value may also indicate the activity of enzymes involved in ripening [Kays, 1997] and facilitate the distribution of essential minerals within the plant [Liu et al., 2018; Jeandet et al., 2022]. Numerous research has documented substantial increases in tomato Brix values in response to SWE applications, suggesting an improvement in tomato fruit quality [Mzibra et al., 2021; Subramaniyan et al., 2023; Iacuzzi et al., 2024].

The soluble solid content of a tomato determines its Brix value, as well as the levels of carbohydrates and amino acids [Anthon et al., 2012; Zhang et al., 2023; Li et al., 2021]. Therefore, an increase in Brix value during tomato ripening indicates an elevated level of soluble carbohydrates. It can be suggested that Brix correlates positively with the sugar content of tomatoes [Zhu et al., 2018; Agius et al., 2018].

The highest and lowest enhancements in Brix and TSC were noted in response to the water and acid extract applications, respectively (**Table 4.17** and **Figure 4.22**). This tendency is also valid for EC results of tomato fruits.

There were increasing and decreasing trends in macro- and micro-mineral concentrations in response to SWE applications (**Tables 4.18** and **Table 4.19**). Higher levels of K were detected in the *C. barbata* extracts (**Table 3.2**), which is the most often studied macronutrient for optimizing tomato quality [Sonntag et al., 2019; Kutman, 2023]. Furthermore, K is significant for human health as a nutritional component in nutritious diets [Townsend et al., 2023], so improving K level of tomato via SWE applications needed to emphasize. However, research showed a more dramatic effect of increasing K levels in tomatoes [Zodape et al., 2011; Di Stasio et al., 2018], which suggests that the increase in K levels achieved using water and alkali extracts in the current experiment was relatively modest. Conversely, a reduction in Ca levels in SWE-treated fruits may arise via an antagonistic relationship between Ca and K during soil uptake or translocation process [Hernández-Pérez et al., 2020].

It has been reported that mineral concentrations are on a dry matter basis. Even though the dry matter ratio of plants wasn't specifically measured after biostimulant applications, earlier publications show a strong positive connection between Brix value and dry matter of plants, which includes both soluble and insoluble compounds [Jauregui et al., 1998]. The elevated Brix values in all biostimulant-applied plants, even those subjected to acid extract, indicate that these fruits possessed much higher K concentration on a FW basis compared to the control plants.

The concentration of S in the tomato fruits changed significantly according to the SWE treatments (**Table 4.18**). The water extract contained K ions and S-containing ingredients such as fucoidans naturally present in the seaweed itself. Additionally, the production process of alkali and acid extracts involves the application of K and S through pH adjustments, resulting in higher K and S levels. Along with the primary impacts of S from *C. barbata* extracts, the metabolic changes brought about by their biologically active components like fucoidans, may enhance N and S utilization, as noted and examined by many investigations [Shukla et al., 2016; Bouissil et al., 2020; Nivetha et al., 2020]. Potassium is essential for the translocation of photosynthetic products into the phloem and their subsequent transport to sink organs, including fruits [Daoud et al., 2020]. This indicates that optimal K supply correlates with increased

yields, as reported in cocktail tomatoes [Amjad et al., 2014]. It has also been shown to affect tomato quality in addition to yield, such as fruit color [Hartz et al., 2005] and soluble carbohydrate concentration similar to this study [Sonntag et al., 2019].

Sulfur is significant for the taste of tomato fruit with lots of aromatic S-containing ingredients [Du et al., 2015]. It is well established that fruits abundant in S stimulate the formation of endogenous GSH, which acts as a natural antioxidant [Al-Temimi et al., 2023]. In addition, S is crucial for synthesizing essential amino acids such as cysteine, which, associated with glutamic acid and glycine [Li et al., 2020]. In accordance with this, the increased level of S of tomato fruits in response to *C. barbata* extract-applied plants were associated with much greater GSH levels (Table 4.21). The cycle of ascorbate-glutathione is a major antioxidative metabolic process to eliminate ROS [Decros et al., 2023]. Moreover, GSH is also crucial for ensuring redox equilibrium in the human metabolism as both a diagnostic and therapeutic target because its levels are lower in many chronic diseases [Minich et al., 2019]. However, the bioavailability of GSH is open to discussion. Foods rich in GSH and its precursors have been suggested as supporting human health.

Numerous research documented the biostimulatory effects of brown SWEs applications on the levels of microelements in tomato fruits, including Cu, Fe, Mn, and Zn [Zodape et al., 2011; Mannino et al., 2020]. A minimal fluctuation in micronutrient concentrations occurred following the application of SWEs (Table 4.19), such as modest increase in B via water extract application. Additionally, B fertilization has an importance for fruit quality such as taste in the process of tomato growing [Xu et al., 2021].

The phenolic accumulation in tomato fruit increased significantly after the application of three SWEs up to 22% (Figure 4.18A). Recent research found that tomato plants that were treated with alkali extracts of two different brown seaweeds [Ali et al., 2019; Kumari et al., 2011] had higher phenolic content than control plants. Phenolics are secondary compounds that help plants resist biotic and abiotic stress conditions [Kumar et al., 2023; Khanday et al., 2024], and suggested that consuming phenolic acids is good for people's health and protective against different diseases [Del Rio et al., 2013]. An elevation in TPC is therefore regarded as a desirable outcome. Given that phenolic acids are potential antioxidants present in plants, a robust link between TPC and antioxidant activity can be asserted [Jacobo-Velazquez et al., 2009].

Consistent with the results, all *C. barbata* extracts notably increase tomato's antioxidative activity as measured by three methods (**Figure 4.18B-D**). Reports indicate that biostimulant treatments markedly enhance antioxidant capacity, as assessed by ABTS and DPPH [**Mannino et al., 2020**]. The beneficial biostimulatory effects may stem from the bioactive constituents of SWEs, including alginates, fucoidan, and laminarin, which can activate and enhance antioxidative enzyme activity, hence contributing to phenolic synthesis [**Stirk et al., 2020; Bouissil et al., 2020; Ali et al., 2021b**]. Subsequently, SWEs may possess compatible solutes such as mannitol, which might enhance tolerance mechanisms to water-stress conditions and function as a secondary metabolite [**Di Stasio et al., 2020; Mutlu-Durak et al., 2024**]. It may also be posited that all *C. barbata* extracts are abundant in phenolics, which can be absorbed, altering signaling mechanisms in the plants and resulting in elevated levels of phenolics in treated plants.

The utilization of the water extract markedly increased lycopene concentration in tomato fruits (**Figure 4.19**). While alkali extract application did not produce a significant alteration relative to the control, acid extract resulted in a minor rise. Lycopene, the primary pigment that imparts the red color to ripen tomatoes, acts as an indication of fruit quality [**Li et al., 2024**]. It serves as a powerful antioxidant by scavenging ROS [**Saini et al., 2020**]. Numerous research has indicated that the use of SWEs can notably enhance lycopene level of tomato fruits [**Kumari et al., 2011; Mzibra et al., 2021; Subramaniyan et al., 2023; Layek et al., 2023**]. In addition, the impact of K on fruit quality, particularly in relation to lycopene synthesis, has been extensively documented [**Fanasca et al., 2006a, 2006b**]. It has been reported that there is a positive correlation between increased K concentration and higher lycopene content in tomato (**Table 4.18 and Figure 4.19**). Lycopene, the precursor of all carotenoids, functions as an accessory photosynthetic pigment and serves as the primary dietary source of provitamin A [**Stra et al., 2023**]. In this study, *C. barbata* extracts also led to notable increases in β -carotene levels, particularly in the water and alkali extract applications (**Figure 4.19**). In fruits treated with alkali extract, lycopene may have been redirected toward β -carotene biosynthesis during ripening, rather than toward lutein formation, which may explain the observed reduction in lutein content [**Giorio et al., 2013**]. The boost in lycopene content due to SWE treatments can be attributed to the existence of cytokinin- and auxin-like chemicals, believed to facilitate

the mobilization of essential nutrients within the fruit [Kumari et al., 2011]. Concurrent elevations in lycopene and β -carotene concentrations subsequent to the application of SWE-based biostimulants have also been documented [Sidhu et al., 2017]. The antioxidant qualities of lycopene suggest that consumption of SWE-treated, lycopene-rich tomatoes may aid in the prevention of specific chronic diseases [Tan et al., 2010].

Tomatoes serve as a valuable source of vitamin C which is a crucial quality attribute; numerous studies have demonstrated that SWE based biostimulants markedly enhance total AsA concentrations in tomatoes [Kumari et al., 2011; Subramaniyan et al., 2023; Colla et al., 2017; Ali et al., 2016; Sarinana-Aldaco et al., 2022]. However, no differences were observed in such studies [Di Stasio et al., 2018; Mannino et al., 2020]. Consistent with previous findings, this study demonstrated that all of the SWEs applications increased total AsA levels (**Figure 4.20**). With the water extract having the greatest impact, the concentration of reduced AsA was further increased by the SWEs treatments.

It has been known that optimal production conditions are not always consistent with quality, leading to a potential imbalance between the two factors which are yield and quality [Kutman, 2023]. Nevertheless, there are examples of SWEs being used as biostimulants on tomato plants, improving growth, yield and quality simultaneously [Hussain et al., 2021; Mzibra et al., 2021]. Contrary, there is an example of the nutritional quality of tomato fruits can be improved by two distinct brown SWE applications; one did not influence growth or yield, while the other diminished yield [Di Stasio et al., 2018]. This study demonstrated that all evaluated SWEs increased tomato yield and improved various quality metrics. Water extract was the most promising in enhancing essential quality attributes, including Brix value, antioxidant activity, carotenoids and vitamin C levels. However, regarding total yield achievement, it was exceeded by the alkali and acid extract applications under the specified greenhouse circumstances.

5.2.2. Investigation of Biostimulant Effects of *C. barbata* Extracts on Tomato by Foliar Application

The foliar application of alkali and acid extracts of *C. barbata* led to a notable increase in fruit yield of tomato plant (**Figure 4.23**). In a similar study, foliar application of brown SWEs proved to be effective in terms of growth and physiological responses in the cultivation of tomato [Villa e Vila et al., 2023]. The foliar application of SWE has been demonstrated to enhance tomato growth, yield and quality parameters in the literature [Kumari et al., 2011; Yao et al., 2020; Subramaniyan et al., 2023]. Although foliar applied SWE can be absorbed through the stomata or cuticle of plants, the effectiveness can be impaired by environmental factors [Battacharyya et al., 2015]. Consistent with current literature, soil drenching was more effective compared to foliar application of *C. barbata* extracts. Environmental conditions may limit the effectiveness of foliar treatments, which might help demonstrate why soil application yielded better outcomes [Mattner et al., 2018]. In addition, tomato plants yielded better fruit production when the commercial *A. nodosum* extract was given to the soil rather than through foliar application, demonstrating the higher efficacy of soil drenching [Ahmed et al., 2022].

The observed yield increase in tomato plants following foliar application of *C. barbata* extracts in this study can be ascribed to several interacting physiological factors. Foliar biostimulants enhance nutrient uptake in plants by augmenting the activity of specific genes involved in nutrient absorption and also microbial activity in both roots and leaves (Shukla et al., 2023). Although there were slight decreases in Ca and Zn, and an increase in S with SWEs applications, no dramatic changes were observed in the concentrations of the measured mineral elements in general (**Table 4.24** and **Table 4.25**), the findings suggest a potential enhancement in C and N metabolism. Elevated concentrations of chlorophyll and photosynthesis associated with seaweed bioactive substances may enhance plant growth and C absorption. Moreover, foliar SWEs have been documented to facilitate the endogenous accumulation of osmoprotectants including glucose and sucrose, thus boosting osmotic adjustment and aiding in enhanced fruit retention [Goñi et al., 2018]. The detected elevation in Brix readings in SWE-treated plants further corroborates this postulated mechanism (**Table 4.23**). Additionally, SWEs encompass phytohormones may enhance flowering and fruit growth [Khan et al., 2009].

5.3. Broccoli Experiment

5.3.1. Investigation of Biostimulant Effects of *C. barbata* Extracts on Plant Growth and Quality of Broccoli by Drench Application

In this chapter, it has been sought to verify whether SWEs activate distinct physiological processes in broccoli. In general, SWE-treated broccoli plants showed better growth performance at an early stage of growth compared to control plants (**Figure 4.25**). Broccoli is particularly sensitive to extreme environmental conditions [**Prohens et al., 2008**], which can change the color from green to purplish because of anthocyanin accumulation [**Raiola et al., 2017**]. The recent growing periods for broccoli experienced mild stress due to unexpected highlight and temperature conditions, resulting in the detection of purple coloration in the lower leaves, particularly more pronounced in control plants.

The yield of numerous crops rarely reaches its optimum production potential, thereby diminishing this yield gap, which is crucial for enhancing food security [**Smith and Merchant, 2018**]. In accordance with literature, it has been demonstrated that SWEs can enhance the commercial value of broccoli. Indeed, it has been revealed that there has been up to a 46% increase in broccoli head FW, particularly in alkali and acid extract applications (**Figure 4.26**), which is also visually evident in **Figure 4.27**. The application of biostimulants in sustainable agriculture systems results in enhanced biomass growth and production [**Drobek et al., 2019**]. Numerous studies have shown that SWEs positively influence plant growth, yield, and physiological traits in a range of agricultural crops. The SWEs obtained from kelps enhanced early plant growth parameters and biomass of broccoli by in greenhouse conditions [**Mattner et al., 2013**]. It has been also observed that soil application of SWE, derived from a blend of two brown seaweeds, significantly improved growth and yield characteristics broccoli [**Mattner, 2018**]. Likewise, *A. nodosum* extract application enhanced early shoot and root growth of wild cabbage (*Brassica oleracea*) by up to 50% [**da Silva et al., 2012**], and early shoot growth of pea [**Yusuf et al., 2012**]. It has been stated that physiological characteristics influence photosynthesis, given that high efficiency of photosynthesis correlates with enhanced plant growth, biomass, and agricultural output [**Calzadilla et al., 2022**]. These findings pointed out the potential of *C. barbata* extracts in enhancing

physiological and morphological characteristics of plants as evidenced by this research.

It is well established that seaweeds naturally accumulate various minerals in marine environments [Delaunois et al., 2014] and this mineral-rich composition is believed to play a central role in the beneficial effects of SWEs on crops. Consistent with previous findings and tomato results, particularly water and alkali extracts, led to a modest increase in K concentration in SWE-treated broccoli plants (Table 4.29). Similar enhancements in N and K uptake have been reported in globe artichoke [Elsharkawy et al., 2021], pea [Elsharkawy, 2019], strawberry [Consentino, 2022], and spinach [Rouphael et al., 2018], which are largely attributed to SWE-induced modifications in root morphology, number and size of xylem and phloem structure, and enhanced transport systems [Billard et al., 2014; Kossak and Dyki, 2008].

Potassium plays a pivotal role not only in the regulation of osmotic balance and stomatal conductance but also in the biosynthesis of essential metabolites such as carotenoids indirectly [Bramley, 2002]. In broccoli, an increase in K concentration following SWE treatments was accompanied by elevated carotenoid levels in this study (Table 4.32), supporting the biochemical role of K in pigment metabolism. Beyond its metabolic influence, K contributes to the development of fibrous tissues, which may enhance mechanical strength of broccoli [Costa et al., 2018]. Furthermore, K serves as a principal counter-ion in the transfer of nitrate and photosynthates, thus influencing nitrogen utilization efficiency and the carbon-nitrogen balance [Alvarado-Camarillo et al., 2018]. The observed increase in K concentration can be considered a valuable indicator of enhanced sink strength and improved nutrient translocation throughout the plant and contribute overall quality in broccoli.

The decrease in S concentration in the leaves, accompanied by an increase in the head tissues of broccoli following *C. barbata* applications (Table 4.27 and Table 4.29), may be attributed to enhanced S remobilization within the plant. This shift is likely caused by biostimulant-induced enhancement of mineral assimilation, which promotes the translocation of photoassimilates via the phloem to sink organs [Ertani et al., 2009]. Photoassimilates are allocated to sink tissues based on their capacity to metabolize or store sugars, and this partitioning strongly influences yield formation [Stitt et al., 2010; Braun et al., 2014].

Sulfur itself can be translocated via the phloem in various chemical forms such as GSH, S-methylmethionine, or glucosinolates. In some broccoli cultivars, S fertilization has been shown to specifically enhance the accumulation of S-rich secondary metabolites, particularly glucosinolates, in the head tissues [Aghajanzadeh et al., 2014]. The GSH results also demonstrate this, with a clear enhancement observed with *C. barbata* extracts applications (Figure 4.29). Since S concentration is closely linked to crop quality [Fuentes-Lara et al., 2019; Li et al., 2020], its efficient assimilation and directed transport toward generative tissues such as the head may represent a physiological strategy to prioritize metabolic advantage in yield and nutritional value.

Additionally, SWE applications rich in organic matter, carbohydrates, and plant hormones have been associated with enhanced plant growth by modulating key physiological and metabolic pathways [Arioli et al., 2015]. Notably, the savory flavor profile of *Brassicaceae* vegetables is also affected by soil S availability, with increased S supply resulting in elevated levels of GSH-like compounds and other sulfur-containing metabolites [Kopsell et al., 2007].

It has been shown that a 26% increase in carotenoid concentration in the broccoli head has been achieved by the application of alkali extract (Table 4.32). In the literature, brown seaweed has been reported to contain various pigments such as chlorophyll a, b, β -carotene and fucoxanthin [Peng et al., 2011]. The application of brown SWEs was reported to increase chlorophyll and carotenoid concentrations in spinach seedlings [Fan et al., 2013]. The application of SWEs exhibited a significant increase in the carotenoid concentration of soybeans [Mathur et al., 2015] and tomato seedlings [Karthik and Jayasri, 2023]. The carotenoids found in SWEs serve as potent antioxidants [Delaunois et al., 2014]. Carotenoids absorb excess sunlight not captured by chlorophylls and safeguard photosynthetic pigments from detrimental reactions in an abundance of oxygen [Collini, 2019]. Moreover, high concentrations of carotenoids enhance agronomic benefits; they are essential for photosynthesis, serve as fundamental constituents of photosystems, provide precursors for the synthesis of plant hormones, transmit signals, and regulate plant growth [Sun et al., 2022]. Carotenoids in spring-grown broccoli may have contributed to protection against excess solar radiation following SWE application. Their levels are known to vary

depending on agricultural practices and climatic conditions. [Nuñez-Gómez et al., 2020].

The SWEs contain a diverse array of phenolic compounds, including flavonoids, phenolic acids, and isoflavones [Delaunois et al., 2014]. The yield and phenolic content of pepper were significantly increased by using SWEs, which are rich in phenolics, ascorbic acid and antioxidants [Ashour et al., 2021]. Conversely, the TPC of broccoli heads was not changed with the SWE applications, but there are significant differences in terms of antioxidant capacity (Table 4.28). It has been indicated that the application of biostimulants significantly enhances antioxidant activity, particularly DPPH levels, with notable increases observed in ABTS and FRAP assays in the edible parts of the plants [Mannino et al., 2022]. Increased antioxidant activity in spinach was observed in response to *A. nodosum* extracts, attributed to enhanced phytohormonal activity stimulated by the biostimulant [Fan et al., 2013]. It has been also shown that the use of brown SWE significantly increased the levels of phytohormones in tomatoes. This suggests that SWE applications contain signaling compounds that trigger hormone biosynthesis, thus promoting growth and increasing fruit yield [Khedia et al., 2020].

Biostimulant applications have been demonstrated to maintain fruit quality variables, including AsA [Koleška et al., 2017]. AsA is a vital component of plants, present in high concentrations in chloroplasts and other cellular compartments. It plays a significant role in photosynthesis, acting as a cofactor for enzymes involved in the biosynthesis of phytohormones and in the regulation of plant cell growth [Smirnoff and Wheeler, 2000]. Total AsA in the broccoli head did not show significant difference with the SWE treatments, but the reduced AsA concentration showed a significant difference with the alkali and acid extract compared to the control (Table 4.33). Similar to recent results, it has been shown that plant based biostimulants were found to enhance the AsA content in both the leaves and roots of turnip in a systematic investigation [Godlewska et al., 2021]. Similarly, the application of legume-derived protein hydrolysate extracts led to an increase in bioactive compounds, particularly total phenols and ascorbic acid, thereby improving the functional quality of spinach [Rouphael et al., 2018]. Furthermore, the foliar application of a commercial brown SWE was reported to enhance the yield and ascorbic acid concentration in pepper [Arthur et al., 2003]. Although the most bioavailable and physiologically active form

of AsA is the reduced form, the oxidized form of AsA can be easily converted to the reduced form, providing a stable supply of AsA in the human body [Padayatty et al., 2003]. On the other hand, the reduced form of ascorbic acid states for the most active form of vitamin C and is capable of scavenging ROS [Arrigoni and De Tullio, 2002]. Increased levels of reduced AsA confer notable benefits in managing stress conditions, enhancing photosynthetic efficiency, and optimizing growth metabolism, which collectively contribute to biomass accumulation and yield [Smirnoff, 2000; Foyer and Noctor, 2011; Smirnoff, 2018]. Furthermore, reduced AsA is important for the cycle of ascorbate-glutathione, which is crucial for maintaining the balance of redox in the plant cells [Foyer and Halliwell, 1976]. Therefore, the observed increase in reduced AsA concentration following *C. barbata* extracts applications may represent a key physiological response that enhances both the antioxidant capacity, stress resilience, and quality of broccoli plants.

The GSH is a potent antioxidant and has the capability to detoxify reactive oxygen species in the plants [Kopriva et al., 2019; Li et al., 2020]. Broccoli is a profitable and high-quality commercial crop with a significant amount of glucoraphanin and aliphatic glucosinolate present in the inflorescence [Tarafder et al., 2023]. It has been indicated that SWEs derived from *Laminaria japonica* exhibited elevated amounts of phenolic activity and S-containing compounds, such as glucosinolates and their derivatives, in broccoli [Flores et al., 2021]. As shown in Table 4.29, it has been found that the S concentration and in parallel, GSH concentration also increases in broccoli head (Figure 4.29). The largest proportion of sulfhydryl components was detected as GSH in the plants [Speiser et al., 2018]. Glutathione represents a key non-protein S source involved in redox balance [Jahan et al., 2019]. Thus, the improvement in GSH as a major sulfhydryl compound may reflect activation of sulfur-dependent metabolic pathways, contributing to the accumulation of health-promoting phytochemicals in broccoli [Minich and Brown, 2019].

The best biostimulant applications in terms of production and quality were found to be alkali and acid extracts. These findings all point to the potential of biostimulants to enhance the physiological and biochemical characteristics of plants, particularly by facilitating the accumulation of bioactive chemicals. As a result, integrating biostimulants into cultivation practices could provide a long-term way to preserve crop quality and yield while reducing reliance on conventional macronutrient fertilization.

5.4. Pepper Experiment

5.4.1. Investigation of Biostimulant Effects of Selected *C. barbata* Extracts and a Commercial Product on Plant Growth and Quality of Pepper by Drench Application

Utilizing *C. barbata* extracts significantly increased fruit number and yield rather than average fruit weight (**Figure 4.30**). Notably, water and Acadian extract treatments led to the most substantial improvements, highlighting the biostimulant potential of *C. barbata* in enhancing pepper productivity. These results were comparable to those obtained with the commercial formulation, further supporting its practical relevance for agricultural use. In addition, Türkiye has a diverse range of pepper genetic resources, particularly sweet pepper varieties, which are typically harvested before reaching full ripeness to meet consumer preferences [O'Donoghue et al., 2018; Karataş et al., 2024; Toker et al., 2025]. Therefore, improving both production and nutritional quality is strategically important from an agronomic perspective.

Previous investigation has shown that foliar application of brown SWEs greatly improves sweet pepper growth, fruit number, yield, and nutritional quality, which is comparable with our results [Arthur et al., 2003; Rajendran et al., 2022; Mohamed et al., 2021]. Plant hormones like auxins and cytokinins, along with bioactive substances like amino acids and vitamins, increase the activity of genes and help with physiological processes like cell division and growth, which can lead to these advantages [Ali et al., 2019; Prasad et al., 2010; Rokayya et al., 2016]. It has been demonstrated that SWE application upregulates genes involved in cytokinin production in sweet pepper [Ali et al., 2019]. In a similar study, two different brown seaweed-based biostimulants were used and their effects were compared with the commercial product Acadian [Ali et al., 2022]. It has been shown that all applications improved pepper development characteristics, including chlorophyll content, biomass, and yield, and exhibited elevated levels of beneficial microorganisms in both root- and leaf-associated regions compared to control.

The improving nutritional quality in terms of vitamin C, carbohydrate, and carotenoid concentration observed in previous research further supports the biostimulant role of SWEs [Mohamed et al., 2021; El Boukhari et al., 2020]. In this work, TPC, antioxidant activity, GSH, chlorophyll, and carotenoid concentrations showed similar

results with the application of water, acid, alkali extracts compared to both control and Acadian (**Figure 4.31**, **Figure 4.33**, and **Figure 4.34**). All extracts improved the level of total AsA of pepper (**Figure 4.32**). The quality and consumer appeal of sweet peppers are directly influenced by nutritional parameters such as vitamin C and carbohydrates [Howard et al., 1994; Navarro et al., 2006]. Vitamin C is essential for protecting plant tissues from oxidative stress and also benefits human health through dietary consumption [Mellidou and Kanellis., 2024]. In addition, the AsA concentration determined in this study (up to 600 mg kg⁻¹ FW) was found be close to the range from approximately 700 to 2000 mg kg⁻¹ FW for sweet pepper, depending on cultivar, ripening stage, and growing conditions [Howard et al., 1994; Tarrago-Trani et al., 2012; NIH, 2023]. These findings demonstrate that *C. barbata* extracts are equally effective as commercial algae products at increasing sweet pepper productivity and some fruit quality parameters. Acadian application reported to improve drought tolerance in soybean by regulating ABA levels and maintaining leaf temperature and turgor pressure, thereby enhancing physiological stability [Shukla et al., 2018]. It also stimulated the release of root exudates such as flavonoids, which attracted beneficial microbes to the rhizosphere and supported plant resilience [Khan et al., 2012]. These consistent findings demonstrate the seaweed-based biostimulant's broad-spectrum effectiveness in a variety of plant species and environmental settings.

The rise in soluble carbohydrate levels in pepper fruits following alkali extract, despite no notable yield variations different from other SWEs (**Figure 4.30** and **Figure 4.35**), indicates improved photoassimilate translocation and sink activity. It is also evident in the growth of plants, which is closely correlated with source and sink balance [Li et al., 2015]. The source strength of a plant is defined as the rate at which it produces photoassimilates, and also the rate of photosynthesis. On the other side, sink organs depend on the delivery of sucrose (or other carbohydrates) by the phloem for growth and development [Lemoine et al., 2013]. A study indicated that the application of foliar *A. nodosum* extract increased the levels of endogenous glucose and sucrose (soluble sugars) in tomato leaves [Goñi et al., 2018]. This outcome indicates enhanced fruit quality and energy storage, likely leading to nutritional value and postharvest longevity. This also highlights the persistent discourse in agronomy, wherein enhancing quality may be as crucial as optimizing yield, depending on production objectives.

Furthermore, all three treatments, except the alkali extract, significantly outperformed the control, and yield improvements following water and acid extract treatments were on par with those from the commercial product Acadian. All of the extracts increased the amount of vitamin C in pepper fruits. Interestingly, only the alkali extract considerably raised the amount of soluble carbohydrates in comparison to the control; yet, this effect did not vary statistically from that of the Acadian or water treatments. Together, water and acid extracts perform similarly to Acadian when yield and quality metrics are taken into account



6. CONCLUSION

Global agriculture is under pressure to satisfy the nutritional requirements of growing human populations while struggling with climate-related concerns, including increased droughts and resource depletion. Drought significantly threatens crop yield by altering water availability and compromising plant physiological systems. In response to these challenges, the integration of biostimulants into agricultural practices has emerged as a sustainable approach to improve plant growth, stress resilience, and food quality.

This thesis evaluated the biostimulant potential of water, alkali, and acid extracts of *C. barbata* on both soil-grown cereal and horticultural crops grown under greenhouse conditions. The hypothesis was that each extract, due to its distinct biochemical composition, would exert distinct biostimulant effects on plant performance, yield, and quality parameters of plants. Biochemical analyses verified compositional diversity among the extracts, and the findings from experiments indicated that the application methods (foliar and drench) and crop species markedly affected the biostimulant efficacy.

Drench applications of water and acid extracts have been found to be more effective in wheat growth, particularly under drought conditions, by eliciting physiological responses such as enhanced antioxidant enzyme activity, increased accumulation of compatible solutes, and stimulation of root microbial activity. These effects are attributed to the presence of bioactive compounds such as alginates, mannitol, fucoidans, carbohydrates in the extracts. On the other hand, drench applications of acid and alkali extracts led to the significant improvements particularly in tomato and broccoli, with yield increases ranging from 40% to 60%. These treatments enhanced several quality parameters, including antioxidant activity, vitamin C, soluble carbohydrate, and GSH concentrations. While the water extract exhibited relatively modest effects on yield, it contributed notably to improvements in fruit quality attributes, potentially resulting in a trade-off between productivity and crop quality.

Mineral analysis revealed increases in S and K concentrations across the treatments. Although the extracts modestly contributed to fruit mineral concentrations, these changes were not directly associated with the intrinsic nutrient content of the extracts. This suggests that the observed effects may be mediated indirectly, potentially through enhanced nutrient uptake mechanisms or improvements in soil physicochemical properties. Overall, the findings imply that the biostimulant effects cannot be solely attributed to nutrient supplementation but are likely driven by synergistic interactions among multiple bioactive compounds present in the SWEs.

Foliar applications, particularly in wheat, demonstrated that water and acid extracts could enhance grain yield without significantly altering mineral concentrations. However, in both wheat and tomato, foliar treatments were generally less effective compared to drench applications. In tomato, the most pronounced yield responses were observed with drench applications of alkali and acid extracts, whereas in wheat, water and acid extract treatments promoted both early-stage and long-term growth.

In pepper, the most notable positive effect was a significant increase in fruit yield following the application of water and acid extracts. These treatments produced results comparable to those obtained with the commercial seaweed-based biostimulant (Acadian), reinforcing the potential of *C. barbata*-derived formulations as competitive alternatives in the market. Overall, quality attributes remained largely unaffected across treatments, except for increases in vitamin C and soluble carbohydrate concentrations.

Drench and foliar applications each present distinct advantages and limitations. Drench treatments offer prolonged effects on the rhizosphere, promoting deeper physiological responses under stress conditions, but they require higher extract volumes, and their efficacy may vary with soil properties. In contrast, foliar applications allow for rapid, localized effects with lower application rates; however, their impact on yield may be limited, particularly in crops with reduced foliar absorption through their leaves.

Overall, the findings confirm the promising biostimulant potential of *C. barbata* extracts in enhancing plant growth, productivity, and quality under both optimal and drought-stressed conditions. These results underscore that the efficacy of seaweed-

based biostimulants is highly dependent on extract composition, application method, and species-specific crop responses.

Despite these encouraging results, certain factors must be considered prior to commercial implementation. Economic viability, standardization of extraction processes, selection of appropriate level of application, and large-scale manufacturing persist as significant obstacles. Additionally, sustainable sourcing must be ensured, as *C. barbata* is a protected species in the Marmara Sea. Establishing regulated seaweed aquaculture may offer a viable solution. Future research should focus on long-term field trials under diverse environmental conditions, explore molecular mechanisms underlying biostimulant action, and assess the compatibility of SWEs with integrated nutrient and pest management systems. Furthermore, collaboration between researchers, government agencies, farmers, and industry stakeholders will be crucial to unlock the full bioeconomic potential of this local bioeconomic resource.

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BIOGRAPHY

Yağmur ARIKAN ALGÜL completed her bachelor's degree in Bioengineering at Yıldız Technical University in 2017 and participated in the Erasmus exchange program in Biochemical Engineering at TU Dortmund. She later joined the Integrated PhD Program in Biotechnology at Gebze Technical University, where she conducted her doctoral research within Kutman Lab, focusing on the biostimulant effects of *Cystoseira barbata* extracts on soil-grown crops under stress conditions. Her research interests include plant physiology, plant nutrition, sustainable agriculture, and biostimulants. She has been involved in two TÜBİTAK-funded research projects (3501 and 2244) and was awarded the Council of Higher Education (YÖK) 100/2000 PhD Scholarship in the field of Sustainable Agriculture. She has co-authored peer-reviewed publications in Agronomy, Physiologia Plantarum, and Phytomedicine Plus. Throughout her PhD, she worked as an R&D Assistant at GÜBRETAS, gaining extensive experience in greenhouse experimentation, stress physiology, agricultural quality evaluation, and statistical analysis. Her practical skills include the use of advanced analytical techniques such as ICP-OES, HPLC, UV-spectrophotometry, and small-scale bioreactors. She has contributed to the advancement of seaweed-derived biostimulants as sustainable agricultural inputs and remains dedicated to promoting environmentally friendly innovations for advanced agricultural systems.

PUBLICATIONS AND PRESENTATIONS FROM THE THESIS

Arıkan-Algül Y., Mutlu-Durak H., Kutman Ü. B., Yıldız Kutman B., (2025), "Biostimulant extracts obtained from the brown seaweed *Cystoseira barbata* enhance the growth, yield, quality, and nutraceutical value of soil-grown tomato", *Agronomy*, 15(5), 1138.

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Kutman Ü. B., Mutlu Durak H., Arıkan Y., Haznedaroğlu B., Yıldız Kutman B., (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) 2022, 2–5 October 2022, Oral presentation.

Arıkan Y., Mutlu Durak H., Kutman Ü. B., Yıldız Kutman B., (2023), "Potential Biostimulant Effect of Brown Seaweed Extracts (*Cystoseira barbata*) on Tomato Growth and Quality", V. International Conference on Agricultural, Biological and Life Science (AGBIOL 2023), 18–20 September 2023, Oral presentation.

Arıkan Y., Mutlu Durak H., Yıldız Kutman B., Kutman Ü. B., (2023), "The Biostimulant Effects of Brown Seaweed (*Cystoseira barbata*) Extracts on Tomato", International Biotechnology Congress (BioTurkey), 28–30 September 2023, Oral presentation.

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Arıkan Y., Mutlu Durak H., Kutman Ü. B., Yıldız Kutman B., (2024), "Foliar application of brown seaweed (*Cystoseira barbata*) extracts stimulates durum wheat growth and yield", 1st International Congress on Biotech Solutions for Sustainability (Bio4sus), 7–10 May 2024, Poster presentation.