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Diana Medeiros Pacheco

SEAWEEDS AS PLANT HEALTH PROMOTERS

Master Thesis Dissertation within the scope of the Master in Biodiversity and Plant Biotechnology supervised by Professor Dr. Leonel Pereira (University of Coimbra) and by Professor Dr. Kiril Bahcevandziev (Polytechnic Institute of Coimbra) and presented to the Faculty of Sciences and Technology of the University of Coimbra – Department of Sciences of Life.

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Resumo

Atualmente, o aumento exponencial da população humana levou à massificação de práticas agrícolas, sendo a produtividade e sustentabilidade das culturas agrícolas uma das maiores preocupações. Paralelamente, a intensificação do uso de fertilizantes e pesticidas sintéticos tem levado a impactos nocivos na saúde humana e ao meio ambiente. Sendo por isso uma das tendências atuais, a busca por novos produtos estimulantes e pesticidas naturais. Neste contexto, as algas surgem como um elemento-chave, sendo que é evidenciado na literatura o potencial dos seus extratos e alguns compostos (exemplo: polissacarídeos) para contribuir para um futuro mais sustentável da prática agrícola. Com este objetivo, avaliou-se o impacto de polissacarídeos sulfatados na germinação e desenvolvimento de plântulas de couve (*Brassica oleracea*). Como tal, os principais polissacarídeos das algas (agar, carragenana e alginato) foram extraídos, caracterizados quimicamente por espectroscopia infravermelha transformada de Fourier (FTIR-ATR) e aplicados em sementes de *B. oleracea* (1 mg/ mL). Entre os polímeros testados, a carragenana-iota (de *Calliblepharis jubata*), a carragenana-kappa/ iota (do gametófito feminino de *Chondracanthus teedei* var. *lusitanicus*) e agar (de *Gracilaria gracilis*) apresentaram efeitos positivos no crescimento da couve, particularmente no comprimento e peso da parte aérea. De seguida, foram realizados ensaios em estufa com o extrato aquoso da alga castanha *Saccharina latissima* (1,2% v/v) e o biofertilizante à base de bactérias BlueN (0,03% m/v), tanto aplicados isoladamente como em conjunto. Verificou-se que o extrato de alga isolado e em conjunto com o BlueN afetaram o crescimento e as características nutricionais da alface (*Lactuca sativa* var. *crispa* L.) positivamente, produzindo folhas de alface mais pesadas (74,13 ± 3,07 e 74,25 ± 6,86 g, respetivamente) e enriquecidas com micronutrientes, como o manganês e o zinco. Com uma abordagem estratégica e integrada em mente, os polissacarídeos previamente extraídos (1 mg/ mL) e os seus extratos aquosos (1,2 % v/v) foram testados quanto ao seu potencial antifúngico contra *Botryosphaeria dothidea* (Ascomycota) através do método de difusão de disco de agar. Apesar de neste estudo, não se ter observado inibição do crescimento fúngico, a literatura indica que os compostos de algas podem estimular respostas de defesa da planta e proteger as culturas agrícolas contra vários fito-patógenos.

Em suma, este estudo demonstra que os extratos aquosos e os polissacarídeos das macroalgas têm potencial no estímulo do desenvolvimento e crescimento de plântulas/ plantas, no entanto, não se verificou atividade antifúngica.

Palavras-chave: Agricultura sustentável; Bioestimulante; Compostos bioativos algais; Macroalgas vermelhas e castanhas; Potencial antifúngico

Abstract

Currently, the exponential growth of the human population has resulted in the massification of agricultural practices, with agricultural productivity and sustainability being among the most pressing concerns. Similarly, the increased use of fertilizers and synthetic pesticides has resulted in negative effects on human health and the environment. As a result, one of the current trends is the search for new stimulants and natural pesticides. In this context, algae emerge as a key element, with literature demonstrating the potential of its extracts and some compounds (for example, polysaccharides) to contribute to a more sustainable agricultural practice. With this goal in mind, the impact of sulfated polysaccharides on the germination and development of kale (*Brassica oleracea*) seedlings was evaluated. As a result, the main algal polysaccharides (agar, carrageenan, and alginate) were extracted, characterized chemically by infrared spectroscopy (FTIR-ATR), and applied to *B. oleracea* seeds (1mg/ mL). Among the polymers tested, iota-carrageenan (from *Calliblepharis jubata*), kappa/iota-carrageenan (from the female gametophyte of *Chondracanthus teedei* var. *lusitanicus*) and agar (from *Gracilaria gracilis*) showed positive effects on kale seedlings growth and development, particularly on the length and weight of the aerial part. Afterwards, experiments in glasshouse were conducted using an aqueous extract of the brown seaweed *Saccharina latissima* (1.2% v/v) and a biofertilizer based on bacteria, BlueN (0.03% m/v), both separately and in combination. The seaweed extract alone and combined with BlueN affected the growth and nutritional characteristics of lettuce (*Lactuca sativa* var. *crispa* L.) positively, resulting on heavier lettuce leaves (74.13 ± 3.07 e 74.25 ± 6.86 g, respectively) and enriched with micronutrients, such as manganese and zinc. With a strategic and integrated approach in mind, previously extracted polysaccharides (1 mg/ mL) and their aqueous extracts (1.2 % v/v) were tested for their antifungal potential against *Botryosphaeria dothidea* (Ascomycota) using the agar disk diffusion method. Although no inhibition of fungal growth was observed in this study, the literature indicates that algae compounds can stimulate plant defense responses and protect agricultural crops against several phytopathogens.

In short, this study demonstrates that seaweed aqueous extracts and polysaccharides have potential in plant growth stimulation and seedling development, however, they do not show antifungal activity.

Keywords: Algal bioactive compounds; Antifungal potential; Biostimulant; Red and brown seaweeds; Sustainable agriculture.

General Index

List of publications and scientific communications related directly to this thesis

Articles

Cotas, J., Pacheco, D., Araujo, G. S., Valado, A., Critchley, A. T., & Pereira, L. (2021). On the health benefits vs. risks of seaweeds and their constituents: the curious case of the polymer paradigm. *Marine Drugs*, *19*(3), 164. https://doi.org/10.3390/md19030164

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de Melo, P. C., Sousa, T., Teixeira, R., Cotas, J., Pacheco, D., Gonçalves, A. M. M., Bahcevandziev, K., & Pereira, L. (2022). Seaweeds and their derivates as a multirole tool in agriculture. In J. Sangeetha & D. Thangadurai (Eds.), *Seaweed Biotechnology: Biodiversity and Biotechnology of Seaweeds and Their Applications* (pp. 201–227). Apple Academic Press - CRC Press, New Jersey, USA.

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Pacheco, D., Miranda, G., Pato, R. L., Cotas, J., Santos, S. M. D., Pereira, L., Bahcevandziev, K. (2021) *Nutritional evaluation of Laminaria ochroleuca harvested in the North of Portugal*. [Poster presentation]. Seaweed4Health Virtual Conference.

Pacheco, D., Rocha, C. P., Cotas, J., Bahcevandziev, K., Gonçalves, A. M. M., Pereira, L. (2021) *Fatty acid and polysaccharide evaluation of the non-native brown seaweed Undaria pinnatifida located in two geographical sites of the Portuguese coast*. [Poster presentation]. Seaweed4Health Virtual Conference, 2021.

Figures Index

Abbreviation List

AOAC: Association of Official Analytical Chemists

°C: Degree Celsius

CIRAD: Agricultural Research Centre for International Development

CFU: colony-forming unit

cm: centimeter

DW: dry weight

EC: Eletrical conductivity

EU: European Union

FAO: Food and Agriculture Organization

FW: fresh weight

FTIR-ATR: Fourier-Transform Infrared - Attenuated Total Reflectance

g: gram

GAE: Gallic acid equivalent

GP: Germination percentage

h: hour

I: iota

k: kappa

λ: lambda

mg: milligram

ml: milliliter

mm: millimeters

m/v: mass/ volume

rpm: Revolutions per minute

UV/VIS: Ultraviolet/ Visible

v/v: volume/ volume

WHO: World Health Organization

1. Introduction

1.1. Societal challenges

Crop plants are important sources of nutrients (such as potassium, iron, and zinc) for a healthy human diet (Schreinemachers et al., 2018). However, our world will confront two main challenges in the future: climate change and a growing global population. The significant rise in food demand must be met while reducing agriculture's global environmental footprint, and this at a time when agriculture is already under pressure due to climate changes (Schreinemachers et al., 2018). Due to the exponential growth of the human population, it is estimated that by 2050, the world would require 60% more food than it does today, with around 80% of this increase coming from already under cultivated land (FAO, 2018). Agriculture applies agrochemicals (synthetic fertilizers and pesticides), intense tillage, and over-irrigation to meet the current food demand (Kopittke et al., 2019; Petersen & Snapp, 2015). Although optimal agrochemicals utilization leads to higher returns, it can have hazardous and long-term negative consequences on the environment and humans, particularly when used incorrectly or excessively. These disadvantages may result in increased pathogens pesticide resistance, pesticides residues run-off into the environment generating major problems like as eutrophication, water contamination, harming humans and animals, and causing overall increases in the production costs (Dubey, 2010).

The uncontrolled and excessive use of synthetic chemical inputs to enhance agricultural productivity is degrading the soil and threatening the environment, with it being estimated that 40% of global arable land suffers from decreasing fertility (Foley et al., 2011).

Aside from these environmental impacts, researchers highlights that the persistent misuse of pesticides and chemical fertilizers has resulted in the extinction of non-target beneficial organisms, affecting the entire food chain, and causing biodiversity loss (Ratnadass et al., 2012).

The World Health Organization (WHO) reports about 25 million cases of acute occupational pesticide poisoning in developing nations (Thundiyil, 2008). Thereby, many of these synthetic pesticides and fertilizers are now strictly regulated, particularly in industrialized nations, due to research demonstrating their persistence in the environment and highly harmful effects on humans' health. Thus, raising concerns in the agriculture industry (Nisha et al., 2021).

For these reasons, along with the 2030 Global Agenda, the Sustainable Development Goal #12 (European Parliament, 2019) seeks for an efficient and environmentally friendly management of the agricultural resources and practices. Considering integrated management strategies, scientists and farmers alike are looking for environmentally friendly alternatives, in which algal biomass and its extracts as biofertilizer and biostimulant products are in the research forefront (Caradonia et al., 2019).

1.2. Seaweeds: an important marine resource to address and promote Sustainable Development Goals

Seaweeds play an important ecological role, particularly as nursery areas that protect the shoreline while also sequestering carbon and promoting primary production (Araújo et al., 2016; Grebe et al., 2019; Schoenrock et al., 2018; Zhu et al., 2020). However, currently, the marine environment is being endangered by the overgrowth of non-indigenous seaweed species, threatening coastal fauna, flora, and the ecosystem services they provide (European Union, 1981; van Kleunen et al., 2010). For instance, the Iberian Peninsula has been targeted by several non-indigenous seaweeds, where the red seaweeds *Asparagopsis armata*, *Grateloupia turuturu* and the brown seaweeds *Sargassum muticum*, *Undaria pinnatifida* and *Colpomenia peregrina* have been considered serious threats to the environment, due to their overgrowth (Pacheco et al., 2020). In fact, when viewed from an ecosystem perspective, non-native seaweed harvesting/ removal can be an important approach for maintaining ecosystem stability, as the biomass can be used to produce fertilizer, as seen in Canada with the non-native red seaweed *Mazzaella japonica* (Pardilhó et al., 2022).

From another perspective, native seaweed cultivation can be seen as a profitable, sustainable, and environmentally friendly solution (Grebe et al., 2019; Zhu et al., 2020). Due to the low investment required to establish seaweed farms, aquaculture is a particularly robust strategy for developing coastal nations to contribute for climate change mitigation while protecting their coasts and marine ecosystems from some effects of the climate change (Duarte et al., 2017). Seaweed native population protection and cultivation, for example, has the potential to mitigate the effects of climate change and ocean acidification through carbon dioxide sequestration (Araújo et al., 2016; Schoenrock et al., 2018). Hence, seaweeds can help to achieve the targets of Sustainable Development Goal #13 (Climate Action) in several ways. Thus, the assessment of the biotechnological applications and economical value of algal resources should be conducted, not only on native seaweed species, but also on non-indigenous seaweed species.

The biotechnological potential of seaweeds reveals the ability to contribute to meet the requirements of the objectives of Sustainable Development Goal #12. Achieving economic growth and sustainable development requires that we urgently reduce our ecological footprint by changing the way we produce and consume resources. Agriculture is the world largest water consumer, and irrigation now accounts for nearly 70% of all freshwaters. Currently, high food demand has resulted in widespread agricultural practices, with crop productivity being one of the primary concerns for producers. However, the increased use of synthetic fertilizers has had serious negative effects on human health and the environment. The search for novel and natural agricultural products is a current trend, and seaweed polysaccharide bioactivity can help in this endeavor (Pacheco et al., 2021). Seaweeds are marine resources that produce

a variety of primary and secondary metabolites that have a significant positive impact on agricultural crops (Mzibra et al., 2018). Several studies have shown that different seaweed extracts can improve seed germination, plant growth, and development (Demir et al., 2006; Di Filippo-Herrera et al., 2019; Hernández-Herrera et al., 2016; Hernández Carmona, 2018; Khan et al., 2009; Sousa et al., 2020). Furthermore, it has been demonstrated that seaweed extracts can help plants cope with biotic and abiotic stresses (such as drought and salinity), while also improving crop chemical characterization, such as minerals and pigments (Bharath et al., 2018; Bonomelli et al., 2018; Khan et al., 2009; Martynenko et al., 2016; Michalak et al., 2017). Improving seed germination and, therefore, plant growth and production for food security is a challenging task (van den Burg et al., 2016). Because abiotic and biotic stressors can have a significant impact on seed and plant germination, growth, and productivity, several biotic elicitors can be used to induce crops productivity (Tallman et al., 2018). Still, organic agriculture, frequently results in yield losses of 20% or more when compared to conventional cultivation due to increased biotic pressure and nutrient constraint (de Ponti et al., 2012; Ponisio et al., 2015). Hence, biotechnological-driven solutions research that enables efficient resource management, particularly of water, nutrients, and soil, while maintaining high yields and high-quality products, will be essential in the future years for agricultural intensification to be sustainable (Colla et al., 2015; Craigie, 2011; Petersen & Snapp, 2015).

1.3. Biostimulant and biofertilizers contribute towards a sustainable agriculture

Plant biostimulants and biofertilizers have captivated the interest of the scientific community and agrochemical companies (du Jardin, 2015; Yakhin et al., 2017). Biofertilizers are organic substances (e.g., seaweeds) or products which contains organisms (e.g., fungus, bacteria) that colonize the rhizosphere or plant tissue when applied to the seed, plant surface, or soil, stimulating growth, yield, and improving the supply or availability of primary nutrients to the host plant, without deleterious environmental side effects (Adjanohoun et al., 2012; Dasgupta et al., 2021; Lokya et al., 2019). One of the oldest applications of seaweeds is as a biofertilizer in agriculture (Illera-Vives et al., 2020; Sousa et al., 2020). Because seaweeds are a source of macro and micronutrients, their use has been optimized to enhance soil quality while increasing crop yield. It has been stated by Battacharyya et al. (2015) and Illera-Vives et al., (2020) that ancient Romans employed algae to retain the moisture and freshness of the seedlings and to fertilize the coastal soils along the Atlantic populations. In Portugal, the use of seaweeds to fertilize soils has been practiced since the 14th century, though it has since fallen into disuse and is now only practiced in the country's northern region (Pereira et al., 2019). As a result, a variety of seaweeds are employed as soil conditioners to enhance organic matter while also preserving soil moisture and mineral content (Mathur et al., 2015).

Concurrently, free-living bacteria can also be employed as external nitrogen source, promoting plant growth (Lugtenberg & Kamilova, 2009; Murugan et al., 2020). For example, *Methylobacterium* is a bacteria genus noted for its eco-friendly ability to improve plant growth through atmospheric nitrogen fixation, phosphate solubilization, and the production of plant growth promoters (Kennedy et al., 2004; Murugan et al., 2020). Research on the potential of this bacteria led to development of a commercially available biofertilizer, BlueN, a foliar treatment containing *Methylobacterium symbioticum*. This bacterium contains an enzyme nitrogenase, which converts atmospheric nitrogen (N_2) into ammonia (NH_4^+), used for glutamine production, an essential amino acid for plant needs. This bacterium colonizes the plant during its early growth stages, providing a supplementary and direct nitrogen source to the plant without the risk of volatilization or leaching. Hence, this product has been shown to improve vegetable, extensive, and ligneous crop cultures (Corteva, 2022).

According to Regulation number (EU) 2019/1009, biostimulants are biologically derived products that, even in small amounts, can stimulate nutrient absorption by acting indirectly on soil structure and nutrient availability, or by directly affecting plant physiology while increasing its quality and chemical composition (Halpern et al., 2015; Yakhin et al., 2017). Biostimulant compounds are a wide range of products that include seaweed extracts. The mechanisms of biostimulant effects are influenced by several elements, beginning with the raw materials and the manufacturing procedures used to create the final commercial product (Calvo et al., 2014; Muscolo et al., 2013; Shukla et al., 2019). Regardless of product type, the presence of active molecules (i.e.: peptides, algal polymers) that mimic and/ or trigger the production of phytohormones and plant growth regulators, stimulates plant growth and productivity (Ali et al., 2021; Colla et al., 2017; Shah et al., 2018; Shukla et al., 2019). Hence, plant biostimulants elicit a cascade of messages that result *in vivo* by regulating the plants' main and secondary metabolism (Rouphael & Colla, 2020).

1.4. The diversity of algal bioactive compounds and their agricultural potential

Seaweed extracts have long been utilized as foliar sprays in Canadian and European coastal agricultural areas to promote crop growth, yield, and productivity (Pardilhó et al., 2022; Silva et al., 2019). Biostimulants derived from seaweed are among the most effective current biological plant growth enhancers. Moreover, they have been shown to mitigate plant diseases and abiotic stressors, resulting in increased yields (Hassan et al., 2021). Thus, seaweed extracts as biostimulants are among the best current sustainable biological plant growth promoters since they are biodegradable and nontoxic, with no synthetic chemical residues or hazards (Hassan et al., 2021). Hence, seaweeds and its compounds could be used to create novel products that enhance seed germination, plant growth and development, while mitigating the consequences of climate change by replacing chemical products application and lowering

production costs (Di Stasio et al., 2018; Ertani et al., 2018; Khan et al., 2009; Senthuran et al., 2019; Silva et al., 2019). In fact, seaweed-based biostimulants commercially available, such as Maxicrop, Algifert, Goemar, Kelpak, Seaspray, Seasol, Cytex, Profertil and Seacrop can be found on the market (Hernández, 2018; Pise & Sabale, 2010). Being most of them based on brown seaweed extracts based on *Ascophyllum nodosum*, *Fucus* spp., *Laminaria* spp., *Sargassum* spp., *Ecklonia maxima*, and *Durvillaea* spp. (Craigie, 2011). As they contain higher levels of organic matter, macro- and microelements, vitamins, fatty acids, betaines, carotenoids, phenolic compounds and plant growth regulators, these seaweed-based products are thought to be better when compared to synthetic ones (Nabti et al., 2017; Shukla et al., 2021), because all these bioactive compounds elicit and directly promote plant growth and defense reactions (Khan et al., 2009). In fact, biostimulants based on seaweed extracts have been shown to influence crop plants photosynthetic rate, nucleic acid synthesis and ion uptake (Craigie, 2011; Khan et al., 2009). As a result, these products can enhance nutrient availability, as well as chlorophyll and antioxidants production, enhance water-holding capacity and plant metabolism (Khan et al., 2009). The components in the algal extracts, however, differ depending on the class and species of seaweed (Table 1). Besides that, the effect of these substances on crops depends on the type of plant, its receptor mechanism, and the type of application used, specifically whether foliar, root feeding, or a combination of both (Ali et al., 2016), which is why it is critical to study and capitalize on this marine resource in order to make agricultural crops more efficient, sustainable, cost-effective, and healthier.

Table 1 - Main bioactive compounds of red (Rhodophyta) and brown (Phaeophyceae) seaweed extracts with agricultural potential.

Bioactive Compounds	Red (Rhodophyta)	Brown (Phaeophyceae)	Mechanism of Action	Reference
Polysaccharides	Agar Cellulose Mannan Carrageenan Furcellaran Floridean starch Xylan Rhodymanan	Alginate Cellulose Heteroglucan Fucose Fucoidan Laminaran	-Metal ion chelators -Coenzyme's regulation -Stimulate plant defense responses	(Kraan, 2012; Mercier et al., 2001; Vera et al., 2011)
Plant growth regulators	Cytokinin Auxin Gibberellin Abscisic acid Indole-3-aceticacid Ethylene Brassinosteroid Jasmonate Salicylic Acid Strigolactone Zeatin	Cytokinin Auxin Gibberellin Abscisic acid Indole-3-aceticacid Ethylene Brassinosteroid Jasmonate Salicylic Acid Strigolactone Zeatin	-Promote cell division -Control root and shoot elongation -Initiation of flowering and other metabolic functions -Induces defense and stress responses	(Crouch & van Staden, 1993; Stirk & Staden, 2014)

Table 1 – (Continuation)

Bioactive Compounds	Red (Rhodophyta)	Brown (Phaeophyceae)	Mechanism of Action	Reference
Betaines	Glycine γ-Aminobutyric acid δ-Aminovaleric acid Laminine	Glycine γ-Aminobutyric acid δ-Aminovaleric acid Laminine	-Protection against abiotic stress (i.e.: salinity, drought)	(Blunden et al., 2010; MacKinnon et al., 2010; McNeil et al., 1999).
Carotenoid	β-carotene α-carotene Zeaxanthin Lutein	Fucoxanthin β-carotene Violaxanthin	-Oxidative stress protection -Enzyme activators	(Christaki et al., 2013; Esserti et al., 2017; Othman et al., 2018; Poojary et al., 2016)
Minerals	Macro (C, Cl, Fe, Mg, P, K, Na and S) Micro (B, Cr, Co, Cu, F, Gr, I, Mn, Mo, Ni, Se, Si, S, Tn, W, V, Zn)	Macro (C, Cl, Fe, Mg, P, K, Na and S) Micro (B, Cr, Co, Cu, F, Gr, I, Mn, Mo, Ni, Se, Si, S, Tn, W, V, Zn)	-Plant nutrition and physiology -Enzyme activators	(Circuncisão et al., 2018; Ocean Fresh Seaweeds, 2010; Senn, 1987)
Phenolic compounds	Bromophenols Flavonoids Phenolic terpenoids Mycosporine-like amino-acid	Bromophenols Flavonoids Phenolic terpenoids Phloroglucinol Eckol Dieckol	-Oxidative stress protection -Stimulate plant defense responses	(Cotas et al., 2020; Plouguerné et al., 2006; Wijesekara & Kim, 2015)

As the major constitution of seaweed biomass is composed by polysaccharides, this led to a growing interest on their commercial exploitation, particularly in agar, carrageenan, and alginate. Meanwhile, seaweed polysaccharides structural and molecular diversity is not yet fully elucidated (Ermakova et al., 2015), being different according to the species and their life cycle, abiotic and biotic conditions.

For instance, seaweeds belonging to phylum Rhodophyta, class Florideophyceae, have a triphasic life cycle, exhibiting a gametophyte (female and male, this generation is mostly macroscopic), carposporophyte generation (first sporous generation, which is attached in female gametophyte until the release of the carpospores, which will lead to the development of the tetrasporophyte generation) and tetrasporophyte (which give gametophyte generation, through the production of tetraspores) (García-Jiménez & Robaina, 2015). Red seaweeds are typical carrageenan producers; however, the same species may not synthesis the same type of carrageenan throughout the life cycle, resulting in distinct types of carrageenan on gametophytes and in tetrasporophyte generation (Pereira, 2004). Several studies show that the red seaweeds *Grateloupia turuturu* and *Chondrachantus teedei* var. *lusitanicus* different phases of their life cycle can be differentiated by morphological characteristics, as well as by the type of carrageenan that they synthesize (Cardoso et al., 2019; Pereira, 2004; Soares et al., 2016), caused by the differential gene expression in the different phases of seaweeds life cycle (Bi & Zhou, 2014; Lipinska et al., 2020). Thus, the blade of the tetrasporophyte *G. turuturu* exhibits a smooth texture. While the non-fructified gametophyte presents spherical cystocarps at the blade level, the fructified gametophyte contains prominent cystocarps and cruciate tetrasporangia (Cardoso et al., 2019; Katsanevakis et al., 2014). While, in the species *C. teedei* var. *lusitanicus* the tetrasporophyte exhibits dark tetrasporangial sori on lateral branches, while the female gametophyte exhibits along the thallus, spherical and multiaxial cystocarps (Pereira & Silva, 2021). Hence, it is highlighted the presence of florid starch inside the tetrasporocysts and cystocarps (Pereira, 2004). Henceforth, polysaccharides bioactivity is strongly related with its chemical composition and structure, so there is a need to characterize algal polysaccharides to understand their bioactivity (Mzibra et al., 2018).

Carrageenans have already showed potential as a biostimulant product, encouraging sustainable land plant growth by influencing several physiological and biochemical processes. For instance, λ-carrageenan significantly increased *B. oleracea* var. *italica* microspore-derived embryos when combined with heat stress (Lemonnier et al., 2001). From another viewpoint, k-carrageenan improves chickpea and maize development by stimulating the formation of secondary metabolites (Bi et al., 2011).

Other red seaweeds species, such as *Gracilaria* spp. produces agar as a polysaccharide, with also proven benefic effects on crop plants, such as amaranth (*Amaranthus aritis*) due to their

biostimulant properties that expand plant tolerance to abiotic stresses, such as drought (Mahusook et al., 2021).

While alginate, a brown seaweed polysaccharide, is highlighted for its agricultural potential due to its water retention and cation-exchange capacity (Illera-Vives et al., 2020), by reducing water surface tension, forming a film on the plant's surface, increasing contact area, and making it easier for water-soluble substances to enter the plant cell through the surface cell membrane of the stem and leaf, the plant can absorb the nutrients more effectively, promoting plant growth (Wang et al., 2020). Moreover, several studies give evidence of alginate biostimulant properties, by stimulating defense responses in crop plants (Akimoto et al., 2000; Cai et al., 2012; Chandía & Matsuhiro, 2008).

1.5. Seaweeds as biopesticides: antifungal potential

Crop plants are attacked by a variety of phytopathogens, which can reduce plant development and productivity. *Botryosphaeria* spp., for example, belongs to the order Botryosphaeriales, and fungi in this order are among the most frequent and important canker dieback diseases of trees worldwide, with *Botryosphaeria dothidea* being one of the most prevalent species on a wide range of hosts (Marsberg et al., 2017). For many years, *B. dothidea* was thought to be a wound-infecting pathogen. However, over the last few decades, this fungi have been identified predominantly as endophyte that infect healthy tissue of woody plants and remain latent until stress conditions arise (Maresi et al., 2007; Pérez et al., 2010; Sakalidis et al., 2011).

Many plant communities, including trees in natural ecosystems, managed forests, and agriculture, are expected to be pressured due to climate change (Kirilenko & Sedjo, 2007; Lavalle et al., 2009; Sturrock et al., 2011). As a result, the potential impact of *B. dothidea*, a widespread pathogen that is currently established as an endophyte in different plant communities around the world, may be aggravated (Desprez-Loustau et al., 2006).

Even though plants have primary defense mechanisms such as the salicylate, jasmonate, and ethylene signaling pathways, each pathway is triggered differently depending on the type of the attack (Bektas & Eulgem, 2015; Iriti & Varoni, 2015; Mercier et al., 2001). These mechanisms aim to restrict the phytopathogen growth and can lead to induced systemic resistance or systemic acquired resistance, making the plant less susceptible to pathogen attack (Heil, 2002), which involves the production of elicitors, oxidative bursts, and antimicrobial compounds synthesis (Heil, 2002; van Loon et al., 1998). Disease will occur if the pathogen is faster than the induced response if no elicitors are produced or if suppressors prevent the plant defense reactions (Sticher et al., 1997). In this context, elicitors are molecules/ compounds that trigger or stimulate certain defense mechanism in a plant. As a result of the interaction of an elicitor with a receptor of the cell on which it acts as a metabolic

stimulus, called a "signal" (Aziz et al., 2003; Huffaker et al., 2013). The elicitor can trigger diverse plant defense mechanisms, such as calcium flux, mitogen-activated protein kinase activation, and the production of secondary signals such as reactive oxygen species, nitric oxide, and the phytohormones jasmonic acid, ethylene, and salicylic acid (Huffaker et al., 2013). Thus, elicitors are compounds that cause plant defensive reactions, by binding to specific receptors in the cell membrane and triggering defense responses by activating genes involved in defense mechanisms (Vera et al., 2012). Salicylic acid is a phenolic compound, present in plants, with elicitor action by inducing the activation of genes that encode pathogenesis-related proteins and enzymes related to the production of phytoalexins and lignin (Cole, 1999). The increase in the activity of several enzymes, such as peroxidases, polyphenol oxidases, phenylalanine ammonia lyases, lipoxygenases, β-1,3-glucanases and chitinase, in plant tissues is related to the occurrence of resistance induction (Romero & García, 2009). Considering this, seaweed extracts include a diverse range of bioactive compounds, such as polysaccharides, plant growth hormones, fatty acids, sterols, carotenoids, oxylipins, minerals, peptides, amino acids and proteins, lipids, polyphenolics, and phlorotannin's that evoke and directly stimulate plant growth and defense responses (Salah et al., 2018; Khan et al., 2009). Hence, seaweeds isolated compounds or their extracts are prospective biopesticides that could be used to control pests in agriculture sector (Hamed et al., 2018). The mechanism of action of these natural chemicals, however, is not entirely understood, and their effect on pathogens is diverse (Pandit et al., 2022). Nonetheless, they are favored over synthetic products due to their lower environmental effect, and performance, but also because they activate plant defensive responses as elicitors, inhibiting or limiting pathogens development (Cheung et al., 2014; Pandit et al., 2022).

1.6. Objectives

Hence, this thesis is organized into three sections to provide a comprehensive understanding of seaweed extracts and polysaccharides in relevant crop plants.

The first assay of this thesis aims to assess how different algal polysaccharide extracts affect plant growth (biostimulant potential). For this reason, it was used a crop plant important for global food demand, *Brassica oleracea* L. (kale), which is planted globally as a human food source (Rakow, 2004), producing over 105 million tons of crop vegetables in the genus *Brassica* each year (Cartea et al., 2011; Sanlier & Guler, 2018). As a result, algal polysaccharides isolated from both native and non-native five red seaweeds (*Gracilaria gracilis*, *Asparagospis armata*, *Calliblepharis jubata*, *Chondracanthus teedei* var. *lusitanicus* and *Grateloupia turuturu*) and three brown seaweeds (*Colpomenia peregrina*, *Sargassum*

muticum and *Undaria pinnatifida*) from the Portuguese coast can affect seed germination and kale growth (*Brassica oleracea*).

While the second assay aim to investigate the agricultural potential of seaweed extracts by assessing their biostimulant properties on lettuce (*Lactuca sativa* L.), a leafy herbaceous plant and one of the most popular salad crops, in both fresh and ready-to-eat markets in the world, with 27 million tons of lettuce and chicory produced globally in 2020 (Chen et al., 2019; FAO & CIRAD, 2021). Hence, this chapter is focused on analyzing the biostimulant effect of aqueous extracts of the brown seaweed *Saccharina latissima* itself and in combination with a bacteria-based biofertilizer (*Methylobacterium symbioticum*), as a foliar spray on lettuce (*Lactuca sativa* L.) growth and nutritional profile.

Finally, the third assay discusses the antifungal potential of the previous chapters described aqueous and polysaccharide extracts against the phytopathogen *Botryosphaeria dothidea.*

2. Materials and methods

2.1. Seaweeds' carbohydrate polymers as plant growth promoters

To determine how different algal polysaccharide extracts affects *Brassica oleracea* L. (kale) seed germination and development, the main polysaccharides (agar, carrageenan, and alginate) were extracted from five red seaweeds (*Gracilaria gracilis*, *Asparagospis armata*, *Calliblepharis jubata*, *Chondracanthus teedei* var. *lusitanicus* and *Grateloupia turuturu*) and three brown seaweeds (*Colpomenia peregrina*, *Sargassum muticum* and *Undaria pinnatifida*). Following each polysaccharide characterization and solubilization, they were applied to kale seeds to assess their plant growth promoter activity (Figure 1).
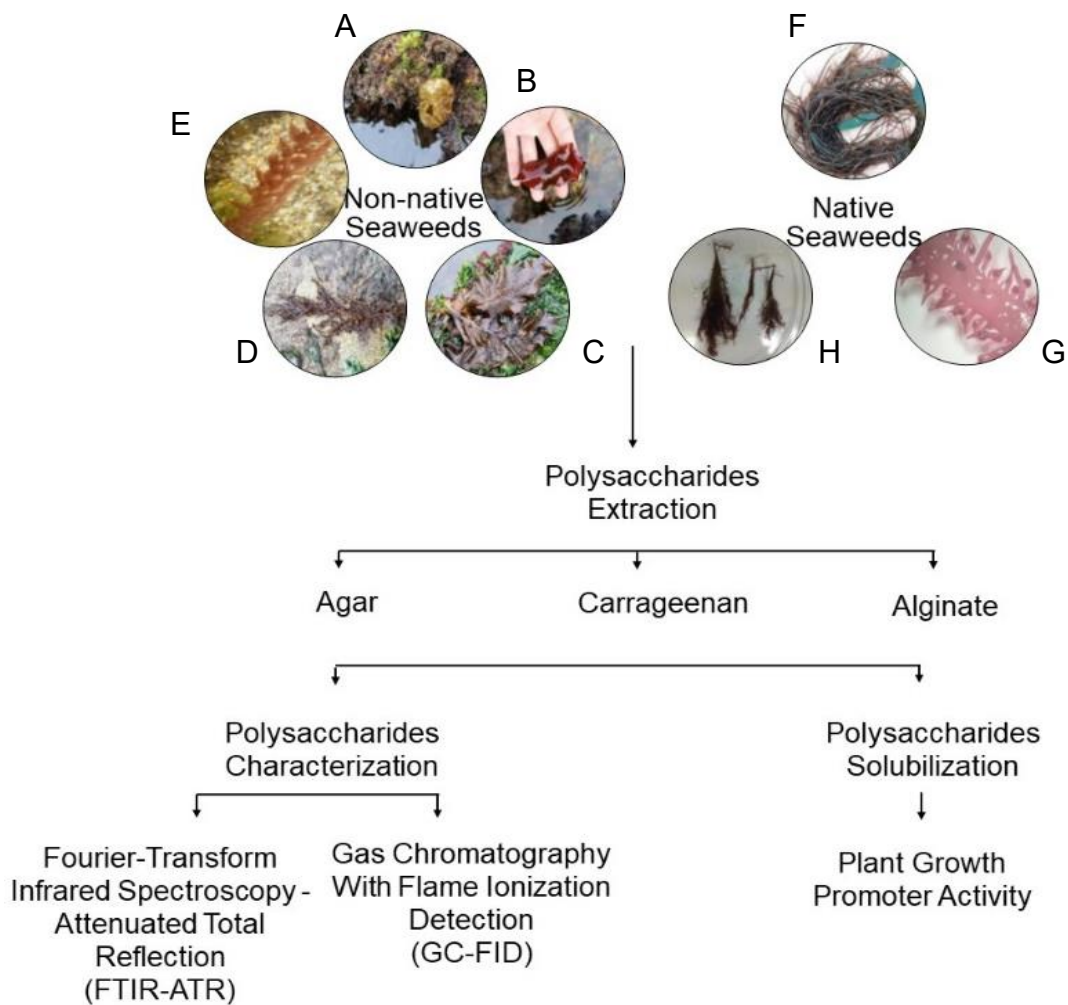
Figure 1 - Schematic representation of the experimental design. A - *Colpomenia peregrina*; B - *Grateloupia turuturu*; C - *Undaria pinnatifida*; D - *Sargassum muticum*; E- *Asparagospis armata*; F - *Gracilaria gracilis*; G - *Chondracanthus teedei* var. *lusitanicus* and H – *Calliblepharis jubata*

2.1.1. Seaweed harvesting and preparation

During January, May, and October 2020, five red seaweeds, namely *Gracilaria gracilis*, *Asparagospis armata*, *Calliblepharis jubata*, *Chondracanthus teedei* var. *lusitanicus* and *Grateloupia turuturu*; and three brown seaweeds *Colpomenia peregrina*, *Sargassum muticum* and *Undaria pinnatifida* were collected in two Portuguese seashores, in Buarcos Bay (Figueira da Foz) and Quebrado Beach (Peniche) (Table 2). Afterwards seaweeds were transported in plastic bags in a coolbox to the laboratory and were frozen at -20 ºC for further utilization, whereas samples that were used for biochemical analysis were stored at -80 ºC.

Table 2 - Seaweed harvesting sites and sampling date.

Seaweed Species	Location	GPS	Harvesting date
Rhodophyta (red seaweed)			
Asparagopsis armata*	Quebrado Beach	39.368258, -9.372303	20/10/2020
Calliblepharis jubata	Buarcos Bay	40.165867, -8.885556	19/10/2020
Chondracanthus teedei var. *lusitanicus*	Buarcos Bay	40.165867, -8.885556	27/05/2020
Gracilaria gracilis	Buarcos Bay	40.165867, -8.885556	19/10/2020
Grateloupia turuturu*	Buarcos Bay	40.165867, -8.885556	13/01/2020
Ochrophyta (brown seaweed)			
Colpomenia peregrina*	Quebrado Beach	39.368258, -9.372303	20/10/2020
Sargassum muticum*	Buarcos Bay	40.165867, -8.885556	19/10/2020
Undaria pinnatifida *	Buarcos Bay	40.165867, -8.885556	13/01/2020

Note: * non-indigenous seaweed species

Afterwards, the seaweeds were washed with filtered seawater to remove sand, epiphytes, and other detritus from the seaweed biomass. Due to carrageenan type variation, the red seaweed *G. turuturu* and *C. teedei* var. *lusitanicus* were separated according to their life cycle generations, using a binocular magnifying glass (Kern & Sohn GmbH, Germany). Then, the

biomass was washed with distilled water to remove the salt content of seawater, placed in plastic trays, and dried in an air-forced oven (Raypa DAF-135, R. Espinar S.L., Barcelona, Spain) during 48 h at 60 ºC. After this procedure, the biological samples were milled (< 1 cm) with a commercial grinder (Taurus aromatic, Oliana, Spain) and stored in sterile flasks in a dark and dry place at room temperature (23 °C).

2.1.2. Polysaccharide extraction

Each polysaccharide (agar, carrageenan, and alginate) was extracted according to the methods mentioned bellow, and the polysaccharide extraction yield was calculated as follows (Wang et al., 2018):

$$Y\,(\%) = \frac{W1}{W2} \times 100$$

Where $W1$ is the weight of the dried polysaccharide and $W2$ is the weight of the sample.

2.1.2.1. Agar

Agar extraction was based in the method described by Li et al. (2008) with modifications. The extraction was done using dried seaweed (20 g) and it was added to 600 ml distilled water. Agar extraction was performed in an electric pressure cooker (Aigostar 300008IAU, Aigostar, Madrid, Spain) at a temperature of 115 °C with an air pressure of 80 Kpa, for 2 h. The solution was hot filtered, under vacuum, through a cloth filter supported in a Buchner funnel. After that the extract was filtered under vacuum with a Gooch funnel (porosity: G2). The filtrated solution was allowed to gel at room temperature (23 °C), frozen overnight and thawed. Then, the thawed gel was dried in an air-forced oven (60 °C, 48 h) (Raypa DAF-135, R. Espinar S.L., Barcelona, Spain).

2.1.2.2. Carrageenan

Carrageenan extraction was performed according to the method described by Pereira & van de Velde (2011). Before extraction, the milled seaweed (1 g) was pre-treated with an acetone (Fisher Chemicals, Portugal): methanol (VWR Prolabo Chemical, Portugal) (1:1) solution in a final concentration of 1 % (m/v) (final volume: 100 ml; 50 mL acetone: 50 mL methanol) for 16 h, at 4 °C, to eliminate the organic-soluble fraction. The liquid solution was decanted, and the seaweed residues obtained were dried in an air-forced oven (Raypa DAF-135, R. Espinar S.L., Barcelona, Spain) at 60 °C before the extraction.

The dried samples were placed in 150 mL of NaOH (Applichem Panreac, USA) (1 M) (1 g of initial seaweed: 150 mL of NaOH solution) in a hot water bath system (GFL 1003, GFL, Burgwedel, Germany), at 85–90 °C, for 3 h. The solutions were hot filtered, under vacuum

(Laborport N820, Lisbon, Portugal) through a cloth filter supported in a Buchner funnel. After that, the extract was again filtered under vacuum with a Gooch funnel (porosity: G2). The extract was evaporated (rotary evaporator model: 2600000, Witeg, Germany) under vacuum to one-third of the initial volume (50 mL). The carrageenan was precipitated by adding twice its volume of 96% ethanol (José Manuel Gomes dos Santos, Portugal) (100 mL). The carrageenan precipitated was washed with ethanol 96%, 48 h at 4 °C before dry in an air force oven (60 °C, 48 h) (Raypa DAF-135, R. Espinar S.L., Barcelona, Spain).

2.1.2.3. Alginate

The alginic acid was extracted using the modified method of Sivagnanavelmurugan et al. (2018). The milled seaweed were added to a solution of HCl (Fisher Chemicals, Portugal) at 1.23 % (1:30 v:v) (3 mL of HCl: 87 mL of distilled water per 3 grams of dried seaweed) was added and kept at room temperature (23 °C) for 48 h. After 48 h, the solution was removed by filtration, under vacuum with a Gooch funnel (porosity: G2). The residue was washed with distilled water for 2 to 3 times. Then, the residue was alkali extracted in a 2 % sodium carbonate (Fisher Chemicals, Portugal) (90 mL for the initial weight of the dried biomass; 1:30 m:v) for 48 h and the extract was filtered through a cloth filter supported in a Gooch funnel (porosity: G2), under vacuum to remove the residues from the alginate solution. Then 37 % HCl (Fisher Chemicals, Portugal) was added to the filtrate for precipitation of alginic acid (1 ml of 37 % of HCl: 30 ml of the final solution). The precipitate was separated by centrifugation (Christ Universal Junior II, Christ, Osterode/ Harz, Germany) (4000 rpm, for 15 min) and then the alginate was dried in an air force oven (60 °C, 48 h) (Raypa DAF-135, R. Espinar S.L., Barcelona, Spain).

2.1.3 Carbohydrate characterization

2.1.3.1. Carbohydrate and uronic acids analysis

Carbohydrate analysis from the dried algal biomass comprised the quantification of polysaccharides (analysed in the form of monosaccharides) and uronic acids. Samples were subjected to a simultaneous initial hydrolysis for both sugars' determinations, and then, to different procedures, depending on the type of sugar.

For uronic acids analysis, hydrolysis was stopped after 1 h and an aliquot of 0.5 mL was retrieved from each sample to a new tube. Samples were then subjected to the procedures described by Selvendran et al. (1979) and Coimbra et al. (1996), consisting of the reduction of the samples to neutral sugars and proceeding to total uronic acids quantification using a Biochrom EZ Read 2000 Microplate reader, reading at an absorbance of 520 nm wavelength. Galacturonic acid (Merck KGaA, Darmstadt, Germany) was used to create the calibration

curve for the measurements and the colorimetric reagent used was 3-phenylphenol (Merck KGaA, Darmstadt, Germany).

Polysaccharide samples were run through a Thermo Scientific Trace 1310 chromatography equipment equipped with a flame ionization detector (GC-FID). A TG-WAXMS A (30 m length, 0.32 mm i.d., 0.25 µm film thickness) gas chromatography column was used, and the oven was programmed to an initial temperature of 180 ºC, following a linear temperature increase of 5 ºC min^{-1} until the final temperature of 230 ºC, maintaining this temperature for 12 min. The carrier gas was Helium at a flow rate of 2.5 mL min^{-1}. The monosaccharides were identified by retention time comparison with standards. Quantification of sugars was performed by comparison of the sugar chromatographic peaks to the peaks obtained for the internal standard used (2-desoxyglucose). So, the standard was 2-desoxyglucose (Merck KGaA, Darmstadt, Germany).

2.1.3.2. Carbohydrate FTIR-ATR analysis

The Fourier-Transform Infrared Spectroscopy - Attenuated Total Reflection (FTIR-ATR) analysis is a method of infrared spectroscopy technique, which is widely used to study and characterize carbohydrates present in seaweeds (among other compounds) and it was based on the protocol described by Pereira (2013).

For FTIR-ATR analysis, the dried polysaccharides samples from the previous polysaccharide extraction stages, were powdered using a commercial mill, and subjected to direct analysis without any further preparation. This technique only needs dried milled (<1 mm) sample (without humidity) to be analysed.

FTIR-ATR spectra were recorded on a Perkin Elmer Spectrum 400 spectrometer (Waltham, MA, USA), with no need for sample preparation, since these assays only required dried samples (Pereira, 2013). All spectra are the average of two independent measurements from 650 to 1500 cm^{-1} with 128 scans, each at a resolution of 2 cm^{-1}.

2.1.4. Seed germination assay

Polysaccharide solution preparation was done by milling the dried polysaccharide and adding distilled water (1 mg/ mL), under constant agitation (Labinco Model L34, Breda, Netherlands) until the complete dissolution of the polysaccharide. Afterwards, the polysaccharide solution was immediately used in the germination assay.

A pH meter (pH meter 3310 Jenway, Staffordshire, UK) and a portable electric conductivity meter (Portable conductivity meter ProfiLine Cond 3310 WTW, Oberbayern, Germany) were used to determine the pH and electric conductivity of the polysaccharide solution.

Kale seeds were disinfected through emersion for 1 minute in a solution of sodium hypochlorite (José Manuel Gomes dos Santos, Portugal) (NaClO) 2% and rinsed for 3 min in a volume of 250 mL of distilled water (Rayorath et al., 2008). Previously, sterilized Petri dishes (15 cm x 2.5 cm) were prepared with cotton and filter paper. Subsequently, 70 mL of each polysaccharide solution, was added. The control was done with addition of distilled water in the same volume. Then, 25 disinfected kale seeds were sown in each Petri dish, sealed with parafilm, and incubated (Heraeus B5090E Incubator, Thermo Scientific, Osterode, Germany) at 25 ± 1 °C in darkness, for 9 days.

The plant growth parameters evaluated were:

- Germination percentage (GP): calculated according to Hernández-Herrera et al. (2014)
  GP = (number of germinated seeds/total number of seeds) × 100
- Aerial part (measured from the cotyledon base to the apical bud) and radicular length, using a ruler.
- Fresh weight of the cotyledon's aerial and radicular parts, using an analytical scale (Kern, Germany).

2.1.5. Statistical analysis

All the polysaccharide extraction and characterization methods were done in triplicated; the seed germination was done with four replicas.

Macronutrient profiles of the algae species studied were statistically analyzed and compared through non-metric multidimensional scaling (nMDS), associated to Analysis of Similarities (ANOSIM) and Similarity Percentage Analysis (SIMPER), as well as Analysis of Variance (ANOVA), to assess differences in the studied components between species.

The seed germination assay statistical analysis was performed with the software Sigma Plot v.14. It was employed an ANOVA analysis to assess statistically differences between the germination percentage. While, Bonferroni multiple comparison t-test was used after the rejection of the ANOVA null hypothesis, to discriminate the differences between radicular and aerial part length and weight. The statistical analysis was performed comparing the different treatments with the control, being considered statistically different when p-value <0.05.

2.2. Biostimulant effect and biochemical response in lettuce plants treated with algal extracts

This assay aims to investigate the agricultural potential of a brown seaweed aqueous extract by assessing their biostimulant properties on lettuce (*Lactuca sativa* var. *crispa* L.).

Hence, this chapter is focused on analyzing the biostimulant effect of aqueous extracts of the *Saccharina latissima* used as a foliar spray on lettuce (*Lactuca sativa* var. *crispa* L.) growth

and nutritional characteristics in combination with a bacteria-based biofertilizer (*Methylobacterium symbioticum*).

2.2.1. Seaweed harvesting and processing

The brown seaweed *Saccharina latissima* (Fig. 2) was harvested in the entrance of Viana do Castelo harbor (Lima River mouth) (41°41'17.7"N 8°50'11.4"W), on the 24 of July 2020. Afterwards seaweeds were transported in plastic bags in an electric cool box (to maintain the biomass collected at the least 5 ºC) to the laboratory and kept frozen at -20 °C for further use.

Figure 2 - Photographic record of *Saccharina latissima* (Phaeophyceae) on Viana do Castelo harbor (Lima River mouth) (41°41'17.7"N 8°50'11.4"W).

Later, the seaweed specimens were washed with filtered seawater, collected from the sampling site, to remove the sand, epiphytes, and other detritus from the seaweed biomass. Then, the biomass was washed rapidly with distilled water to remove the salt content from the seaweed surface. After washing seaweeds were placed in plastic trays, and dried in an air-forced oven (Raypa DAF-135, R. Espinar S.L., Barcelona, Spain) during 48 h at 60 °C. After this procedure, the biological samples were milled (<1 mm diameter) with a commercial grinder (Taurus aromatic, Oliana, Spain) and stored in sterile flasks in a dark and dry place at room temperature.

2.2.2. Extract preparation

The aqueous extracts of *S. latissima* were prepared as described by Sousa et al. (2020) through the weighting (Radwag WLC 1/2, Radom, Poland) of 12 g of milled dried seaweed mass and then wetted with 100 mL of distilled water and added into a blender (Moulinex LM811D11, SEB, Selongey, France) for 3 min (automatic programs: auto clean and smoothie).

Afterwards, the solution was filtered in a Buchner funnel (Linex, Barcelona, Spain), with a nylon net set (mesh dimension: 1 mm), connected to a kitasato flask, under vacuum; followed by another vacuum filtration with a Gooch funnel (porosity: G2). The aqueous extract was previously diluted, with tap water, to a concentration of 1.2% (v/v).

Apart from *S. latissima* extracts, for this study, commercially available products, "Profertil" (Adubos de Portugal, Alverca, Portugal), and "BlueN" (Hubel Verde, Faro, Portugal) were used. They were provided by the companies and the aqueous extracts were prepared accordingly with the technical sheets, taking in account the crop culture stage and the field area. The Profertil extract with a concentration of 1.5% (v/v) was performed with tap water. While the BlueN treatment was prepared a dilution of 0.03% (m/v) with distilled water.

The pH and electrical conductivity of the aqueous extracts were measured with a pH and electrical conductivity meter (Hanna Instruments, Vöhringen, Germany).

2.2.3. Experimental conditions

A commercial curled lettuce variety (Pombal Verde, Leiria, Portugal) was employed in this experiment (*Lactuca sativa* var. *crispa* L.). The current experiment was performed to evaluate the impact of *S. latissima* aqueous extract (1.2 %) as a leaf treatment, to assess its growth biostimulant activity, as well as its promoting activity of the bacteria present in BlueN (*Methylobacterium symbioticum*) as a NPK (nitrogen, phosphorus, and potassium) mineral fertilizer for *Lactuca sativa*. As a positive control was used a commercially leaf fertilizer (Profertil) at a concentration of 1.5% (v/v), while water was used as a negative control. The treatments with the algal extract were applied 21 days after potting with a sprayer (Isolab BTPTI20500001, Eschau, Germany), and the treatments with BlueN were applied one day after (day 22).

Plants were grown in pots (with a diameter of 18 and height of 13 cm), in conditioned substrate (SIRO, Coimbra, Portugal) under greenhouse conditions, and watered every day with equal doses of ± 1.5 L (Figure 3). Abiotic parameters, namely the temperature (maximum and minimum) and relative air humidity, were monitored daily with a thermohygrometer (Meter8 TA298, Shenzhen, China).

Figure 3 - Apparatus of the experimental design.

In Table 3 is briefly represented the treatments used in the experiment. Each treatment was applied in twelve plastic pots organized in a randomized block design; thus, each treatment was represented by a 0.3 m^2 field area. The experiment lasted 80 days, from May to July 2021. Due to the soil and climatic conditions, all plants of each treatment were sprayed with Profertil (1.5% v/v) 38 and 45 days after the lettuce potting and the substrate was fertilized twice, with 1 g of Agriazul (Deiba, Setúbal, Portugal), 55 and 62 days after the lettuce potting.

Table 3 - Description of the treatments employed in the experimental design. Each treatment was applied in twelve plastic pots, in a randomized block design.

ID	Designation	Treatment
CP	Positive control	12 mL of Profertil 1.5 % (v/v)
CN	Negative control	No treatment
E	Algal extract	12 mL of *S. latissima* aqueous extract 1.2% (v/v)
EB	Algal extract + BlueN	12 mL of *S. latissima* aqueous extract (1.2% v/v) + 30 mL of BlueN 0.03% (m/v)
B	BlueN	30 mL of BlueN 0.03% (m/v)

2.2.4. Algal biomass and extract characterization

2.2.4.1. Moisture and Ashes Content

According to the international standard method 930.04 of Official Methods of Analysis of AOAC International (Cunniff, 1997), the moisture content was assessed through the fresh weight of the algal samples and, after oven-drying (Memmert, Büchenbach, Germany) at 60 °C during

48 h. Afterwards, the samples were milled (< 1mm) and, approximately, 2 g of each sample was placed in crucibles and dried at 105 °C for 2 h. Then, the samples were placed in a desiccator until constant weight, being again weighted (Mettler Toledo, Columbus, OH, USA), to calculate the moisture content. In accordance with the AOAC method 930.05 (Cunniff, 1997), the dried samples at 105 °C were placed in an incineration muffle during 2 h at 550 °C (Induzir, Leiria, Portugal) and further cooled in a desiccator and weighted to assess the ashes amount.

The moisture at 65 °C was calculated according to standard method 930.04 of AOAC (Cunniff, 1997):

$$Moisture\ at\ 60°C\ (\%) = \frac{(P2 - P3)}{(P2 - P1)} \times 100$$

Where, P1—weight of the tray (g); P2—weight of the tray + sample (g); P3—weight of the tray + dried sample (g).

The moisture at 65 to 105 °C was calculated according to standard method 930.04 of AOAC (Cunniff, 1997):

$$Moisture\ (60°C - 105°C)\ (\%) = \frac{(P5 - P6)}{(P5 - P4)} \times 100$$

Where, P4—crucible weight (g); P5—crucible weight + sample (g); P6—crucible weight + dried sample (g).

The moisture content was calculated according to standard method 930.04 of AOAC (Cunniff, 1997):

$$Moisture\ (\%) = \frac{\frac{(P5 - P4)\ \times (P2 - P1)}{(P3 - P1) - (P6 - P4)}}{\frac{(P5 - P4) \times (P2 - P1)}{P3 - P1}} \times 100$$

Where, P1—weight of the tray (g); P2—weight of the tray + sample (g); P3 weight of the tray + dried sample (g); P4—crucible weight (g); P5—crucible weight + sample (g); P6—crucible weight + dried sample (g).

The ashes content was calculated according to standard method 930.05 of AOAC (Cunniff, 1997):

$$Ashes\ (\%\ db) = 100 \times \frac{(P5 - P6)}{(P5 - P4)}$$

$$Ashes\ (\%\ fb) = \frac{ashes\ (\%\ db) \times (100 - H)}{100}$$

Where, % db—percentage of dried biomass; % fb—percentage of fresh biomass; P4—crucible weight (g); P5—crucible weight + sample (g); P6—crucible weight + ashes (g); H—moisture (%).

2.2.4.2. Crude Lipids

The total lipids content was gravimetrically quantified following a continuous extraction process with diethyl ether in a Soxhlet apparatus (Behr Labor-Technik GmbH, Düsseldorf, Germany), as it follows the international standard AOAC method 930.09 (Cunniff, 1997). The distillation flasks were previously dried at 105 °C for 2 h, cooled in a desiccator and weighted in an analytical scale (Sartorix, Göttingen, Germany). Afterwards, the distillation flasks were filled (2/3 of their capacity) with diethyl ether (Panreac, Darmstadt, Germany). Then, approximately 2 g of the algal samples were packed in filter paper and placed into the thimble. After 16 h of extraction, all the solvent was collected and evaporated (BÜCHI Labortechnik AG, Flawil, Switzerland). The distillation flasks were then dried at 105 °C for 2 h and weighted (Sartorix, Göttingen, Germany) when cooled down.

Crude lipids were calculated according to the formula presented by the standard method of AOAC 930.09 (Cunniff, 1997):

$$Crude\ lipids\ (\%\ \text{db}) = 100 \times \frac{P3 - P1}{P2}$$

Where, % db—percentage of dried biomass; P1—distillation flask weight (g); P2—sample weight (g); P3—distillation flask weight + lipids (g).

2.2.4.3. Total Nitrogen/ Protein

The total nitrogen/ protein content was determined by Kjeldhal method (AOAC method 978.04) (Cunniff, 1997), whilst it was used multiplication factor of 5 as a protein conversion factor on the formula for total protein determination (Angell et al., 2016). In a Kjeldhal tube, was added approximately 0.5 g (Mettler Toledo, Columbus, OH, USA) of the previously dried algal sample, and then it was added a selenium catalyst (PanReac AppliChem, Darmstadt, Germany) and 12 mL of sulfuric acid (Chem-Lab NV, Zedelgem, Belgium). The tubes were then placed into the Kjeldhal digester (VELP Scientifica, Usmate Velate MB, Italy) at 400 °C for 2 h. The samples were allowed to cool in the fume cupboard, and it was added 50 mL of distilled water in each tube and placed into the Kjeldhal distiller. Concurrently, it was placed 30 mL of boric

acid (Chem-Lab NV, Zedelgem, Belgium) in an Erlenmeyer (one for sample), being further placed into the Kjeldhal distiller as well (VELP Scientifica, Usmate Velate MB, Italy). To the Kjeldhal tube was added 50 mL of distilled water and 50 mL of sodium hydroxide (NaOH) at 40% (m/v) (JMGS - José Manuel Gomes dos Santos, Odivelas, Portugal). The distilled solution was collected and titrated with hydrochloric acid (HCl) 0.1 M (Chem-Lab NV, Zedelgem, Belgium).

Total protein was calculated according to the formula (Cunniff, 1997):

$$Total\ protein\ (\%\ db) = factor \times 100 \times \frac{0{,}01401 \times [HCl] \times (V - V0)}{P1 \times 10}$$

Where, % db—percentage of dried biomass; P1—sample weight (g); [HCL]— hydrochloric acid concentration (M); V—volume of titrant spent in sample titration (mL); V0—volume of titrant spent in control sample titration (mL).

2.2.4.4. Crude Fiber and Total Carbohydrates/ Nitrogen-Free Extractives

According to the standard method 930.10 of AOAC (Cunniff, 1997), the crude fiber was analyzed through the weighting of 2 g (Sartorix, Göttingen, Germany) from the algal samples, previously oven dried (Memmert, Büchenbach Germany) at 105 °C for 2 h and placed in a 600 mL goblet. It was then added 200 mL of sulfuric acid (H_2SO_4) 12.5 g/ L (Chem-Lab NV, Zedelgem, Belgium) and the samples were placed in a fiber analyzer (Labconco Corporation, Kansas City, United States of America) for 30 min. After this procedure, the samples were filtered with a filter crucible G2 (Robu, Hattert, Germany), under vacuum (General Electric, Boston, United States of America). The residue was then placed into the goblet with 250 mL of sodium hydroxide (NaOH) 12.5 g/ L (JMGS - José Manuel Gomes dos Santos, Odivelas Portugal) and set into the fiber analyzer for more 30 min. With the same filter crucible G2, the samples were again vacuum filtered and dried at 130 °C for 2 h. After the samples were cooled down in a desiccator, they were weighted in an analytical scale (Sartorix, Göttingen, Germany) and placed into an incineration muffle at 550 °C (Induzir, Leiria, Portugal) for 2 h. Finally, the samples could cool down and were weighted to calculate the crude fiber. Nitrogen-free extractives are the difference for 100 of the remaining constituents (moisture, lipids, protein, crude fiber, and ash), while the total carbohydrates correspond approximately to the difference between 100 and the sum of the moisture, ash, lipids, and protein.

Total fiber was calculated according to the formula (FAO, 2003):

$$Crude\ fiber\ (\%\ db) = 100 \times \frac{P2 - P3}{P1}$$

Where, % db—percentage of dried biomass; P1—sample weight (g); P2—crucible weight + sample dried at 130 °C (g); P3—crucible weight + sample dried at 550 °C (g).

2.2.4.5. Mineral and Trace Element Characterization

With the ashes obtained, the mineral content was analyzed through dry mineralization and assessed by using flame atomic absorption spectrometry (PerkinElmer PinAAcle 900 T, Waltham, MA, United States of America) (Lucas & Sequeira, 1976). For this analysis, it was performed an acid digestion with nitric acid 65% (m/v) (Chem-Lab NV, Zedelgem, Belgium), in a water bath at 100 °C around 30 min. Finally, the samples were filtrated to a volumetric flask and the volume adjusted with distilled water. After the necessary dilutions (1:10, 1:100 and 1:500) the analysis was carried out on the atomic absorption spectrophotometer equipped with the cathode corresponding to each element. Except phosphorus analysis that was performed by spectrophotometry (PG instruments T80+ UV/VIS spectrophotometer, Leicestershire, United Kingdom) (Ribas et al., 1988).

2.2.4.6. Total Phenolic Compounds Quantification

For this purpose seaweeds were dried at 40 °C for 48 h, then it was done a crude extract, using distilled water (12:100 m:v), in a Moulinex LM811D11 blender (SEB, Selongey, France) After the liquification of seaweeds, the crude extracts were filtered with a Buchner and Gooch funnel (porosity: G2) (Linex, Barcelona, Spain), under vacuum (Sousa et al., 2020).

The total phenolic compounds were quantified using the Folin-Ciocalteu method and gallic acid was used as standard (GAE - Gallic acid equivalent units). For the analysis, 450 µL of crude extract, 50 µL of Folin-Ciocalteu reagent (Panreac, Barcelona, Spain), 1000 µL of aqueous solution of sodium carbonate (75 g/L m:v) (Chem-Lab NV, Zedelgem, Belgium) and 1000 µL of distilled water were added to tubes. The samples were immediately vortexed for 30 seconds and incubated in the dark for 30 min at room temperature. The absorbance of the supernatant was measured at 750 nm using a Hitachi 2000 (Hitachi 2000, Tokyo, Japan). The total phenolic content (TPC) in the crude extracts was determined in duplicate. To quantify the total phenolic content, a standard curve was performed ($y=0.0168x + 0.0159$; $r^2 = 0.9998$) with different concentrations of gallic acid (0, 4, 6, 8, 10, 20, 40, 60 mg GAE/ L).

2.2.4.7. Carbohydrate FTIR-ATR analysis

The physical-chemical characterization of each polysaccharide was performed as described in the methods chapter 2.1.3.2. Carbohydrate FTIR-ATR analysis.

2.2.5. Physiological and biochemical lettuce's characterization

2.2.5.1. Growth parameters, moisture and ashes content

Lettuces (twelve plants per treatment) were harvested, separated their roots and leaves, and root length and aerial part average diameter were measured by using a ruler. In each plant sample, the leaf and root fresh weight (FW) were separately measured, and the dry weights (DW) were obtained after plants were dried in an oven (60 °C, for 3 days), until the constant weight was reached. Then, each sample was cooled, then the dry leaf and root weights were separately measured. Afterwards, the leaves were milled (< 1mm) and, approximately, 2 g of each sample was placed in crucibles and dried at 105 °C for 2 h. Then, the samples were placed in a desiccator until the constant weight was reached, being again weighted, to calculate the plant moisture content. In accordance with the AOAC method 930.05 (Cunniff, 1997), the dried samples at 105 °C were placed in an incineration muffle during 8 h at 550 °C (Induzir, Leiria, Portugal) and further cooled in a desiccator and weighted to assess the ashes amount.

The moisture at 65 °C was calculated according to standard method 930.04 of AOAC (Cunniff, 1997):

$$Moisture\ at\ 60°C\,(\%) = \frac{(P2 - P3)}{(P2 - P1)} \times 100$$

Where, P1—weight of the tray (g); P2—weight of the tray + sample (g); P3—weight of the tray + dried sample (g).

The moisture at 65 °C to 105 °C was calculated according to standard method 930.04 of AOAC (Cunniff, 1997):

$$Moisture\ (60°C - 105°C)\,(\%) = \frac{(P5 - P6)}{(P5 - P4)} \times 100$$

Where, P4—crucible weight (g); P5—crucible weight + sample (g); P6—crucible weight + dried sample (g).

The moisture content was calculated according to standard method 930.04 of AOAC (Cunniff, 1997):

$$Moisture\ (\%) = \frac{\dfrac{(P5 - P4)\ \times (P2 - P1)}{(P3 - P1) - (P6 - P4)}}{\dfrac{(P5 - P4) \times (P2 - P1)}{P3 - P1}} \times 100$$

Where, P1—weight of the tray (g); P2—weight of the tray + sample (g); P3 weight of the tray + dried sample (g); P4—crucible weight (g); P5—crucible weight + sample (g); P6—crucible weight + dried sample (g).

The ashes content was calculated according to standard method 930.05 of AOAC (Cunniff, 1997):

$$Ashes\ (\%\ db) = 100 \times \frac{(P5 - P6)}{(P5 - P4)}$$

Where, % db—percentage of dried biomass; P4—crucible weight (g); P5—crucible weight + sample (g); P6—crucible weight + ashes (g).

2.2.5.2. Total nitrogen/ protein

The total nitrogen/ protein content was determined by Kjeldahl method (AOAC method 978.04) (Cunniff, 1997), whilst it was used multiplication factor of 6.25 as a protein conversion factor on the formula for total protein determination. In a Kjeldahl tube, was added approximately 0.5 g (Mettler Toledo, Columbus, OH, United States of America) of the previously dried algal sample, and then selenium catalyst was added (PanReac AppliChem, Darmstadt, Germany) and 10 mL of sulfuric acid (ChemLab NV, Zedelgem, Belgium). The tubes were then placed into the Kjeldahl digester (VELP Scientifica, Usmate Velate MB, Italy) at 400 °C for 2 h. The samples were allowed to cool in the fume cupboard, and it was added 100 mL of distilled water in each tube and put into the Kjeldahl distiller. Concurrently, it was placed 20 mL of boric acid (ChemLab NV, Belgium) in an Erlenmeyer (one for sample), being further placed into the Kjeldahl distiller as well (VELP Scientifica, Usmate Velate MB, Italy). To the Kjeldahl tube was added 50 ml of distilled water and 50 mL of sodium hydroxide (NaOH) at 40% (m/v) (JMGS - José Manuel Gomes dos Santos, Odivelas, Portugal). The distilled solution was collected and titrated with hydrochloric acid (HCl) 0.1 M (ChemLab NV, Zedelgem, Belgium).
Total protein was calculated according to the formula (Cunniff, 1997):

$$Total\ protein\ (\%\ db) = factor \times 100 \times \frac{0,01401\ \times [HCl]\ \times (V - V0)}{P1 \times 10}$$

Where, % db—percentage of dried biomass; P1—sample weight (g); [HCL]— hydrochloric acid concentration (M); V—volume of titratant spent in sample titration (mL); V0—volume of titrant spent in control sample titration (mL).

2.2.5.3. Mineral and trace element characterization

The lettuces mineral and trace element characterization of each treatment was performed as described in the methods chapter 2.2.4.5. Mineral and trace element characterization.

2.2.6. Substrate characterization

The initial and final substrate used for lettuces potting was dried in an air-forced oven at 38 °C for 3 days, until it reaches a constant weight. Then, the sample was milled in a soil deagglomerator (FRITSCH GmbH Pulverisette 8, Midland, Canada), through a sieve of 2 mm, separating the thin (< 2 mm diameter) and rough (> 2 mm) material (Laboratório Químico Agrícola Rebelo da Silva, 1977; Póvoas & Barral, 1992).

Then, the soil sample (< 2 mm) was extracted with demineralized water at 22 °C in a proportion of 1:5 (m/v) for 2 h in an agitator at 200 rpm (P Select Rotabit, Lisbon, Portugal). Following this procedure, with the help of a magnetic stirrer (P Select, Agimatic-N, Lisbon, Portugal), the pH was measured in a potentiometer (pH meter 3310 Jenway, Staffordshire, United States of America) and the electric conductivity in a conductometer (WTW 3110, Porto, Portugal) (Chapman, 1965).

For the extraction of phosphorus, sodium, potassium, calcium and magnesium of the soil sample, the previously aqueous extract was filtrated with paper filter (Whatman nº4, Portugal) to volumetric flasks. Then, the phosphorus (P_2O_5) was quantified through the colorimetric method of ammonium molybdate in acidic medium and ascorbic acid in a molecular absorption spectrophotometer (PG instruments T80+ UV/ VIS spectrophotometer, Leicestershire, United Kingdom) at a wavelength of 650 nm. Meanwhile, for the sodium, potassium, calcium, and magnesium measurement was added lanthanum chloride (5%) to the previously filtrated samples and the elements were quantified through atomic absorption spectrophotometry.

For the organic carbon (CO) quantification, 0.5 g of the soil sample (< 2 mm) was weighted into a combustion boat. Then, the sample goes through an oxidative-reduction process in the Carbon/Sulfur analyzer (Leco SC 144 DR, Madrid, Spain) in order to be quantified.

The total nitrogen content was determined by Kjeldahl method. In a Kjeldhal tube, was added approximately 0.5 g of the previously dried soil sample (< 2 mm diameter), and then added a selenium catalyst (PanReac AppliChem, Darmstadt, Germany) and 10 mL of sulfuric acid (ChemLab NV, Zedelgem, Belgium). The tubes were then placed into the Kjeldahl digester (VELP Scientifica, Usmate Velate MB, Italy) at 400 °C for 2 h. The samples were cooled in the fume cupboard, and 100 mL of distilled water was added in each tube and put into the Kjeldahl distiller. Afterwards 20 mL of boric acid was placed (ChemLab NV, Zedelgem, Belgium) in an Erlenmeyer (one for sample), placed into the Kjeldahl distiller as well (VELP Scientifica, Usmate Velate MB, Italy). To the Kjeldhal tube was added 50 mL of distilled water and 50 mL

of sodium hydroxide (NaOH) at 40% (m/v) (JMGS - José Manuel Gomes dos Santos, Odivelas, Portugal). The distilled solution was collected and titrated with hydrochloric acid (HCl) 0.1 M (ChemLab NV, Zedelgem, Belgium) (Bremner, 1979; Póvoas & Barral, 1992).

2.2.7. Statistical analysis

The statistical analysis was performed with the software Sigma Plot v.14. Data was checked for normality (Shapiro–Wilk test) and homogeneity (the equal variance test Brown-Forsythe). One-way analysis of variance (ANOVA) was then performed to assess statistically significant differences between each growth and elemental characterization within the lettuce's treatment. The statistical analysis was performed comparing the different treatments, being considered statistically different when p-value < 0.05. The Tukey multiple comparison t-test was used after the rejection of the one-way ANOVA null hypothesis (Holm-Sidak method).

2.3. Algal bioactive compounds as biopesticides

Finally, the third chapter discusses the antifungal potential of the previous chapters described aqueous and polysaccharide extracts against the phytopathogen *Botryosphaeria dothidea* (Fig. 4).
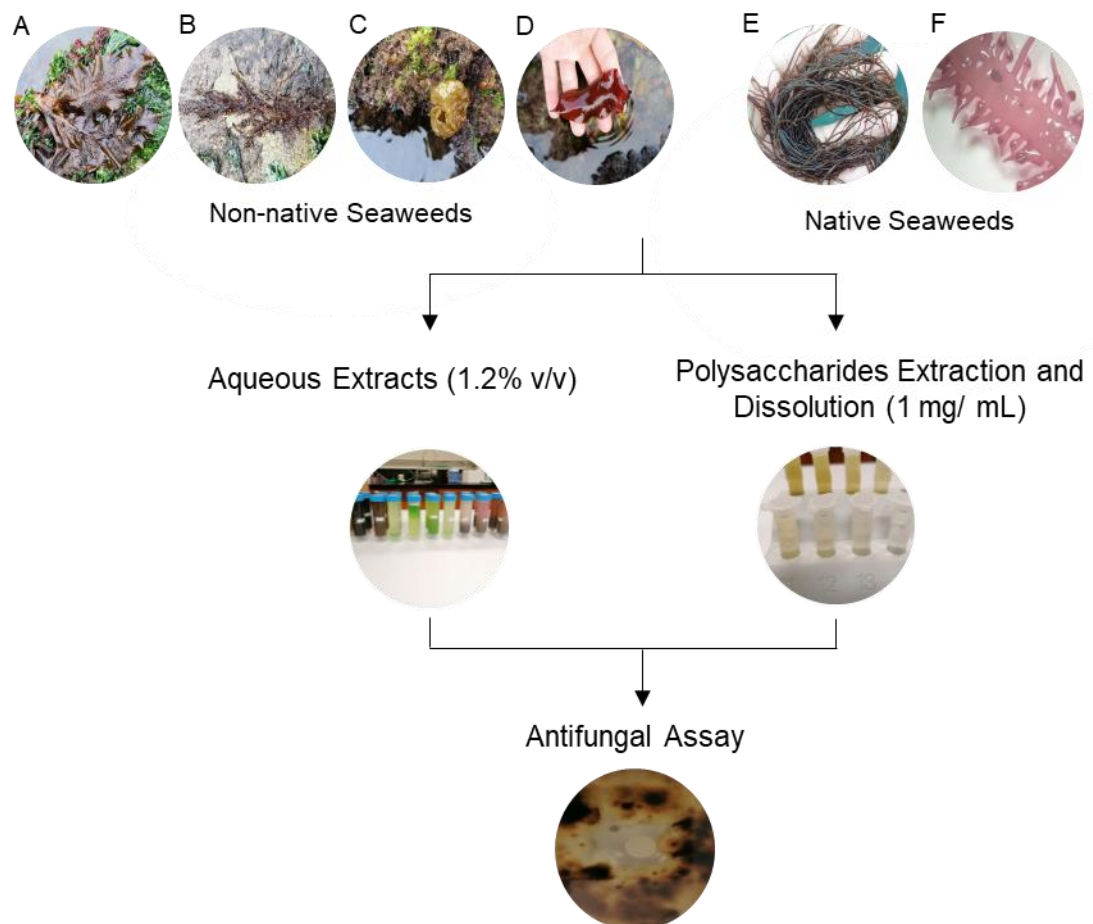
Figure 4 - Schematic representation of the experimental design. A – *Undaria pinnatifida*, B - *Sargassum muticum*, C - *Colpomenia peregrina*, D - *Grateloupia turuturu*, E - *Gracilaria gracilis* and G - *Chondracanthus teedei* var. *lusitanicus*.

2.3.1. Seaweed harvesting and preparation

Seaweeds used in this assay were the same as previously described in the chapter 2.1.1. Seaweed harvesting and preparation, following the same processing preparation.

2.3.2. Polysaccharide extraction, solution preparation and characterization

The carbohydrate studied were extracted according to the methods previously described in the chapter 2.1.2. Polysaccharide extraction. The preparation of the polysaccharide solution was done by milling the dried polysaccharide and adding distilled water (1 mg/ mL), under constant

agitation (Labinco Model L34, Breda, Netherlands) until its complete dissolution. Afterwards, the polysaccharide solution was immediately used in the antifungal assay.

The physical-chemical characterization of each polysaccharide was performed as described in the methods chapter 2.1.3.2. Carbohydrate FTIR-ATR analysis.

2.3.3. Extract preparation and characterization

The aqueous extracts of the red seaweed species *Grateloupia turuturu*, *Gracilaria gracilis*, and *Chondracanthus teedei* var. *lusitanicus*, as well as the brown seaweeds *Colpomenia peregrina*, *Sargassum muticum* and *Undaria pinnatifida*, were performed and characterized according to the methodology reported in the chapter 2.2.2. Extract preparation and characterization.

2.3.4. Fungal strain and culture media

The fungi strain *Botryosphaeria dothidea* was provided by Plant Health Laboratory (FITOLAB) and cultivated in Petri dishes (90 mm) during 7 days at 20 ± 1°C in Potato Dextrose Agar medium (Difco, USA), under complete dark conditions.

2.3.5. Antifungal activity

The fungal growth inhibitory potential of the seaweed aqueous extracts and the extracted polysaccharides were determined by using the agar disc-diffusion method (CLSI, 2012; Karpiński & Adamczak, 2017). As a screening assay, only one replicate was done per treatment. In brief, a spores and mycelia suspensions were prepared by adding 2 mL of a NaCl solution (0.9% v/v), transferred to a sterile tube, and spread 100 µL (8.5×10^3 cells, as counted with a Neubauer chamber) of this suspension on Potato Dextrose Agar medium on a Petri dish (90 mm) under aseptic conditions.

In a final concentration of 1 mg/ mL, a total of 40 µL of each polysaccharide solution was transferred onto sterile filter papers (Whatman, 9 mm). As a negative control, no treatment was employed on the Petri dish, only the fungi, and as a positive control Biotin® (CTS, Europe) was used. Next, the Petri dishes (90 mm) were sealed with Parafilm and incubated at 20 °C (± 1 °C) in the dark. After 7 days, a photographic record of fungal development of each treatment was taken. To evaluate the antifungal potential, it was measured the diameter of the growth halo, with the percentage of inhibition being calculated by comparing the treatments with the positive control.

3. Results and Discussion

3.1. Seaweeds' carbohydrate polymers as plant growth promoters

3.1.1. Carbohydrate characterization

Carbohydrate characterization was essential to analyse chemical and structural differences between the polysaccharides analysed, allowing the comprehension of its overall impact on seedlings development.

3.1.1.1. Polysaccharide yield and solution characterization

In Table 4, it is demonstrated the carbohydrate extraction yields for each sample and the corresponding main polysaccharide present (alginate/ agar/ carrageenan), based on the literature (Imeson, 2009; Pereira, 2013). The extraction procedure was based in the previous literature and from small scale extractions before this work, when it was optimized the methods for each species, mainly the hybrid polysaccharide seaweeds. In this case, it was choosen the polysaccharide extraction technique, according to the polysaccharide in higher concentration. The highest yield of polysaccharide was from the female and male gametophyte of *C. teedei* var. *lusitanicus*, (40.9% and 42.1%, respectively), when compared with all the samples analyzed.

Regarding the pH, it varied between the polysaccharide's solutions, in which alginophytes have an acidic pH, likewise the agarophyte (*G. gracilis*) and the agar/ carrageenan hybrid (*A. armata*). On the alkaline pH, we had the carragenophytes fraction of both, female and male gametophyte of *C. teedei* var. *lusitanicus*. Regarding the conductivity, alginophytes presented a higher conductivity; while the carragenophytes had low conductivity demonstrating variations between the polysaccharides analyzed.

Table 4 - Extraction yield and polysaccharides solution (1 mg/ mL) characterization. The extraction yield results are expressed in mean ± standard deviation (n=3). NA – Not applicable. AA- *Asparagopsis armata*, GG- *Gracilaria gracilis*, GTT- *Grateloupia turuturu* (tetrasporophyte), GT_GNF- *G. turuturu* (non-fructified gametophyte), GT_GF- *G. turuturu* (fructified gametophyte), CJ- *Calliblepharis jubata*, CT_GF- *Chondracanthus teedei* var. *lusitanicus* (female gametophyte), CT_GM- *C. teedei* var. *lusitanicus* (male gametophyte), CT_T- *C. teedei* var. *lusitanicus* (tetrasporophyte), CP- *Colpomenia peregrina*, SM- *Sargassum muticum*, UP- *Undaria pinnatifida*.

Treatments	Polysaccharide described in the literature (Imeson, 2009; Pereira, 2013)	Extraction technique	Extraction yield (%)	pH	EC (µS cm^{-1}; 25°C)
GG	agar	agar	27.0±2.2	3.1	349
AA	agar/ carrageenan	agar	10.6±3.3	2.6	906
GT_GNF	carrageenan/ agar	carrageenan	15.0±3.4	7.5	270
GT_GF	carrageenan/ agar	carrageenan	7.6±0.3	5.8	283
GT_T	carrageenan/ agar	carrageenan	23.0±3.9	6.7	269
CJ	carrageenan	carrageenan	10.4±0.3	4.4	272
CT_GM	carrageenan	carrageenan	42.1±4.5	9.0	244
CT_GF	carrageenan	carrageenan	40.9±1.5	8.7	256
CT_T	carrageenan	carrageenan	28.1±8.1	7.3	210
CP	alginate	alginate	13.0±0.6	3.1	975
SM	alginate	alginate	15.1±0.2	2.9	758
UP	alginate	alginate	8.7±1.3	3.1	667
NA	distilled water	NA	NA	7.0	1.9

3.1.1.2. Polysaccharide and uronic acids profile

Polysaccharide analysis identified six different residues (Table 5), namely ribose, arabinose, xylose, mannose, galactose, and glucose, although the content of galactose and glucose was provided jointly. Galactose and glucose (Gal + Glc) content was, in general, the most abundant residue, apart from *S. muticum* and *U. pinnatifida*, where ribose was the most abundant residue. Significant differences regarding the content of each residue were assessed between the species studied. The polysaccharide profile of *S. muticum* stands out from the remaining species due to the species' ribose and mannose content, particularly higher than those of the remaining species, and because this species does not present arabinose in its profile. *Undaria pinnatifida*, *G. gracilis* and *C. peregrina* also stand out due to their relatively high mannose content, while there is a clear dominance of the residue xylose in the species *C. jubata*, *A. armata* and the three phases of the life cycle of *C. teedei* var. *lusitanicus* and *G. turuturu*.

Uronic acid analysis (Table 5) allowed the observation of significant differences among the groups, with *G. gracilis* standing out from the remaining species, exhibiting a considerable higher content of uronic acids. The female gametophyte of *C. teedei* var. *lusitanicus*, on the other hand, presented the lowest content in uronic acids.

Table 5 - Uronic acids and polysaccharide residues profile and content of each residue of the algae species studied. Rib – ribose, Ara – arabinose, Xyl – xylose, Man – mannose, Gal + Glc – joint content of galactose and glucose. The results are expressed in mean ± standard deviation. Statistically significant differences found in the content of a residue among the studied species are expressed by letters. AA- *Asparagopsis armata*, GG- *Gracilaria gracilis*, GTT- *Grateloupia turuturu* (tetrasporophyte), GTGNF- *G. turuturu* (non-fructified gametophyte), GTGF- *G. turuturu* (fructified gametophyte), CJ- *Calliblepharis jubata*, CTGF- *Chondracanthus teedei* var. *lusitanicus* (female gametophyte), CTTNF- *C. teedei* var. *lusitanicus* (male gametophyte), CTT- *C. teedei* var. *lusitanicus* (tetrasporophyte), CP- *Colpomenia peregrina*, SM- *Sargassum muticum*, UP- *Undaria pinnatifida*.

Seaweed species	Uronic acids (µg.g^{-1} of dried weight seaweed)	Polysaccharide (mg.g^{-1} of dried weight seaweed)					
		Rib	Ara	Xyl	Man	Gal + Glc	N
AA	204.00 ±8.37[a]	0.70±0.14[c]	0.36±0.04	2.69±0.58[a]	0.73±0.08[d]	32.86±1.35	5
CJ	313.97±45.99[c]	0.16±0.09[c]	0.35±0.01	4.80±0.30[b]	0.00±0.00	44.74±4.58	4
CT_GM	295.13±12.94[c]	0.46±0.16[c]	0.22±0.16	4.79±1.47[b]	0.00±0.00	74.99±9.06	4
CT_GF	57.54 ± 11.63[d]	0.72±0.28[c]	0.22±0.16	4.27±1.07[b]	0.00±0.00	74.99±9.06	4
CT_T	295.34±11.58[c]	0.36±0.08[c]	0.13±0.02	5.38±0.20[b]	0.08±0.11[c]	45.10±15.21	5
CP	214.14±13.47[a]	6.26±0.31[d]	0.29±0.02	1.52±0.42[a]	2.34±0.64[b]	9.56±3.21	5
GT_GNF	147.85±50.96[a]	0.55±0.08[c]	0.44±0.05	20.99±0.11[b]	0.29±0.01[c]	24.48±3.27	5
GT_GF	143.24±50.29[a]	0.45±0.03[c]	0.24±0.03	6.01±0.27[b]	0.27±0.01[c]	19.74±3.03	5
GT_T	156.64±50.44[a]	0.49±0.09[c]	0.30±0.05	4.23±2.29[d]	0.23±0.11[c]	24.80±11.72	5
GG	612.02±90.28[b]	0.38±0.06[c]	0.66±0.28	3.13±1.85[d]	3.25±0.72[b]	74.40±18.92	5
SM	147.23±50.04[a]	15.05±1.88[a]	0.00±0.00	1.63±0.16[a]	20.71±4.59[a]	4.37±0.38	4
UP	143.34±89.76[a]	4.04±0.25[b]	0.41±0.03	0.42±0.02[c]	4.02±0.20[b]	3.53±0.26	5

3.1.1.3. Polysaccharide profile

The extracted polysaccharides were analyzed by FTIR-ATR. This spectroscopic technique allowed the polysaccharide characterization in a rapid, nondestructive manner, demanding low amounts of sample (Pereira & Mesquita, 2003). The obtained spectra were reviewed with bibliographic support (Chandía et al., 2004; Pereira, 2013; Pereira et al., 2009; Rupérez et al., 2002). Due to the three main types of polysaccharides (which have different FTIR-ATR spectra), the spectra were divided into different divisions, based in the polysaccharide profile (agar/ hybrids; carrageenan; alginate) (Fig. 5: agar and hybrid agar/carrageenan, Fig. 7: carrageenan and Fig. 9: alginate). Moreover, the idealized structure of the chemical unites of agar and the different main type of carrageenan is presented in the Figure 6. Due to the similarity of FTIR-ATR peaks in the red seaweeds, Table 6 presents the FTIR-ATR bands identification and characterization peaks of red seaweeds, while Table 7 is for brown seaweeds.
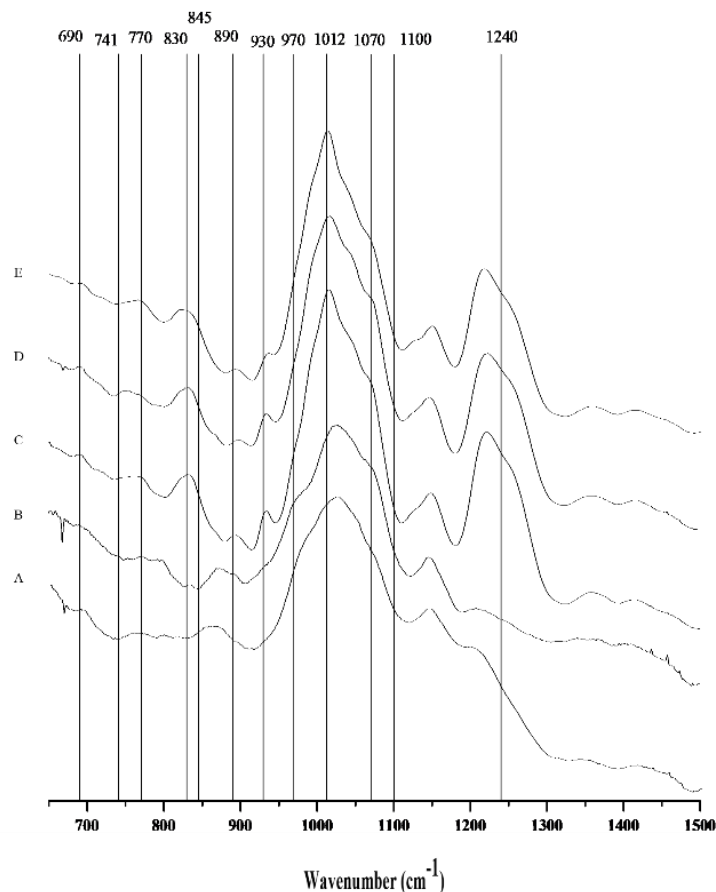
Figure 5 - FTIR-ATR spectra of the agarophyte and hybrids agar/carrageenan: (A) *Asparagopsis armata*, (B) *Gracilaria gracilis*, (C) *Grateloupia turuturu* tetrasporophyte, (D) *G.* t*uruturu* non-fructified gametophyte and (E) *G. turuturu* fructified gametophyte.

Hence, it is emphasized that *A. armata* (Fig. 5 a) and *G. gracilis* (Fig. 5 b) are more similar than *G. turuturu* (tetrasporophyte, non-fructified and fructified gametophyte) samples (Fig. 5 c, d, e), which have higher sulphate esters content (1240 cm^{-1}) and two different peaks (830 and 845 cm^{-1}). However, the region between 690 and 800 cm^{-1} is similar within all the samples.
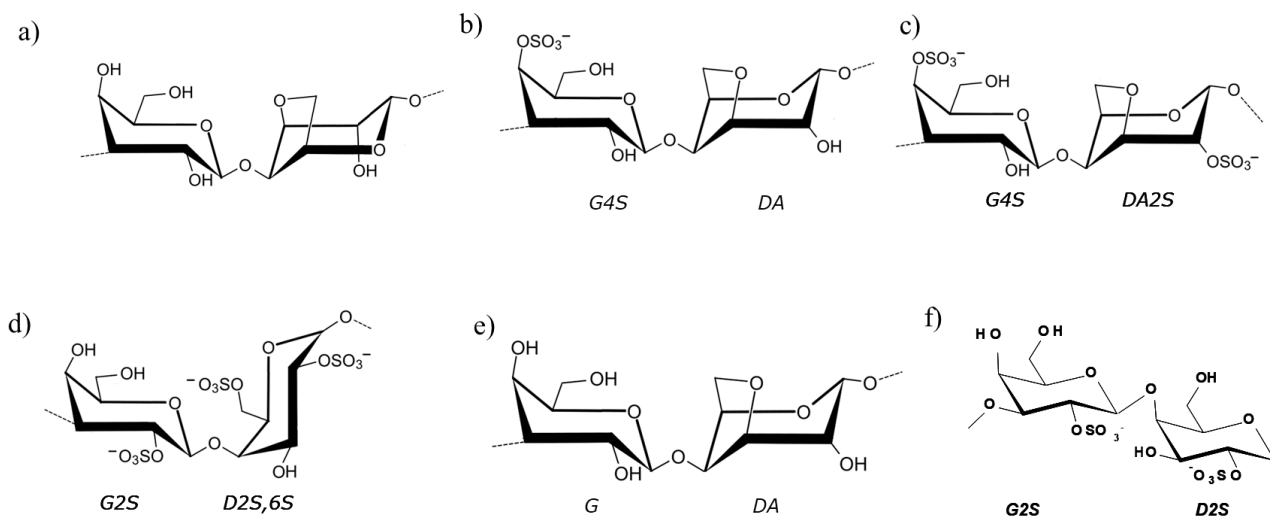
Figure 6 - Idealized structure of the chemical units of a) agar and the different main types of carrageenan b) kappa-carrageenan; c) iota-carrageenan; d) lambda carrageenan, e) beta-carrageenan and f) xi-carrageenan.
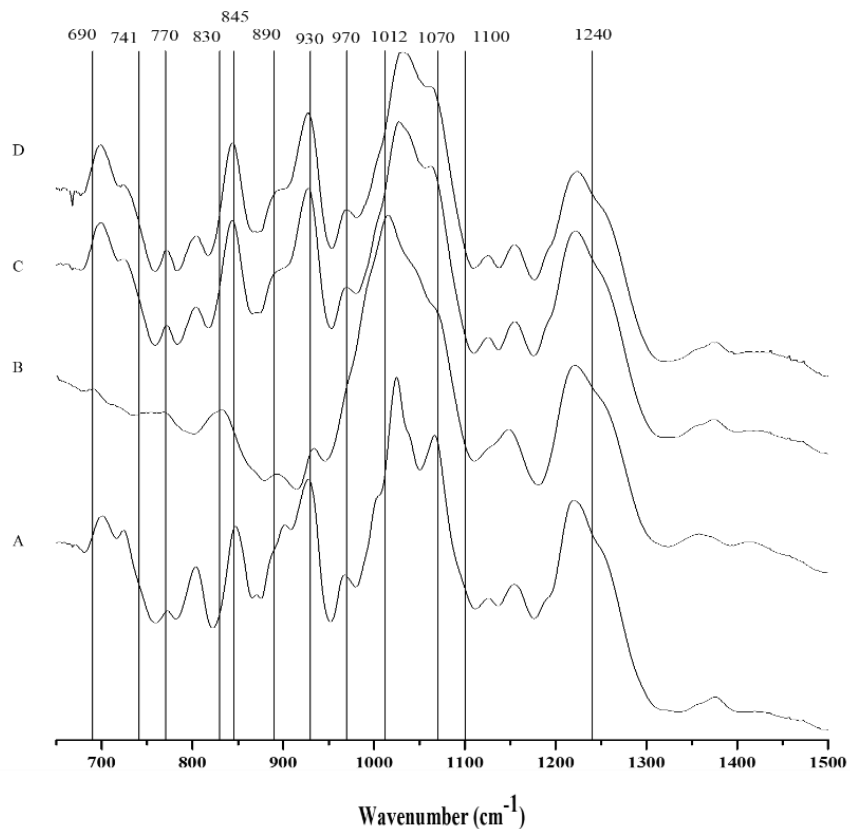
Figure 7 - FTIR-ATR spectra of the carrageenophytes: (A) *Calliblepharis jubata*, (B) *Chondracanthus teedei* var*. lusitanicus* tetrasporophyte, (C) *C. teedei* var*. lusitanicus* male and (D) *C. teedei* var. *lusitanicus* female gametophytes.

In Figure 7 there are two identical spectra, corresponding to both gametophytes of *C. teedei* var. *lusitanicus* (Fig. 7 c, d). However, between them and other carrageenan there is a high dissimilar spectrum, demonstrating different types of carrageenan. The similar peaks are 1012 cm^{-1} region peak, 930 cm^{-1} and 1240 cm^{-1}.

Table 6 - FTIR-ATR bands identification and characterization of the red seaweed polysaccharides (agar and carrageenan), based on literature (Pereira et al., 2009; 2013). AA- *Asparagopsis armata*, GG- *Gracilaria gracilis*, GTT- *Grateloupia turuturu* (tetrasporophyte), GTGNF- *G. turuturu* (non-fructified gametophyte), GTGF- *G. turuturu* (fructified gametophyte), CJ- *Calliblepharis jubata*, CTGF- *Chondracanthus teedei* var. *lusitanicus* (female gametophyte), CTGM- *C. teedei* var. *lusitanicus* (male gametophyte), CTT- *C. teedei* var. *lusitanicus* (tetrasporophyte).

Wave number (cm⁻¹)	Bound	Compound	AA	GG	GTT	GTGNF	GTGF	CJ	CTGF	CTGM	CTT
690	3,6- anhydro-L-galactose (agar)	Agar	+	+	+	+	+	-	-	-	-
741	C-S/C-O-C bending mode in glycosidic linkages of agars	Agar	+	+	+	+	+	-	-	-	-
790	Characteristic of agar-type in second derivative spectra	Agar	+	+	+	+	+	-	-	-	-
805	C–O–SO3 on C2 of 3,6-anhydrogalactose	DA2S	+	+	-	-	-	+	+	+	-
815-820	C–O–SO3 on C6 of galactose	G/D6S	+	-	-	-	-	-	-	-	-
825–830	C–O–SO3 on C2 of galactose	G/D2S	-	-	+	+	-	-	-	-	+
845	D-galactose-4-sulfate	G4S	-	+	+	-	+	+	+	+	-

Table 6 – (Continuation)

Wave number (cm⁻¹)	Bound	Compound	AA	GG	GTT	GTGNF	GTGF	CJ	CTGF	CTGM	CTT
867	C–O–SO3 on C6 of galactose	G/D6S	+	-	-	-	-	-	+	+	-
890–900	Unsulphated b-D-galactose	G/D	-	+	-	+	+	-	+	sh	sh
905	C–O–SO3 on C2 of 3,6-anhydrogalactose	DA2S	+	-	-	-	-	+	sh	sh	sh
930	C–O of 3,6-anhydrogalactose (agar/carrageenan)	(DA)	-	sh	+	+	+	+	+	+	sh
970–975	Galactose	G/D	-	-	-	-	-	+	+	+	-
1012	Sulphated esters	S=O	+	+	+	+	+	+	+	+	+
1070	C–O of 3,6-anhydrogalactose	DA	-	sh	sh	sh	sh	+	+	+	sh
1100	Sulphated esters	S=O	+	+	+	+	+	+	+	+	+
1240-1260	Sulphated esters	S=O	+	+	+	+	+	+	+	+	+

Sh- shoulder (where peak demonstrate intensity, but not enough to be considered a peak due to the surrounding peak intensities)

Gracilaria gracilis FTIR-ATR (Fig. 5 b) demonstrates peaks indicating agar linkages with low sulphate esters as *A. armata* sample (Fig 5 a) (agar peaks: 690, 741 and 790 cm^{-1}), unlike *G. turururu* samples which showed higher sulphate ester content (Fig. 5 c, d, e). Thus, the FTIR-ATR analysis of *A. armata* (Fig. 5 a) and *G. turururu* (in all the life cycle) (Fig. 5 c, d, e) demonstrates a hybrid polysaccharide consisting in agar/ carrageenan or carrageenan/ agar form, respectively. The red seaweed *G. turururu* (Fig. 5 c, d, e) demonstrated that the three phases had the same hybrid polysaccharide. The peaks presented in the spectra support the presence of a hybrid kappa/ iota/ theta-carrageenan with some vestigial presence of agar (agar: 690, 741 and 790 cm^{-1}; kappa: 930 and 845 cm^{-1}; iota: low peak at 805 cm^{-1}; theta: low shoulders at 905, 930, and 1070 cm^{-1}). These results demonstrate that *G. turururu* has a high percentage of kappa-carrageenan, with low content of agar, theta carrageenan and iota-carrageenan. On the other hand, the *C. teedei* var. *lusitanicus* tetrasporophyte (Fig. 7 b) has a hybrid xi/ theta-carrageenan, due to the presence of three shoulder peaks at 905 cm^{-1}, 930 cm^{-1}, and 1070 cm^{-1} (DA) in the FTIR spectrum, which is related to the presence of theta-carrageenan (Pereira et al., 2009; Soares et al., 2016) (similar to *G. turururu* spectra). On the other hand, *C. teedei* var. *lusitanicus* tetrasporophyte (Fig. 7 b) does not have peaks in the agar typical bonds, which demonstrate that this species does not have the presence of agar as the *G. turururu*. In other hand, the *C. teedei* var. *lusitanicus* tetrasporophyte was wide peak in 830 cm^{-1} which is typical of two main peaks near, from the xi-carrageenan. In this case, the wide and standout peak demonstrates that the *C. teedei* var. *lusitanicus* tetrasporophyte (Fig. 7 b) has a xi/ theta-carrageenan (Soares et al., 2016). The male and female *C. teedei* var. *lusitanicus* gametophytes (Fig. 7 c, d) presented similar FTIR-ATR spectra, which corresponds to a hybrid kappa/ iota-carrageenan (presence of the peaks: kappa: 930 and 845 cm^{-1}; iota: 805 cm^{-1}). The FTIR-ATR analysis of *C. jubata* (Fig. 7 a) has predominance of bounds that indicates the presence of iota-carrageenan with a low content in kappa-carrageenan (presence of the peaks: iota: 805 cm^{-1}, and kappa: low intense peaks at 930 and 845 cm^{-1}). Also, the FTIR-ATR analysis demonstrates the inexistence of glucose typical bonds between 1106 and 1150 cm^{-1}, which demonstrate that there is a low hypothesis of glucose presence in the polysaccharides, although as demonstrated by the FTIR-ATR analysis there is a high content in galactose units (Bartošová et al., 2013; Li et al., 2017; Pereira et al., 2009).

The spectra of alginophytes have differences between the seaweed analyzed, with *U. pinnatifida* (Fig. 9 c) being the most different, demonstrating that the alginate structure can be different as observed, particularly, by peaks at 950 and 788 cm^{-1} and by the sulphate esters at 1232 cm^{-1}. Moreover, in the Figure 8 is presented the idealized chemical structure of the a) alginic acid and b) fucoidan.
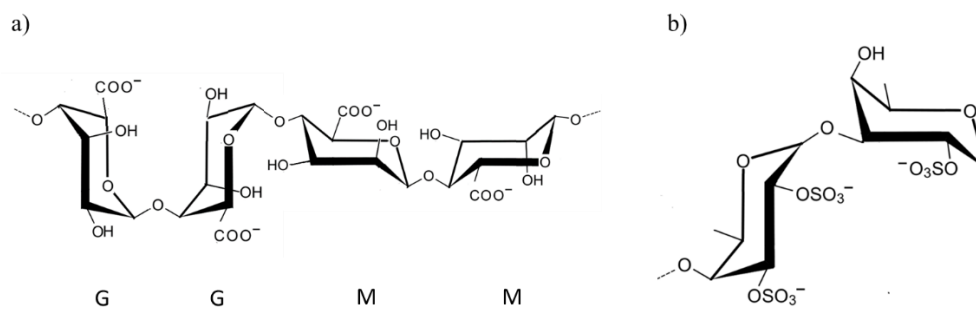
Figure 8 - Idealized structure of the chemical units of a) alginic acid and b) fucoidan.
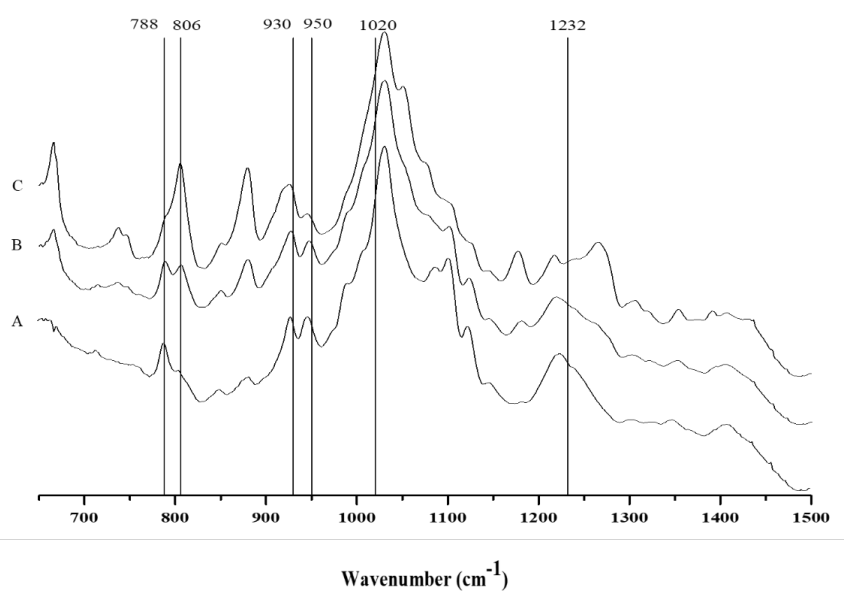
Figure 9 - FTIR-ATR spectra of the alginophytes: (A) *Colpomenia peregrina*, (B) *Sargassum muticum* and (C) *Undaria pinnatifida*.

Table 7 - FTIR-ATR bands identification and characterization of the brown seaweed polysaccharides (alginate), based on literature (Pereira, 2013) CP- *Colpomenia peregrina*, SM- *Sargassum muticum*, UP- *Undaria pinnatifida*.

Wave number (cm^{-1})	Bound	CP	SM	UP
788	Mannuronic acids residues	+	+	+
806	Guluronic acids residues	+	+	+
1020	Alginic acid	+	+	+
1232	Fucoidan	+	+	+
930-950	C-O stretching vibration of uronic acids	+	+	+

The brown seaweeds FTIR-ATR spectra showed typical alginic acid peaks, which indicates more units of mannuronic than guluronic acid (ratio m/g, 788>806). Only *S. muticum* presented an identical concentration of these two uronic acids (Chandía et al., 2004), the *C. peregrina* have more mannuronic acid and *U. pinnatifida* have more guluronic acid (Pereira, 2013).

3.1.2. Seed germination assay

After 9 days of incubation, it was evaluated the growth parameters of the germination assay (Fig.10). It is possible to see a dark colour in one of the four replicates of alginate from *S. muticum*, that could be caused by the hydrolyzation of the seed capsule. Contrarily, the polysaccharide from *A. armata* did not promote seed germination, demonstrating an inhibitory effect.
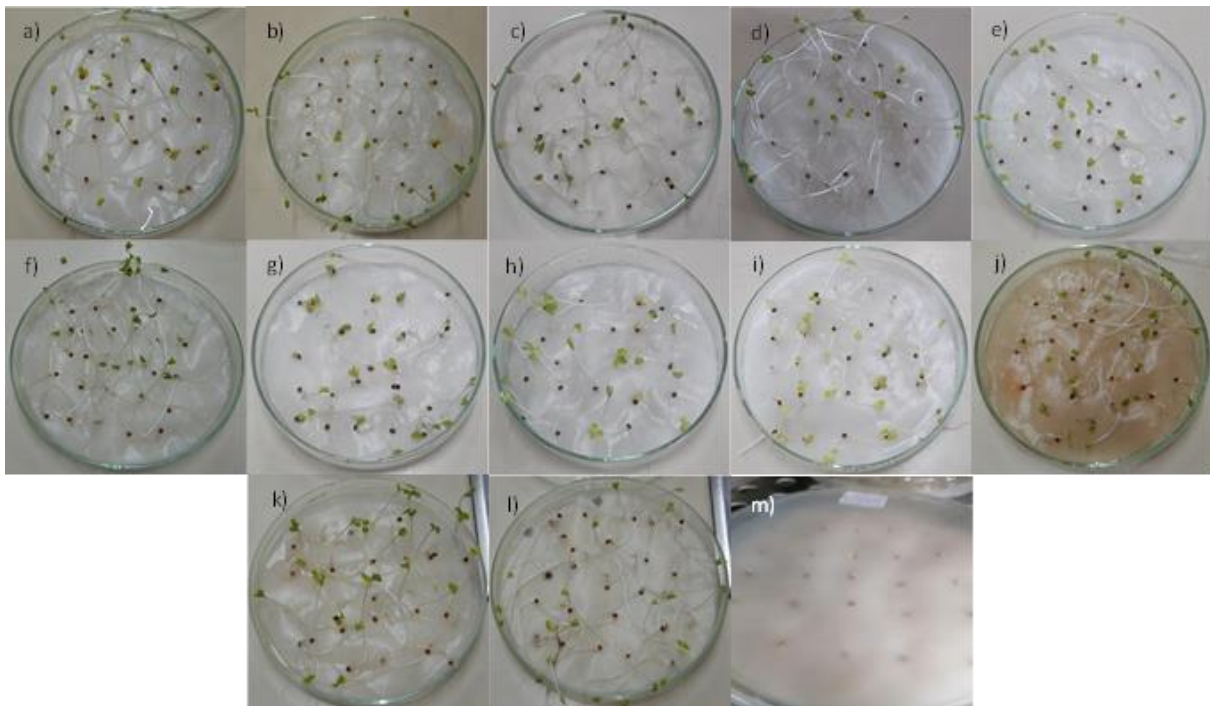
Figure 10 - Photographic record of a) control, b) *Calliblepharis jubata*, c) *Chondracanthus teedei* var. *lusitanicus* (male gametophyte), d) *C. teedei* var. *lusitanicus* (female gametophyte), e) *C. teedei* (tetrasporophyte), f) *Gracilaria gracilis*, g) *Grateloupia turuturu* (non-fructified gametophyte), h) *G. turuturu* (fructified gametophyte), i) *G. turuturu* (tetrasporophyte), j) *Sargassum muticum*, k) *Colpomenia peregrina*, l) *Undaria pinnatifida* and m) *A. armata* after 9 days of incubation

Regarding germination percentage, it was not observed statistically differences between the treatments (Fig. 11 a). Meanwhile, all the polysaccharides' solutions seemed to cause a negative effect on the radicular growth and weight, presenting average values lower than the

control (Fig. 11 b and 11 c). Only, the female gametophyte of *C. teedei* var. *lusitanicus* showed differences from the control, regarding the radicular length and weight.

The positive effect that *C. jubata, G. gracilis* and the female gametophyte of *C. teedei* var. *lusitanicus* revealed on the aerial part length, as they exhibited higher average values when compared with the control (Fig. 11 d). This positive effect is also reflected on the aerial part weight, while the female gametophyte of *C. teedei* var. *lusitanicus* stands out positively from the control (Fig. 11 e).
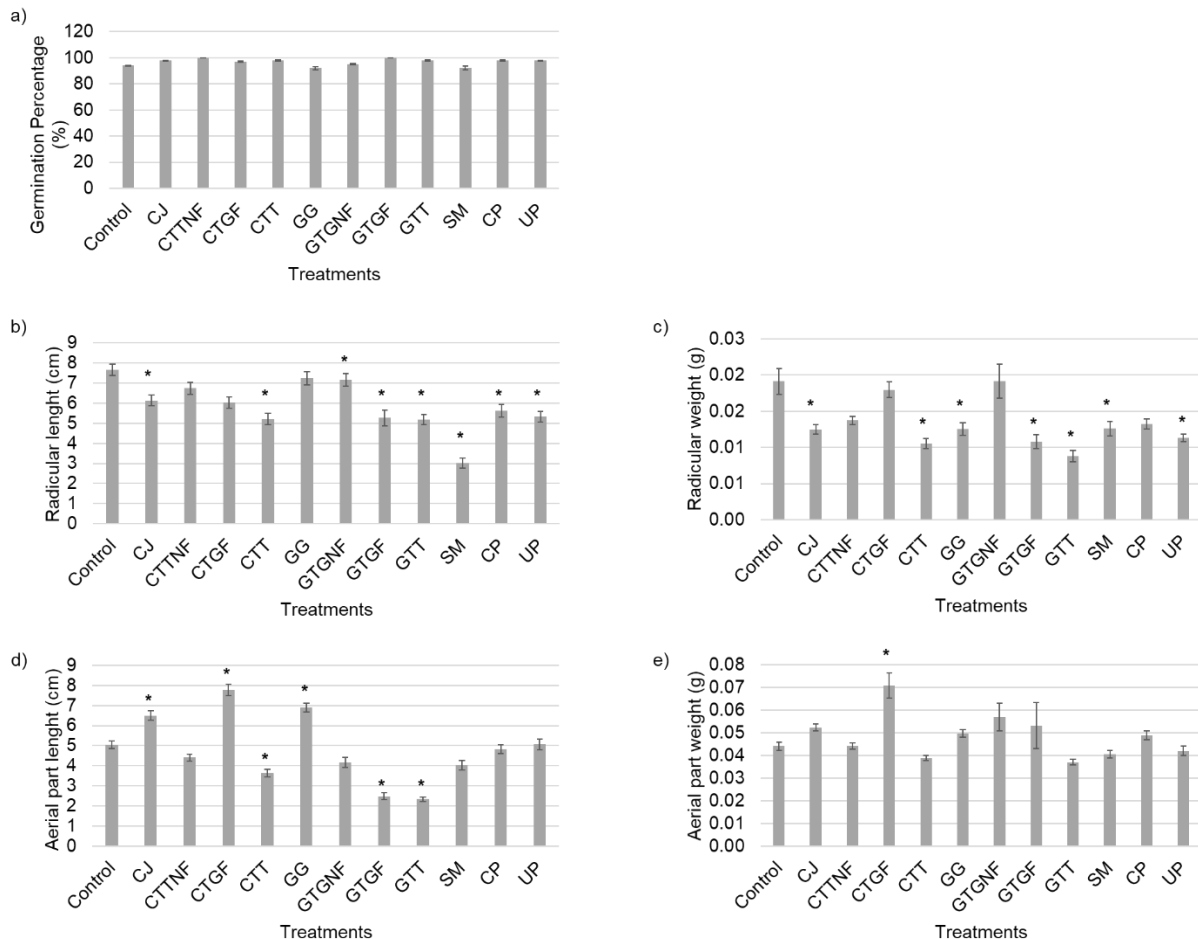
Figure 11 - a) Germination percentage; b) radicular length and c) weight; d) aerial part length and e) weight. * *p*-value < 0.05, comparing with the control. The graphs present average values and standard error (n=4). CJ- *Calliblepharis jubata*, CTTNF- *Chondracanthus teedei* var. *lusitanicus* (male gametophyte), CTGF- *C.* t*eedei* var. *lusitanicus* (female gametophyte), CTT- *C. teedei* (tetrasporophyte), GG- *Gracilaria gracilis*, GTGNF- *Grateloupia turuturu* (non-fructified gametophyte), GTGF- *G. turuturu* (fructified gametophyte), GTT- *G. turuturu* (tetrasporophyte), SM- *Sargassum muticum*, CP- *Colpomenia peregrina*, UP- *Undaria pinnatifida*.

Agar extraction from *G. gracilis* demonstrates a slightly lower percentage than found from other authors, such as Marinho-Soriano & Bourret (2003) or Martín et al. (2013). However, it is necessary to consider the different geographical locations of the sampling sites. For instance, *G. gracilis* from the study conducted by Marinho-Soriano & Bourret, (2003) was performed with a seaweed collected in the Mediterranean Sea, whereas Martín et al. (2013) harvested the algae in the Patagonian coast of Argentina. Regarding the non-native species, *A. armata* collected from Peniche coast, it was reported by Marcia et al. (2014) a yield of extraction of 16%. While, for *G. turuturu*, there is no bibliographic information regarding their polysaccharide extraction yield with the same methodology employed in this study. The carragenophyte, *C. jubata* demonstrated a lower content of carrageenan when compared to other studies of Araujo et al. (2020) and Zinoun & Cosson (1996), which were conducted in Buarcos Bay (Portugal) and in the Normandy coast (France), respectively. However, the results obtained in the carrageenan extraction yield of *C. teedei* var. *lusitanicus* (female and male gametophyte, tetrasporophyte) is in line with the results of Pereira (2004), whereas the sampling site was the same of this study. Regarding the alginate extracted from *S. muticum*, this study reveals a lower yield when compared with the same species harvested in Morocco (El Atouani et al., 2016). Only *C. peregrina* presented a higher yield when compared to the literature (Beacham et al., 2019; Rostami et al., 2017).

This variance in the yield of polysaccharide within the life cycle phase could be explained by the negative correlation between the seaweed dry weight and the carrageenan content, as well as the hygroscopic properties of carrageenan (Pereira, 2013). Moreover, there are several abiotic and biotic factors (such as, light intensity, temperature, salinity, pH, herbivory, wave exposure and weather conditions) that can affect the polysaccharide yield on seaweeds. Previous research showed that it is on spring and in the beginning of summer that seaweeds synthesize more polysaccharides quantity (Cotas et al., 2019; Pereira & Mesquita, 2003; Zinoun & Cosson, 1996).

The FTIR-ATR analysis of *C. jubata* is in concordance with the analysis of Pereira et al. (2009), which observed an iota-carrageenan with low/ residual content of kappa-carrageenan. The FTIR-ATR analysis of *C. teedei* var. *lusitanicus* (female and male gametophyte, tetrasporophyte), were also similar to the results obtained by Pereira (2004) and Soares et al. (2016).

All the brown seaweeds spectra presented alginate peaks, but it was also detected sulphate esters which can be derived from sulphated polysaccharides, such as fucoidan and laminarin present in the wave number 1220 cm^{-1}. At 790 and 800 cm^{-1}, there is a peak demonstrating sulphate groups of the uronic acids (mainly, from guluronic) (Pereira et al., 2013).

Regarding polysaccharide and uronic acids composition, information in literature is scarce or null about the seaweeds used in this study. However, it was found that *G. gracilis* collected in

the South Africa exhibited 7.03% ribose, 6.89% arabinose, 0.06% xylose, 3.33% galactose and 6.16% glucose (Olasehinde et al., 2019). The results in this study are in accordance with the ones reported by the previous cited work, excluding the xylose concentration which hereby presented lower values.

Regarding the polysaccharide solutions applied in the seed germination assay, all of them presented a conductivity lower than 1000 µS cm^{-1}, which is essential to seed germination (Li et al., 2010). The electrical conductivity (EC) is directly related to salinity; thus the increase of the EC values will have a negative impact on plant cell homeostasis, causing a lower water absorbency, compromising metabolic pathways (Kaya et al., 2006; Uçarlı, 2021; Wong & Wong, 1989). The pH also affects seed germination and development (Shoemaker & Carlson, 1990). For instance, neutral pH is optimal for seed germination, conversely acid or basic pH can inhibit seed germination (Laghmouchi et al., 2017). The pH and EC were different within the samples, demonstrating that, even at the same concentration, but from different seaweed sources, the polysaccharides had affected differently the EC and pH. There are several physico-chemical parameters that affect the polysaccharides pH and their rheological properties, such as the polysaccharide concentration and temperature. For instance, low agar concentrations result in a lower pH (Yu et al., 2020). The different types of carrageenan also present different rheological properties and pH sensitivity, being very stable under pH above 6, while between 3.5 and 6 pH values, some of their bioactivities can be affected (CP Kelco, 2002). As alginate contains carboxylate groups in its backbone that are protonated, forming hydrogen bonds, alginate solutions can reach a pH between 3-3.5 (Lee & Mooney, 2012). Uronic acids are an integral component of polysaccharides, such as pectin and alginate, commonly presenting an acidic pH (Mehtiö et al., 2016).

The presence and location of sulphate groups makes seaweeds polymers, such as agar, alginate, and carrageenan biologically active. However, these bioactivities are affected by the sulphation degree, their concentration and oxidation (Zhong et al., 2020). Typically, alginophytes present the lower content of sulphate groups, while carragenophytes present the higher content (Cunha & Grenha, 2016; Ma et al., 2017; Zhong et al., 2020). Nevertheless, carrageenan chemical structure is very heterogenous, and depending on the seaweeds species and the extraction method employed, there are three main types of carrageenan that can be obtained: kappa, iota, and lambda. However, there are other types of carrageenan reported, such as xi, mu or theta (Cunha & Grenha, 2016). These different types of carrageenan mainly differ on the sulphation degree and the position of the sulphate groups on the molecule. In this context, according to the literature, kappa-carrageenan exhibits 25-30% sulfate content, while iota-carrageenan presents 28-30% and lambda-carrageenan contains the highest sulfate concentration (32-39%) (Cunha & Grenha, 2016; dos Santos & Grenha, 2015). Despite the overall lower radicular length when compared with the control, *G. gracilis*

and both male and female gametophyte of *C. teedei* var. *lusitanicus* achieved the highest results. In these treatments, EC (349, 244 and 256 µS cm^{-1}, respectively) were relatively similar, but the pH (3.1, 9.0 and 8.7, respectively) was different, suggesting that pH may influence the radicular growth. Moreover, the main difference between the male and female gametophytes of *C. teedei* var. *lusitanicus* and *G. gracilis* is the uronic acids concentration (295.34, 57.54 and 612.02 µg.g^{-1}), that can interfere in the plant development, mainly in the cell walls rigidity (Lyczakowski et al., 2017). However, in the mentioned species it was found a similar glucose and galactose concentration, which can be an essential key for plant development (Hu et al., 2012). Regarding the radicular weight, the control presented the best result, followed by the non-fructified gametophyte of *G. turuturu* and both female and male gametophytes of *C. teedei* var. *lusitanicus,* not showing statistically significant differences from the control.

In the aerial part, only *C. teedei* var. *lusitanicus* (female gametophyte) seemed to be the best treatment, achieving the highest seedling growth and weight. The best treatments (kappa/ iota-carrageenan extracted from the female gametophyte of *C. teedei* var. *lusitanicus* (the male gametophyte do not have significant differences from the control) and agar extracted from *G. gracilis*, showed different uronic acids concentration (57.54 and 612.02 µg.g^{-1}, respectively), as well as the pH (8.7 and 3.1) and electrical conductivity (256 and 349 µS cm^{-1}) of the polysaccharides solutions. The uronic acids show that can acidify and also increase the EC values (Meywes, 2020). Also, the polysaccharide chemical structure profile was different, *G. gracilis* has more anhydrogalactose, and the female gametophyte of *C. teedei* var. *lusitanicus* has galactose and sulphated galactoses, even in monosaccharide content is different, only exhibiting a similar ribose content (0.46 and 0.38 mg.g^{-1}, respectively).

Kappa/ iota-carrageenan has a higher potential to promote the radicular and aerial kale growth, when compared with the other polysaccharides assayed. This capacity is possible due to the biochemical profile, pH, and electrical conductivity, being the structure the most probable cause of the carrageenan efficiency and the other polysaccharides lack of efficiency in the kale seed germination. According to the literature, the sulphate content that carrageenan contains, particularly kappa and iota, in comparison with the other polysaccharides, can also have a positive effect on *B. oleracea* development, due to the sulphur requirements of this plant (Ishida et al., 2014; Koralewska et al., 2007).

In the alginate solution it was observed an acidic pH and higher EC values, which directly affects the plant cell homeostasis and water retention by the plant (Laghmouchi et al., 2017; Uçarlı, 2021). This also can be an explanation to why *A. armata* polysaccharide solution does not present seed germination. Only the agarophyte *G. gracilis* and the carragenophytes appear to have similar effects, where the electrical conductivity is identical, demonstrating that the cell homeostasis can be the key to seed development.

The darkish colour, observed in the four replicates from *S. muticum*, can be due to the acidic pH of the solution, that can cause the seed coating phytomelanin hydrolysation and extraction (Glagoleva et al., 2020; Keles & Özdemir, 2018).

However, the polysaccharide uronic acid and monosaccharide content does not appear to have direct impact on seed germination, due to the inexistence of a linear correlation between this compound profile and the seed germination in this assay. In literature, the polysaccharide constitution and uronic acids relationship are not well explored.

Concurrently, the polysaccharide chemical and structural characterization can be a key to promote or inhibit the plant development. Hence, the data about this type of assay demonstrates that carrageenan's can enhance plant development by regulating various plants metabolic pathways, such as photosynthesis and ancillary pathways, cell division, purine, and pyrimidine synthetic pathways as well as metabolic pathways involved in nitrogen and sulphur assimilation (Shukla et al., 2016). This can be, in part, an explanation of the results obtained from the female gametophyte of *C. teedei* var. *lusitanicus*, associated with low uronic acid and high galactose and glucose concentration. However, the male gametophyte of *C. teedei* var. *lusitanicus* presents a higher concentration of uronic acids, which is the only difference between their genotype compositions.

Carrageenan can induce plant cell to produce indoleacetic acid (IAA) (Saucedo et al., 2015), which is an important hormone, vital to regulate the plant development, this compound affects cellular elongation, differentiation, cellular division, apoptosis, and morphogenesis, however, can inhibit the root length growth (Donati et al., 2013; Shukla et al., 2016). Moreover, the glucose content also can enhance the production of IAA and consequently the plant development (Mishra et al., 2009). When *Eucalyptus* trees were treated with kappa-oligocarrageenan displayed a concomitant increase in IAA and gibberellic acid (GA3) levels, which is aligned with previous observations showing a reciprocal and positive interaction among auxin and gibberellin in other plants (Abad et al., 2011; González et al., 2014).

3.2. Biostimulant effect and biochemical response in lettuce plants treated with algal extracts

3.2.1. Algal biomass and extract characterization

The chemical characterization of the brown seaweed *Saccharina latissima* (dry algal biomass) used for the aqueous extracts' preparation was analyzed (Table 8), and showed to be a rich source of carbohydrates, representing 60.64 g 100 g^{-1} of its dried biomass. Moreover, a significant part of its biomass (17.81 g 100g^{-1}) is composed by minerals, being the most representative the nitrogen (2.31 g 100g^{-1}) and sodium (1.2572 g 100g^{-1}). For another perspective, manganese (0.00045 g 100g^{-1}) and copper (0.00053 g 100g^{-1}) were the less abundant minerals.

Table 8 - Chemical characterization of the dried biomass of the seaweed *S. latissima*. Results are expressed in mean ± standard error (n = 2; dry weight (DW) basis). NA – Not available.

g 100 g^{-1} of dry seaweed	Concentration	Literature values	Reference
Ash	17.81 ± 0.049	24.3–27.3	(Bikker et al., 2020; Neto et al., 2018; Tibbetts et al., 2016).
Fat	1.52 ± 0.106	0.10–5.5	
Fiber	6.39 ± 0.127	6.2–7.1	
Protein	13.63 ± 0.021	7.4–11.7	
Total Carbohydrates	60.64 ± 0.092	60.3–66.8	(Neto et al., 2018; Tibbetts et al., 2016)
Energy (Kcal 100 g^{-1})	311 ± 1.223	NA	
Nitrogen	2.31 ± 0.03877	1.63	
Phosphorus	0.11048 ± 0.00026	NA	
Calcium	0.44427 ± 0.01368	0.919	
Magnesium	0.28286 ± 0.00014	0.6111	
Potassium	0.25134 ± 0.01345	3.8694	
Sodium	1.2572 ± 0.02229	3.0483	
Iron	0.06265 ± 0.13051	0.1854	
Copper	0.00053 ± 0.42157	0.00386	
Zinc	0.00285 ± 0.12177	0.00386	
Manganese	0.00045 ± 0.90370	0.00056	
Total Phenolic Content (g GAE 100 g^{-1})	$4.91 \times 10^{-3} \pm 1.08 \times 10^{-4}$	1.11×10^{-4}	(Tibbetts et al., 2016).

Still, this biochemical characterization might differ considerably based on biotic and abiotic conditions. The nutritional, macro, and micro elemental analyses of algal biomass, for example, can vary depending on the harvesting season and geographic region (Bikker et al., 2020; Neto et al., 2018; Tibbetts et al., 2016). For instance, *S. latissima* cultivated by a seaweed aquaculture in Northern France (Brittany) and harvested in April 2015 shown increased concentrations of sodium (3.0483 g 100 g^{-1}), potassium (3.8694 g 100 g^{-1}), calcium (0.9194 g 100 g^{-1}), magnesium (0.6111 g 100 g^{-1}), iron (0.1854 g 100 g^{-1}), manganese (0.00056 g 100 g^{-1}) and zinc (0.00386 g 100 g^{-1}), as well as total carbohydrates (68.9 g 100 g^{-1}) and fiber (40.9 g 100 g^{-1}) (Neto et al., 2018). In contrast, lower concentrations of copper (0.00386 g 100 g^{-1}), total protein (0.0102 g 100 g^{-1}) and fat (0.005 g 100 g^{-1}) were registered (Neto et al., 2018). Meanwhile, *S. latissima* wild-harvested in the intertidal region of Fink Cove, (Nova Scotia, Canada) in April 2010, exhibited a higher mineral content, representing 24.5 g 100 g^{-1} of this seaweed dry biomass, but a lower amount of total protein (8.1 g 100 g^{-1}), fat (5.5 g 100 g^{-1}), carbohydrate (59.8 g 100 g^{-1}) and phenolic compounds (1.11 x 10^{-4} g GAE 100 g^{-1}) (Tibbetts et al., 2016). This heterogeneity in algal chemical characterization is caused by the fact that algae metabolic activity changes according to temperature, pH, and nutrition availability in different regions (Samanta et al., 2020).

This brown seaweed's commercial potential stands out because of its alginic acid content, which can reach up to 20% of the algal dry weight and is currently being explored by a variety of industries, including agriculture (Bixler & Porse, 2011; McHugh, 2003; Sterner & Edlund, 2016). In fact, alginic acid is recognized by the International Federation of Organic Agriculture Movements (IFOAM) as an approved additive (USDA, 2015). For these reasons, this polysaccharide of interest was examined using FTIR-ATR (Fig. 12), revealing spectra identical to those of commercial standards, with similar positions of the distinct bands (Belattmania et al., 2020).
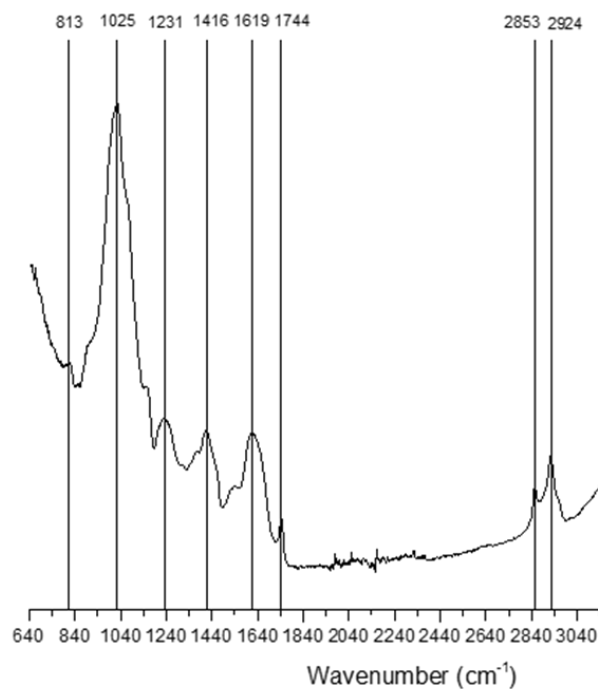
Figure 12 - FTIR-ATR spectrum of *S. latissima*

With this analysis it is possible to identify chemical bounds and functional groups within the analyzed seaweed sample (Table 9). Thus, alginic acid is the main polysaccharide found in this sample, highlighted by the peaks 1020, 1071, 1416 and 1619 cm^{-1} (Cotas et al., 2019; Pereira et al., 2013; Rashedy et al., 2021). Still, other functional groups, such as sulfate groups of the uronic acids (813 cm^{-1}) and C–O stretching vibration of uronic acids (940 cm^{-1}), whereas the presence of sulfate ester groups, which is a distinctive component of fucoidan and sulfated polysaccharides other than alginate in brown seaweeds, is assigned to the broad band on 1231 cm^{-1} (Cotas et al., 2019; Pereira et al., 2013; Rashedy et al., 2021). However, the band between the range 1744 and 2941 cm^{-1} may be indicative of the presence of the pigment fucoxanthin (Cotas et al., 2019).

Table 9 - FTIR-ATR bands identification and characterization of the brown seaweed *S. latissima* polysaccharides (alginate), based on literature (Pereira, 2013).

Wavenumber (cm^{-1})	Chemical bound	Compound
813	Sulfate groups	Sulfate groups of the uronic acids
940	C–O stretching vibration of uronic acids	Uronic acids
1025	Mannuronic units	Alginic acid
1071	Guluronic units	Alginic acid
1231	Sulfate groups	Sulfated polysaccharides
1416	Symmetric stretching vibration of carboxylate group	Alginic acid
1619	Asymmetric stretching vibration of carboxylate O–C–O	Alginic acid
1744	Ketones	Fucoxanthin
2853	O-CH$_3$	Fucoxanthin
2924	Alkanes	Fucoxanthin

Based on these findings and in the literature reviewed, this brown seaweed appears to be a promissory source of alginic acid, which plays an important role in plant nutrition by reducing the surface tension of the water, forming a film on the plant's surface, increasing the contact area, and making it easier for water-soluble substances to enter the plant cell through the leaves and stems, allowing the plant to absorb the nutrients in the seaweed extract most effectively (Guo et al., 2020; Oliveira et al., 2018; Wang et al., 2020).

The nutrition solution used as a foliar spray can have a significant impact on plant growth and development (Ferrón-Carrillo et al., 2021; Hooks et al., 2021; Rusu et al., 2021), and for this reason, extracts physical-chemical characterization is crucial to attain good results.

The physical-chemical assessment of the extracts (Table 10) used on the lettuce plants was carried out, since characteristics such as pH and electric conductivity can inhibit plant development and growth (Kaya et al., 2006; Laghmouchi et al., 2017; Shoemaker & Carlson, 1990; Uçarlı, 2021). Overall, the extracts had similar pH values ranging from 6.70 to 6.93. Despite this, the algal extract (331 µS/cm) had a higher electrical conductivity value than BlueN and the positive control (103 and 117 µS/cm, respectively). The algal extract had a greater concentration of total dissolved solids (165 ppm), but BlueN and the positive control had comparable findings (54 and 59 ppm, accordingly). The pH, electrical conductivity, and total dissolved solids of tap and distilled water, both had a neutral pH, were also measured for

quality control (7.45 and 7.00, respectively). Nevertheless, as it was expected, tap water (106 µS/cm; 54 ppm) presented a higher electrical conductivity and total dissolved solids than distilled water (1.90 µS/cm; 1 ppm).

Table 10 - Extract physical-chemical characterization.

Extract	pH	Electrical conductivity (µS/cm)	Total dissolved solids (ppm)
Profertil (positive control)	6.91	117	59
BlueN	6.70	103	54
Algal extract	6.93	331	165
Tap water	7.45	106	54
Distilled water	7.00	1.90	1

Thus, the electrical conductivity of the tested extracts revealed to be suitable for lettuce growth, because previous research has found that using nutrient solutions with an electric conductivity higher than 1400 µS/cm to lettuce cultivation may cause nutrient imbalance, resulting in decreased leaf number, area, and weight (Huett, 1994; Samarakoon, 2006). Furthermore, high electrical conductivity values in the nutrient solution can lead to excessive vegetative growth, early bolting, and chlorotic and necrotic spots on lettuce's lower leaves (Currey, 2018). Another critical factor on plant development is pH, hence, for lettuce development, nutrient solutions with values between 5.8 and 6 are recommended (El-Nakhel et al., 2020; Ferrón-Carrillo et al., 2021; Hooks et al., 2021; Paradiso et al., 2018; Renna et al., 2018). For instance, researchers found that at pH 5 the weight of shoots and roots was optimal, but with higher pH these values were lower (Roosta, 2011). Moreover, total dissolved solids should also be monitored because high levels can impair plant growth (Sefer & Guuml, 2011).

3.2.2. Abiotic parameters variation

Abiotic parameter variation, such as maximum and minimum temperature and relative air humidity, were also recorded (Fig. 13), revealing a monthly rising trend in maximum and minimum temperatures since the experiment was conducted in late spring. Even though the relative air humidity varies on a daily basis, a similar monthly mean average value was recorded.
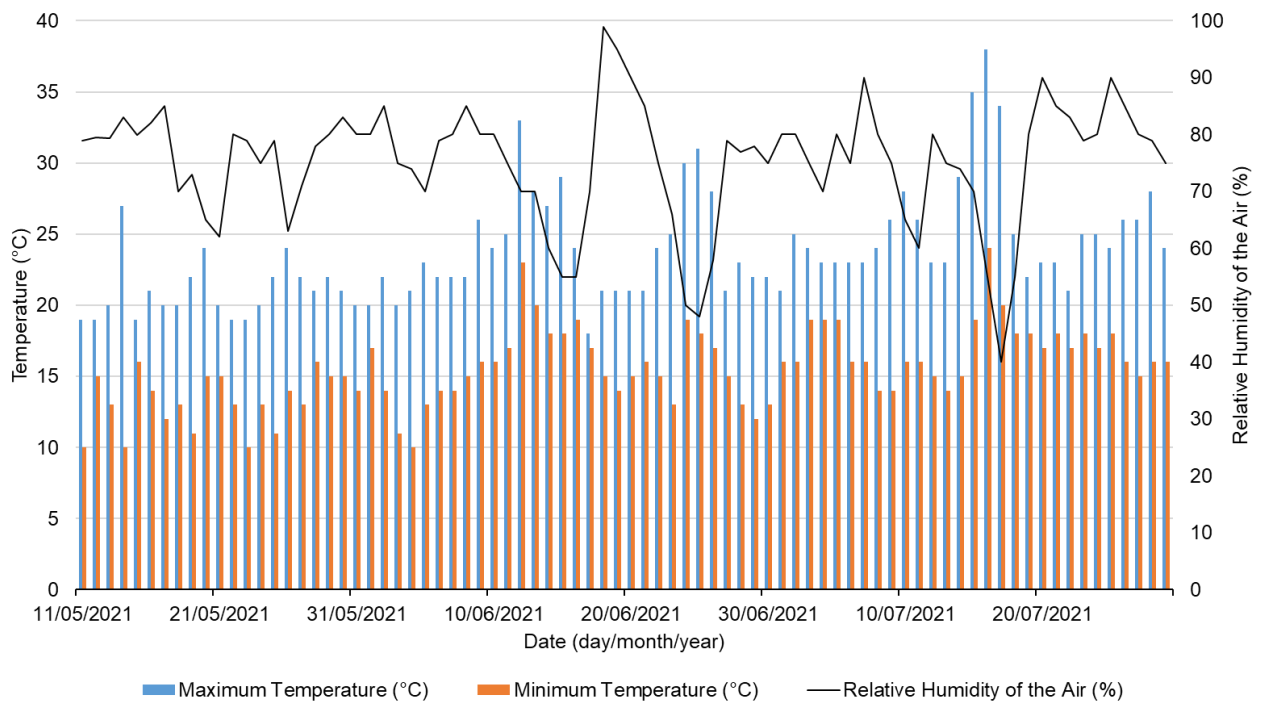
Figure 13 - Register of minimum, maximum temperatures (°C) and relative air humidity (%) recorded in the greenhouse during the bioassay.

Temperatures between 18 to 23° C are ideal for lettuce production, but air temperatures above 23 °C can inhibit plant growth (Currey, 2018). Furthermore, high humidity promotes diseases such as, powdery mildew and *Botrytis*, negatively affecting lettuce cultivation, hence greenhouses should be ventilated to reduce humidity (Currey, 2018).

3.2.3. Physiological and biochemical lettuce's characterization

Both, physical-chemical properties of extracts and the abiotic parameters variation had an impact on lettuce development and nutritional characterization, resulting in distinct growth patterns among treatments. Hence, through the photographic record (Fig. 14), physiological (Fig. 15 and Table 11) and biochemical (Table 12) parameters evaluation in lettuce, it was possible to observe differences among the treatments.

It is clear that the most developed roots were achieved within the positive control (CP) (Fig. 14a), while the less developed roots were registered with the negative control (Fig. 14b) and with Algal extract + BlueN (Fig. 14e). Regarding the leaves (or aerial part) development, it was noted an increased growth in the positive control (Fig. 14a), while the least developed were observed with the BlueN treatment (Fig. 14d).
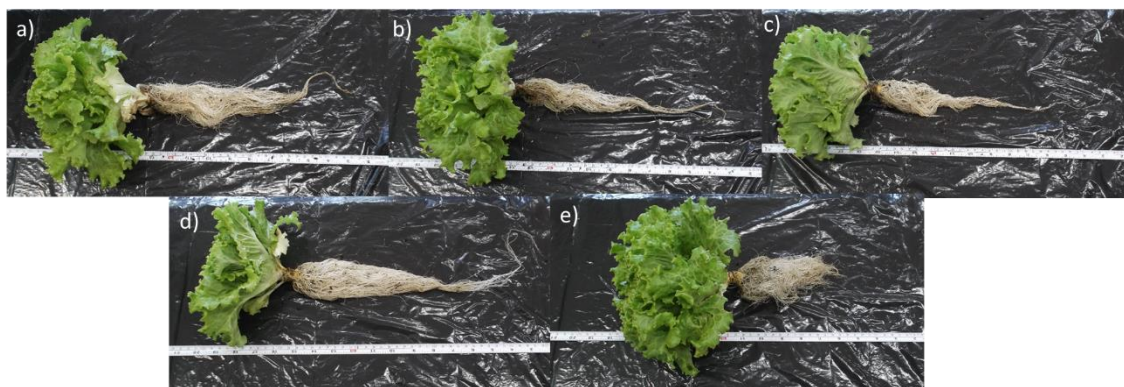
Figure 14 - Photographic record of the lettuce plants in each treatment at the end of the experiment a) CP – Positive control; b) CN – Negative control; c) E – Algal extract; d) B - BlueN; e) EB - Algal extract + BlueN.

Regarding root length and fresh weight (Fig. 15 a and c), no statistically significant differences were found between the treatments. Despite this, the positive control (CP) stands out with the higher root length (37.35 ± 1.73 cm), whereas the treatment with the algal extract (E) and the one with BlueN (B) attained similar root length values (34.49 ± 1.91 and 34.37 ± 2.84 cm, respectively), while the application of both (EB) resulted in the less developed root (28.53 ± 2.83 cm). Herein, it was observed that a longer root has a lower fresh weight, but a shorter root has a higher fresh weight. For instance, lettuces treated with EB attained the highest root fresh biomass (24.35 ± 3.09 g), but quite like the value achieved with BlueN treatment (23.19 ± 3.96 g) and with the algal extract (21.12 ± 2.71 g), while the lowest value was registered on the positive control (16.43 ± 0.89 g).

Also, no statistical differences were found among the different treatments regarding the aerial part diameter (Fig. 15b). Still, the positive control was the treatment that resulted in a longer aerial part (18.42 ± 0.50 cm), whereas the negative control (CN), the algal extract treatment (E), and the Algal Extract + BlueN treatment (EB) all had similar aerial part diameter values (17.91 ± 0.72, 17.73 ± 0.42, and 17.68 ± 0.52 cm, respectively), and the BlueN (B) treatment had the lower aerial part diameter (15.08 ± 0.39 cm).

However, the leaves weight statistically differ between treatments (Fig. 15d), particularly the one with BlueN (B: 49.28 ± 4.07 g), which stands out negatively when compared with the algal extract (E) treatment and both applied together (EB). In fact, the latter achieved the highest fresh leaves biomass (74.25 ± 6.86 and 74.13 ± 3.07 g, respectively).
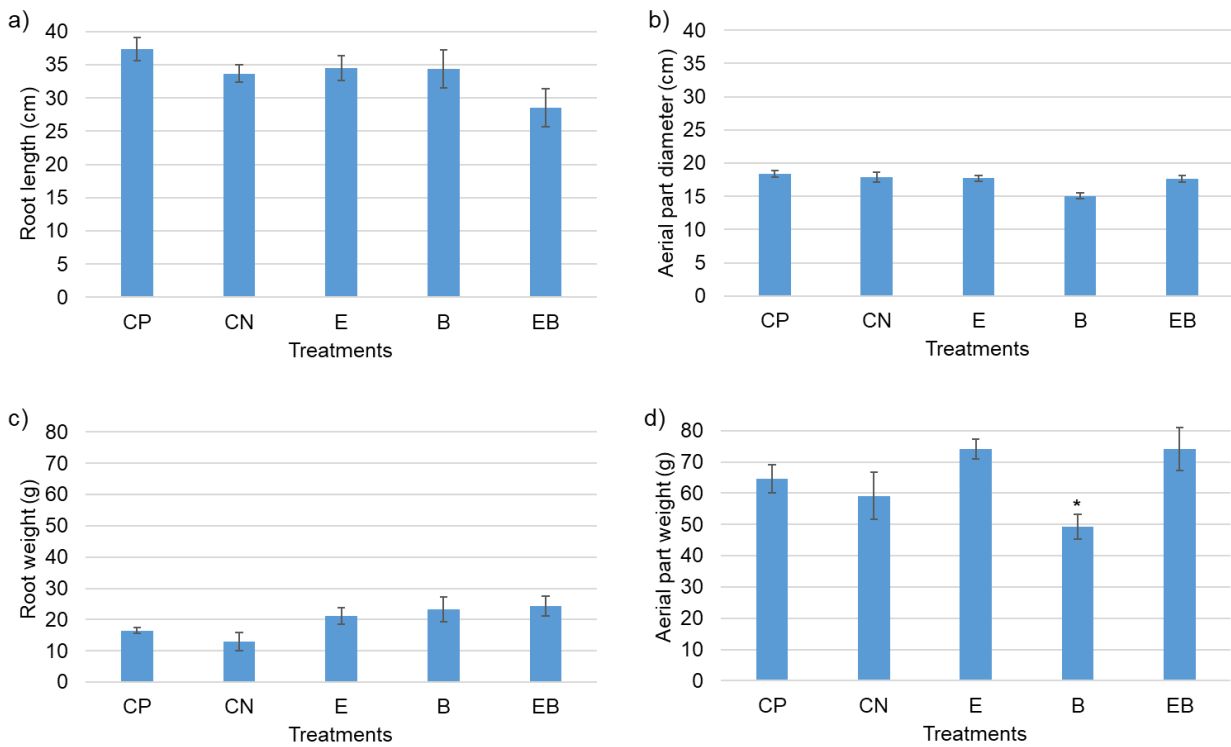
Figure 15 - a) Root length and b) aerial part diameter; c) root and d) aerial part fresh weight of the lettuces on each treatment. The graphs present average values and standard error (n = 12). CP – Positive control; CN – Negative control; E – Algal extract; B - BlueN; EB - Algal extract + BlueN. The symbol * means that there are statistically significant differences (p-value < 0.05).

As a result, both the algal extract (E) and the combination of the algal extract and BlueN (EB) had a good effect on lettuce leaf growth and weight. While the BlueN (B) itself was found to be ineffective for lettuce development.

As water and nutrients are not equally distributed in the soil, the root system's spatial arrangement is important for managing the most efficient use of the resources available (Koevoets et al., 2016). Herein, White (2013) showed that cultivars with higher crop yields have typically been conducted at optimal nutrient concentrations, resulting in the selection of smaller and less plastic roots (less developed, lower specific root length, root demography and biomass allocation within the patch zone) (Hodge, 2004; White et al., 2013). In fact, prior literature, refers that when root architecture contains high number of nodal and lateral roots, the plant yields more and produces higher biomass, because a significant investment in lateral root growth results in the establishment of a shallow root system (Bayuelo-Jiménez et al., 2011). This happens because roots often proliferate when they come upon a nutrient-rich zone, improving their physiological ion uptake capacity (Hodge, 2004). The root system's plasticity or flexibility responses have been proposed has the main mechanism by which plants cope

with soil's naturally occurring nutrient heterogeneity (Hodge, 2004). So, if the plant root is longer and has more biomass than the aerial portion, it means that there are more nutrients in the soil (White, 2013).

The length and weight ratio between the root and the aerial part (Table 11) shows that there is a dependent correlation amongst these variables. Herein, it is possible to observe that the positive control (CP), the algal extract (E) and BlueN (B) treatments exhibited higher ratios (2.04, 1.95 and 2.07, respectively), resulting on a more developed root, than the remaining treatments, whereas the plant focused on the development of leaf growth. In contrast, when compared with the other treatments, the one with BlueN (B) and the combination between BlueN and the algal extract (EB) led to a higher root: aerial part weight ratio (0.47 and 0.37, respectively), indicating that the plant spent more energy on root biomass than leaves.

Table 11 - Length and weight ratio between the root and the aerial part among the different treatments. CP – Positive control; CN – Negative control; E – Algal extract; B - BlueN; EB - Algal extract + BlueN.

Treatment	Ratio root length: aerial part diameter	Ratio root weight: aerial part weight
CP	2.04	0.27
CN	1.89	0.21
E	1.95	0.28
EB	1.59	0.37
B	2.07	0.47

More than the growth characteristics, it is critical to know whether the different treatments nutritionally improved the edible portion of the lettuces (Table 12). Even though the differences in mean values between treatment groups are not large enough to eliminate the possibility that the differences are due to random sampling variability, and thus there is no statistically significant differences, some treatments revealed an enrichment on certain elements. For instance, the algal aqueous extract treatment (E) revealed an enrichment on manganese (80.28 mg/kg), magnesium (0.19%, the same value as the positive control), but the lowest content of sodium (0.20%). The treatment with BlueN (B) reflected the highest mineral content (85.30%), but the lowest moisture and zinc amount (5.08 % and 47.70 mg/kg, respectively). However, the effect of the application of both treatments (EB) resulted on an enrichment of phosphorus (0.41%), sodium (0.28%), copper (6.49 mg/kg) and zinc (58.58 mg/kg); but the lowest content of calcium (1.09%) and magnesium (0.17%). The positive control (CP) stands out critically due to the lowest nitrogen, potassium, and iron values (1.44, 4.61% and 1035.86

mg/kg, correspondingly). From a different perspective, the negative control (CN) had the lowest mineral content (83.80%), which was reflected in the lowest phosphorus (0.38%, the same as the BlueN (B) treatment), copper and manganese tissue accumulation (4.05% and 57.48 mg/kg, respectively). Nonetheless, this treatment resulted in the highest moisture (6.52%), nitrogen (1.77 %), calcium (1.25%), potassium (5.09%), and iron (1451.66 mg/kg) content.

Table 12 - Mineral and trace element characterization of the lettuce leaves within each treatment. The results are presented as mean values and standard deviation (n=3, Dry weight basis). CP – Positive control; CN – Negative control; E – Algal extract; B - BlueN; EB - Algal extract + BlueN. NA- Non available.

Treatment	CP	CN	E	EB	B	Values reported in the literature	Ref.
Moisture (%)	6.30 ±0.18	6.52 ± 0.93	5.46 ±0.61	5.90 ±0.09	5.08 ±0.47	NA	-
Ashes (%)	84.62 ± 0.34	83.80 ±0.11	83.94 ±0.75	84.79 ±0.53	85.30 ±1.8	NA	-
N (%)	1.44 ± 0.05	1.77 ± 0.12	1.73 ± 0.20	1.65 ± 0.08	1.50 ± 0.15	NA	-
P (%)	0.39 ± 0.01	0.38 ± 0.02	0.39 ± 0.02	0.41 ± 0.02	0.38 ± 0.01	0.24	(Kim et al., 2016)
Ca (%)	1.13 ± 0.12	1.25 ± 0.06	1.18 ± 0.13	1.09 ± 0.09	1.12 ± 0.11	0.04-0.81	(Kim et al., 2016; Koudela & Petříková, 2008)
Mg (%)	0.19 ± 0.01	0.18 ± 0.01	0.19 ± 0.02	0.17 ± 0.01	0.18 ± 0.02	0.01-0.50	
K (%)	4.61 ± 0.51	5.09 ± 0.29	5.03 ± 0.21	4.97 ± 0.12	4.91 ± 0.45	0.36-1.89	
Na (%)	0.24 ± 0.02	0.24 ± 0.03	0.20 ± 0.01	0.28 ± 0.03	0.25 ± 0.04	0.01-0.05	
Cu (mg/kg)	5.68 ± 0.95	4.05 ± 0.51	4.96 ± 0.62	6.49 ± 1.06	4.82 ± 0.90	NA	(Kim et al., 2016)
Zn (mg/kg)	51.72 0.184.93	53.88 ± 0.44	58.45 ± 7.83	58.58 ± 4.97	47.70 ± 4.59	22.5	
Fe (mg/kg)	1035.86 ± 226.55	1451.66 ± 558.50	1322.73 ± 54.37	1385.31 ± 438.49	1209.82 ± 108.89	26.8	
Mn (mg/kg)	61.31 ± 3.81	57.48 ± 3.96	80.28 ± 9.92	62.72 ± 13.17	62.23 ± 21.59	14	

When compared with other studies where nutritional characterization of lettuces grown under greenhouse conditions was evaluated (Kim et al., 2016; Koudela & Petříková, 2008), it was possible to observe that in general, the values were lower when compared with the present research. Nonetheless, genetic differences and environmental factors have a direct influence on the phenotypic differences between treatments. influencing the nutritional composition of the edible portion of the lettuce (Kim et al., 2016).

3.2.4. Substrate characterization

Plant biomass production is directly influenced by the composition of the substrate, as it can meet the species' requirements (Schneider et al., 2018). Hence, the initial substrate was physically and chemically characterized, and was analyzed at the end of the experiment per each treatment (Table 13). As a result, when comparing the initial substrate with the final substrate of each treatment, an overall pH slightly decreased and there was observed an increase in electrical conductivity. Regarding the calcium oxide (CaO), magnesium oxide (MgO), potassium oxide (K_2O), phosphorus pentoxide (P_2O_5) and sodium (Na) content, every treatment had a rising effect on the substrate elements. The substrate of the positive control (CP) showed lower potassium oxide (257.1 mg/L) content, while the treatment with BlueN (B) had higher phosphorus pentoxide (504.42 mg/L), in comparison with the initial substrate (305.10 and 440.26 mg/L, respectively). Regarding the nitrogen (N) content, all the substrate samples exhibited similar values.

The importance of substrate fertility is determined by its' ability to sustain plant growth and maximize crop yield. Thus, substrates are divided in fertility classes regarding their phosphorus, potassium, calcium, magnesium, and sodium contents (Table 14) (Laboratório Químico Agrícola Rebelo da Silva, 1977).

The soil substrate exhibited an electrical conductivity lower than 0.50 mS/cm, except for the treatment with BlueN (B: 0.52 mS/cm), which is suitable for lettuce growth, because this species is sensible to high salt concentration (Laboratório Químico Agrícola Rebelo da Silva, 1977). Furthermore, the initial substrate, used in this experiment, for the lettuces potting was considered to have a low calcium content and a very low amount of magnesium. However, this substrate had a very high content of phosphorus and an optimal amount of sodium. Even though the fertility treatment was applied to the lettuce leaves, the chemical characterization of the substrate of each treatment revealed slightly differences, but not statically significant. For instance, the substrate of the treatment with BlueN (B) revealed a higher calcium oxide (176.82 mg/kg), magnesium oxide (35.57 mg/kg), potassium oxide (422.55 mg/kg), phosphorus pentoxide (504.42 mg/kg) and sodium (151.81 mg/kg) content, but lower nitrogen amount (0.82%, w/w). For another perspective, the substrate of the positive control (CP) had

the highest nitrogen (0.91% w/w) concentration, but the lowest potassium oxide (257.10 mg/kg). Regarding the carbon monoxide content (CO), the lowest values (33.90%, w/w) were observed in the substrate of the negative control (CN), and the highest, in the substrate of the positive control (CP) (38.01%, w/w).

Table 13 - Physical-chemical and elemental characterization of the initial and final substrate of each treatment. The results are presented as mean values ± standard deviation (n = 2). SI – Initial substrate; CP – Positive control; CN – Negative control; E – Algal extract; B - BlueN; EB - Algal extract + BlueN.

Physical-chemical parameters	SI	CN	CP	E	EB	B
material <2mm (%, w/w)	79.48	71.00	70.2	73.07	71.89	73.03
pH	6.60 ± 0.01	6.52 ± 0.02	6.55 ± 0.01	6.39 ± 0.01	6.43 ± 0.01	6.42 ± 0.01
EC (mS/cm)	0.20 ± 0.01	0.37 ± 0.03	0.30 ± 0.01	0.45 ± 0.01	0.42 ± 0.01	0.52 ± 0.01
CO (%, w/w)	37.71 ± 0.09	33.90 ± 0.50	38.01 ± 0.61	35.25 ± 0.31	36.56 ± 1.35	36.04 ± 0.20
N (%, w/w)	0.87 ± 0.01	0.85 ± 0.53	0.91 ± 0.01	0.89 ± 0.04	0.84 ± 0.04	0.82 ± 0.01
CaO (mg/kg)	60.10 ± 9.46	113.65 ± 0.01	89.15 ± 7.74	164.89 ± 3.40	149.77 ± 2.70	176.82 ± 4.20
MgO (mg/kg)	3.33 ± 1.08	21.58 ± 2.24	19.13 ± 0.37	31.13 ± 0.58	29.01 ± 0.04	35.57 ± 0.54
K_2O (mg/kg)	305.10 ± 0.38	319.58 ± 15.75	257.10 ± 10.13	340.35 ± 18.98	341.25 ± 0.83	422.55 ± 6.53
Na (mg/kg)	41.69 ± 16.56	123.31 ± 2.19	111.31 ± 5.94	139.81 ± 4.44	125.75 ± 1.75	151.81 ± 10.19
P_2O_5 (mg/kg)	440.26 ± 7.91	279.07 ± 17.12	310.67 ± 2.12	393.38 ± 2.81	333.03 ± 21.94	504.42 ± 17.21

Lettuce is a cool season crop and can have optimal growth at daytime temperatures of 15–20 °C (PlantVillage, 2020). The plant can be grown in a wide range of soils as long as it is fertile and moisture retaining due to the not deep root system of the plant (PlantVillage, 2020). It is often grown in alkaline soil (pH higher than 7.0) but will not tolerate acid soil. Heat tolerant varieties can be grown over the summer months and care should be taken to protect the leaves from strong sun by shading or covering to prevent the plants from bolting (Pardilhó et al., 2021). There are currently no restrictions on the use of seaweeds in agriculture; nevertheless, due to the high salt seaweed content, long-term or excessive use of row and not treated seaweeds may contribute to an increase of salt content in a soil (Nabti et al., 2017).

Table 14 - Substrate fertility classes according with the phosphorus, potassium, calcium, magnesium, and sodium content according to Laboratório Químico Agrícola Rebelo da Silva (1977).

Element	Fertility classes (mg/ kg)				
	Very low	**Low**	**Medium**	**High**	**Very high**
P_2O_5	≤ 10	11 - 20	21 - 30	31 - 60	> 60
K_2O	≤ 20	21 - 59	60 - 120	121 -150	> 150
CaO	≤ 50	51 - 75	76 - 250	251 - 300	> 300
MgO	≤ 17	18 - 34	35 - 50	51 - 83	> 83
Na	Optimal	Medium	High	Very high	
	≤ 50	51 -100	101 - 150	> 150	

BlueN, a commercially accessible product, is composed by *Methylobacterium symbioticum*, an endophyte bacterium that naturally provides nitrogen to plants (Symborg, 2021). Several experiments with this product have shown that it is beneficial to crops, increasing yield and lowering the use of conventional nitrogen fertilization (Symborg, 2021). For example, only one application of BlueN led to a yield increase of 56% in maize crop culture, 40% in grape, and 9% in raspberry (Symborg, 2021). Furthermore, the use of BlueN reduced for a 40% the application of conventional nitrogen fertilizer on wheat crop culture and a 60% reduction in chicory cultivation (Symborg, 2021). Despite the fact that there have been no reports of this product (BlueN) being used on lettuces, earlier research has shown that *Methylobacterium*

spp. can increase the growth and productivity of several important crop cultures, including sugarcane, wheat, corn, peanut, and tomato (Rafique et al., 2021; Schauer & Kutschera, 2011; Zhang et al., 2021).

A patent study that foliar application of a bacteria from the genus *Methylobacterium* ($1x10^6$ CFU/mL to $1x10^{11}$ CFU/mL) has been shown to increase the rate of root and leaf lettuce growth, as well as overall biomass production (Floro et al., 2015). However, because lettuce is a fast-growing crop, BlueN may have a greater impact on annual crops.

Brown seaweeds, such as *Alaria esculenta*, *Ascophyllum nodosum*, *Ectocarpus siliculosus*, *Fucus serratus*, *F. spiralis*, *F. vesiculosus*, *Halidrys siliquosa*, *Laminaria digitata*, *L. hyperborea*, *Saccharina latissima*, *Pylaiella littoralis* and *Ecklonia maxima* have shown to promote plant growth, and as a result, various authors state that a continuous application has more potential than organic manures and synthetic fertilizers in agriculture and horticulture (Abd El-Gawad & Osman, 2014; Battacharyya et al., 2015; Carvalho et al., 2013; Craigie, 2011; Reed et al., 1985). For instance, the brown seaweed *Ecklonia maxima* has shown a positive effect on lettuce growth, while increasing the potassium (46%), magnesium (37%) and calcium (52%) concentration in plant leaves (Crouch et al., 1990). Another example is the commercial *A. nodosum* extract (which was used in this study as a positive control) has also been shown to improve lettuce seedling performance when exposed to high temperatures (Möller & Smith, 1998). Moreover, previous research using Profertil (in the same concentration) on lettuce presented slightly higher results, when compared with this study, achieving an average aerial part diameter of 20 cm and an aerial part weight of 80 g, as well as higher root weight (24 g) (Sousa, 2020).

Nutrients are absorbed by plants through their roots or the surface of their leaves. Thus, the chemical composition of seaweed extract can significantly affect the plant nutrient profile (Battacharyya et al., 2015). Moreover, seaweed extracts alter the physical, biochemical, and biological properties of soil, as well as the architecture of plant roots, leading to a more efficient nutrient uptake (Anderson, 2009). In this context, extensive research regarding the chemical composition of several seaweed extracts indicated that the extracts' nutrient content (usually macronutrients such as nitrogen, phosphorus, and potassium) can affect plant growth and yield (Blunden et al., 1996; Blunden et al., 2010). Furthermore, abiotic factors variation such as, temperature, humidity, and light intensity all influence stomatal opening and the permeability of the cuticle and cell wall, affecting nutrient absorption from the leaf surface (Battacharyya et al., 2015; Calvo et al., 2014; Deshmukh & Bélanger, 2016).

In addition, brown seaweeds contain polysaccharides including alginates and fucoidans, which are beneficial to crop plants, promoting their growth (Pacheco et al., 2021). For instance, alginic acid possesses soil-conditioning properties as well as the ability to bind metal ions and form polymers with high molecular weight (Anderson, 2009; Hegazy et al., 2009). In this

context, the presence of a strongly cross-linked polymeric network promotes soil's capacity to retain water, enhancing root growth (Chen et al., 2003; Lattner et al., 2003; Verkleij, 1992). From another standpoint, alginate can compete with plants for cation uptake, limiting the growth-promoting effect (Chen et al., 2003). For example, the brown seaweed *A. nodosum* had nearly twice the alginate concentration of *Laminaria* species, which could explain why the positive control had a lower development in terms of leaf weight, when compared with the aqueous algal extract of *S. latissima* (previously known as *Laminaria saccharina*). Moreover, phenolic compounds have chelating properties, which may explain why seaweed extracts can release soil components that are otherwise unavailable (Balboa et al., 2013; Reed et al., 1985; Shibata et al., 2003).

Still, not all seaweeds, and thus not all seaweed extracts, are the same; even the same raw material using different methods produces extracts with different qualities (Craigie, 2011). Thus, *S. latissima* ever-changing biomolecular profile is one of the major bottlenecks that hinders this species large-scale seaweed-based biostimulant production (Zhang & Thomsen, 2019).

Furthermore, the concentration of seaweed extract is an important factor for plant growth and deserves additional investigation, as is the timing and frequency of its application to achieve the desired results (Battacharyya et al., 2015; Craigie, 2011).

Moreover, the interaction of the brown algal extract with the bacteria present in the BlueN was investigated in order to determine whether, or not, both products used, had synergistic effects that stimulated plant development.

Previous research has demonstrated that the application of a product composed by plant growth promoting bacteria (*Bacillus licheniformis*, *Bacillus megatherium*, *Azotobacter* sp., *Azospirillum* sp., and *Herbaspirillum* sp.) and by the green microalgae *Chlorella vulgaris* significantly affected the plant weight of both romaine (18.9% at spring) and leaf lettuce (22.7% at summer) (Kopta et al., 2018).

Murugan et al. (2020) investigated the effect of bacterial-algal interaction on tomato (*Lycopersicon esculentum* L.) and red pepper (*Capsicum annum* L.) growth. In this context, the bacteria *Methylobacterium oryzae* and a methanolic extract of the brown seaweed *Sargassum wightii* collected on the Palladam coast (India) were utilized in the investigation. As a result, the extract with the best yield in both crop cultures was the seaweed:methanol ratio of 40:2500, and it outperformed the algal extract and the bacteria alone.

Still, there is very little information about the interaction of *Methylobacterium* sp. with seaweed liquid fertilizers on plant growth, therefore more research is needed.

3.3. Algal bioactive compounds as biopesticides

3.3.1. Algal extracts characterization

Each polysaccharide solution and aqueous extract was examined (Table 15), and it was found that the polysaccharide extract of the tetrasporophyte of the species *G. tururu* and *C. teedei* var. *lusitanicus*, as well as the non-fructified gametophyte of *G. tururu*, had a neutral pH (ranging between 6.7 and 7.5) and similar electrical conductivity values (ranging between 210 and 270 µS cm^{-1}). While the extracts enriched with the polysaccharide alginate (*C. peregrina*, *S. muticum*, and *U. pinnatifida*) and agar (*G. gracilis*) exhibited an acidic pH (ranging from 2.9 to 3.1), but the electrical conductivity differed according to the polysaccharide, with the agar extract revealing a lower value (349 µS cm^{-1}) and the alginate extract presenting higher values (ranging from 667 to 975 µS cm^{-1}). Finally, both female and male gametophytes of *C. teedei* var. *lusitanicus* exhibited similar results of pH (8.7 and 9, respectively) and electrical conductivity (256 and 244 µS cm^{-1}, accordingly).

However, almost all the aqueous extracts had a slightly acidic pH, ranging between 5.7 and 6.5, except for *C. peregrina*, which had a slightly basic pH (8.4). *U. pinnatifida* showed similar electrical conductivity value on the polysaccharide extract (667 µS cm^{-1}) and on the aqueous extract (692 µS cm^{-1}). In overall, lower electrical conductivity values were detected in the aqueous extracts as compared to the polysaccharide extracts (Table 15).

Table 15 - Seaweed polysaccharide and aqueous extracts (1.2% v/v) characterization regarding its pH and electrical conductivity (EC - µS cm^{-1}; 25°C). GG- *Gracilaria gracilis*, GT_T- *Grateloupia turuturu* (tetrasporophyte), GT_GNF- *G. turuturu* (non-fructified gametophyte), CT_GF- *Chondracanthus teedei* var. *lusitanicus* (female gametophyte), CT_GM- *C.* teed*ei* var. *lusitanicus* (male gametophyte), CT_T- *C. teedei* var. *lusitanicus* (tetrasporophyte), CP- *Colpomenia peregrina*, SM- *Sargassum muticum*, UP- *Undaria pinnatifida*.

Seaweed species	Polysaccharide extracts (1 mg/mL)		Aqueous extracts (1.2% v/v)	
	pH	EC	pH	EC
GG	3.1	349	6.1	179
GT_GNF	7.5	270	5.8	147
GT_T	6.7	269		
CT_GM	9.0	244	6.5	198
CT_GF	8.7	256		
CT_T	7.3	210		
CP	3.1	975	8.4	390
SM	2.9	758	5.7	306
UP	3.1	667	6.2	692

The extracts characterization in terms of pH and electrical conductivity is critical for agricultural production effectiveness, and these two indicators can provide significant information on products applied to crops. Electrical conductivity, in a brief, measures the salinity and electrically charged nutrient ions in a solution and the positive effects on crops' yield, quality, and disease resistance are possible with the correct electrical conductivity performance (Taniguchi et al., 2019). For instance, previous research revealed that salt stress is one of the abiotic variables that condition *Botryosphaeria* spp. growth and plant infection, as higher electrical conductivity values are associated to increased salinity levels of the extract (Droby et al., 2011).

Moreover, *Botryosphaeria dothidea* grows best with slightly acidic pH levels, mainly between 5 and 6 (Kim, 1989). This could explain why polysaccharides and aqueous extracts with slightly acidic pH values and higher electrical conductivity values permitted fungal growth.

Because of the numerous compounds with plant growth bioactivity, seaweeds and algal extracts have been widely used as crop production system additives (Khan et al., 2009).

3.3.2. Algal polysaccharides antifungal potential

Biopolymers have recently gained the agrochemical industry's interest due to their ability to improve crop productivity and protection at nano dosages. The use of biopolymers can protect agricultural crops from phytopathogens by regulating genes and enzymes in plants (Sathiyabama, 2019). Thus, the unique features of these biopolymers at the nanoscale level make them suitable for a variety of applications to achieve an agricultural sustainability.

Thus, it was determined whether there was fungal growth inhibition after 7 days of applying the different polysaccharide extracts (Fig. 16, a, b, c, d, e, f, g, h, and i). But, in contrast to the positive control (Fig. 16 k), none of the polysaccharide solutions inhibited *Botryosphaeria dothidea* (Ascomycota) growth, because the inhibition halo was not formed.

Figure 16 - Photographic record of the antifungal effect of the polysaccharides solution extracted from the seaweeds: a) *Grateloupia turuturu* (tetrasporophyte), b) *Grateloupia turuturu* (non-fructified gametophyte), c) *Gracilaria gracilis*, d) *Colpomenia peregrina*, e) *Chondracanthus teedei* var. *lusitanicus* (tetrasporophyte), f) *Chondracanthus teedei* var. *lusitanicus* (male gametophyte), g) *Chondracanthus teedei* var. *lusitanicus* (female gametophyte), h) *Sargassum muticum*, i) *Undaria pinnatifida*, as well as, j) negative control, k) positive control, after 7 days of incubation. The inhibition halo is denoted by an arrow.

Nonetheless, most seaweed polysaccharides and its oligomeric forms have been proven to stimulate defense responses and protection against a wide spectrum of diseases in terrestrial plants (Vera et al., 2011).

For instance, a solution of alginate oligosaccharides (carbohydrate formed from the degradation of sodium alginate) can be used to prolong fruit quality by suppressing cell wall degradation caused by the phytopathogenic fungi *Botrytis cinerea*, *Penicillium expansum*, and *Alternaria alternata* on post-harvest kiwifruit (*Actinidia chinensis*), promoting fruit quality and increasing total phenolic content and flavonoids as well (Liu et al., 2020).

The literature also highlights that the polysaccharide carrageenan, and its oligomeric forms have potential to activate disease and pest resistance in plants (Khan et al., 2009; Mousavi et al., 2018), by modulating the activity of different defense pathways, including salicylate, jasmonate and ethylene signaling pathway (Shukla et al., 2016).

For instance, Mercier et al. (2001) revealed that λ-carrageenan can elicit an array of plant defense responses against *Phytophthora parasitica* var. *nicotianae* (Oomycota) on *Nicotiana tabacum* (tobacco).

While κ-carrageenan (extracted from *Hypnea musciformis*) has showed potential to be used as an elicitor and a strong plant protectant as well as growth promoting agent especially for chickpea plants (Bi et al., 2011).

Other study conducted by González et al. (2013) has showed that κ- and ι-oligocarrageenan, when applied as a foliar spray one time per week, at a dose of 1mg/ mL, increased *Eucalyptus globulus* trunk diameter (58% and 47%, respectively) and enhanced the concentration of compounds with antimicrobial properties, such as genistein, rutin, ellagic acid, morin, luteolin and quercetin.

However, the oligomeric form of the polysaccharides can be more efficient than its native form, as demonstrated by Vera et al. (2011) that showed that at a dose of 1 mg/ mL, native iota carrageenan did not protect against tobacco mosaic virus infection, whereas iota oligo-carrageenan at the same concentration reduced by 79% the number of necrotic lesions in tobacco plants.

Even though agar has been used in several studies as a growth media component for bacterial and fungal growth, as a neutral carrier for nutrients and growth substances (Cregut & Rondags, 2013; Titlyanov et al., 2017), agar extracted from the red seaweed *G. gracilis* contains sulphate esters groups, as previously indicated in chapter 3.1.3. Polysaccharide profile. Sulfate groups in the agar structure could be responsible for the biological activities described in the literature for these macromolecules (Torres et al., 2019; Wijesekara et al., 2011), such as anti-tumor (Sae-lao et al., 2017), anti-inflammatory (de Sousa et al., 2013), and antiviral properties (Andrew & Jayaraman, 2021). More recently, agar extracted from *Gracilaria* spp. also proven benefic effects on crop plants, such as amaranth (*Amaranthus aritis*) due to their biostimulant properties that expand plant tolerance to abiotic stresses (i.e.: drought) (Mahusook et al., 2021).

3.3.3. Algal aqueous extracts antifungal potential

Aqueous extracts were used to determine whether there was fungal growth inhibition 7 days after their application (Fig. 17). But, in contrast to the positive control (Fig. 17 h), none of the treatments inhibited the Ascomycota *Botryosphaeria dothidea* development as the inhibition halo was not formed.
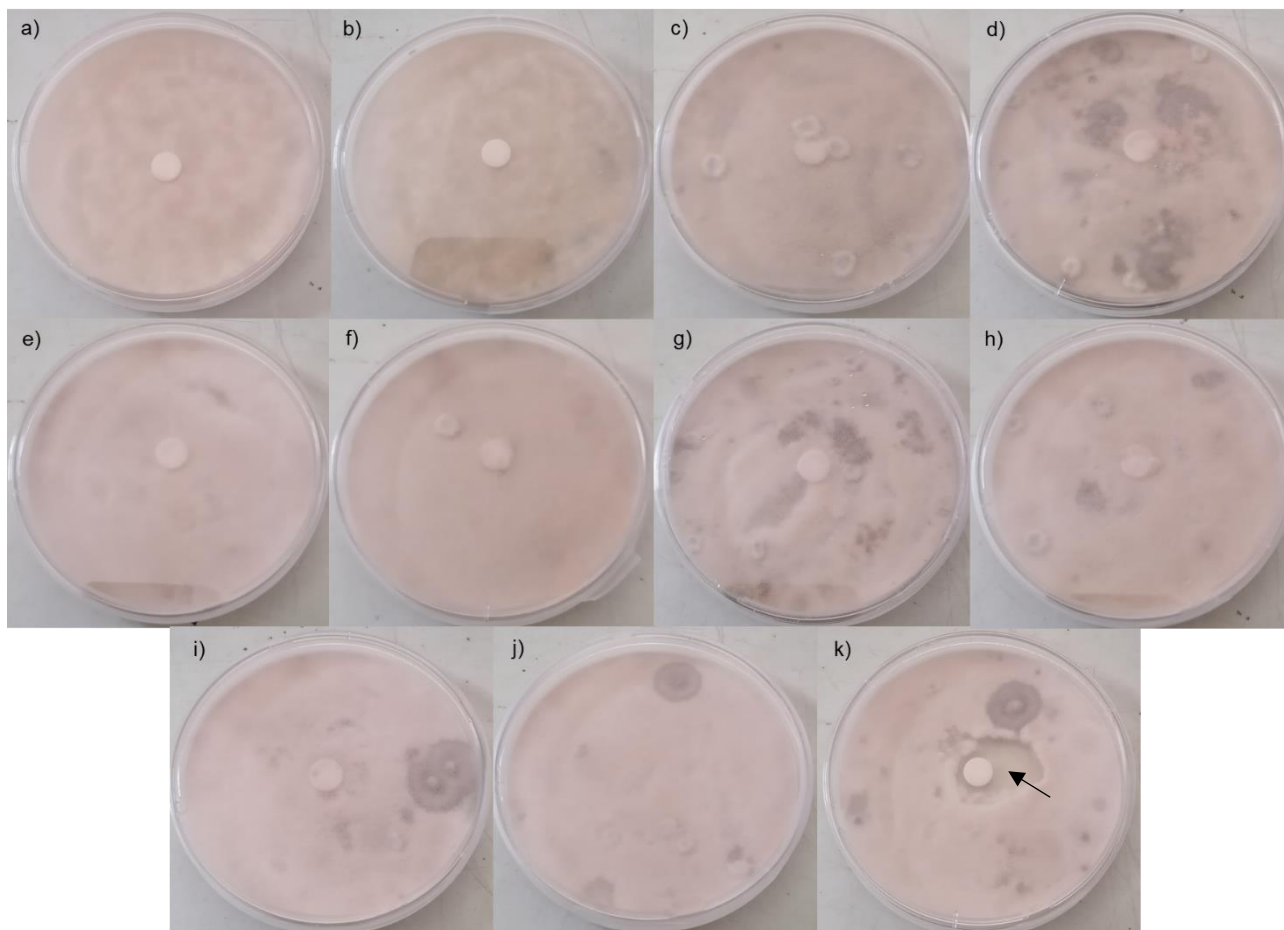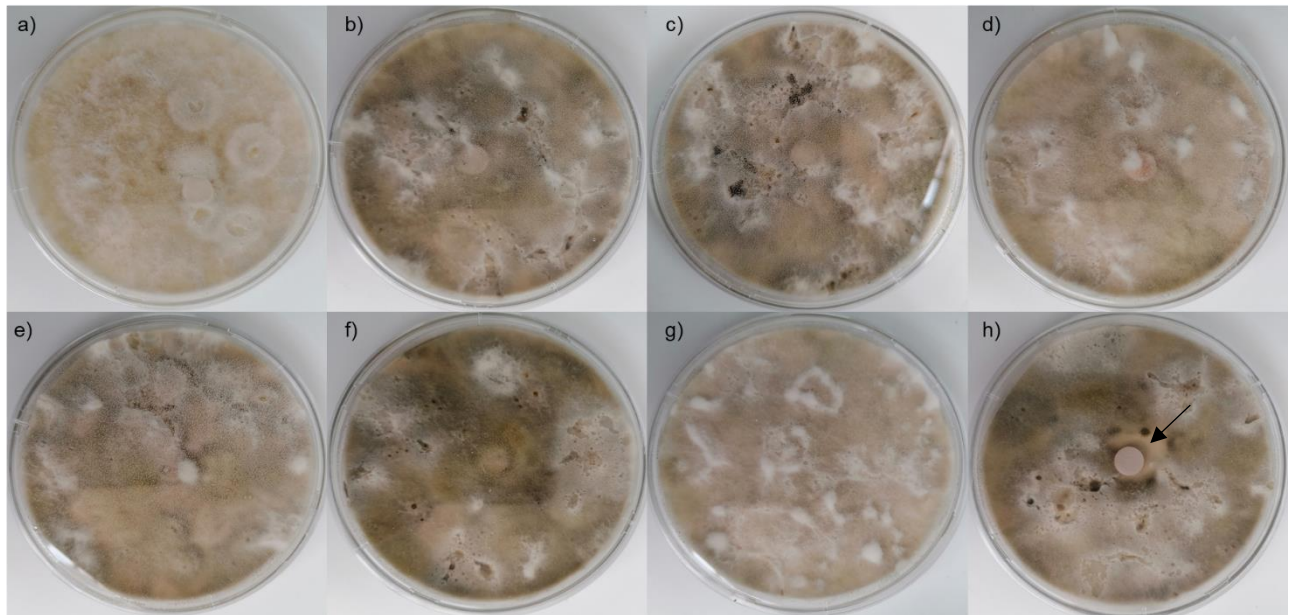
Figure 17 - Photographic record of the antifungal effect of the aqueous extracts from the seaweeds: a) *Grateloupia turuturu*, b) *Gracilaria gracilis*, c) *Chondracanthus teedei* var. *lusitanicus*, d) *Colpomenia peregrina*, e) *Sargassum muticum* and f) *Undaria pinnatifida*, as well as the g) negative control and the h) positive control, after 7 days of incubation. The inhibition halo is denoted by an arrow.

Algal extracts have been employed as agricultural biostimulants due to their beneficial effects on crop plants, including increased productivity, increased photosynthetic activity, and disease resistance caused by fungi, bacteria, and viruses (Sharma et al., 2014). The effectiveness of these algal extracts is based on their trace element, enzyme, and plant growth regulator content, which, when applied to crop plants, activates their physiological processes (Hamed et al., 2018), and thus, offering an effective and non-toxic alternative for plant disease management (Baloch et al., 2013).

Brown algal extracts and algae themselves, such as *Ascophyllum nodosum*, *Fucus* spp., *Laminaria* spp., and *Sargassum* spp., are widely applied in agriculture due to their bioactive compounds that promote crop plant development and productivity (Hamed et al., 2018).

Some research has been done using the brown seaweed *S. muticum* as a plant biostimulant and soil biofertilizer (Flórez-Fernández et al., 2021; Silva et al., 2019). However, researchers discovered that a chloroform:methanol (1:1) *S. muticum* extract (20%) decreased *Rhizoctonia solani* spore germination by 19% (Raj et al., 2016).

Even though the research on the antifungal potential of this seaweed is scarce, some studies highlight the potential of *Sargassum* spp. against phytopathogenic fungi. Acetonic extracts of *Sargassum polyceratium* obtained on Costa Rica's Caribbean Coast, for example, were found

to be efficient against the fungi *Geotrichum candidum* (Borbón et al., 2012). While different concentrations (40 and 50 g/L) of methanolic extracts of *Sargassum vulgare* collected from two different sites of the Tunisian coast exhibited different inhibition percentages of the mycelial growth of the fungi *Pythium aphanidermatum* (34.33 and 31.67%, respectively) (Ammar et al., 2017). An analysis of the methanolic extract in this investigation revealed that the antifungal activity could be related to the presence of phenolic acids and flavonoids (Ammar et al., 2017; Karawita et al., 2005; Kuda et al., 2005; Yildiz et al., 2012).

Another study, suggested that the fatty acid content of *U. pinnatifida* may contribute to its antifungal capability, possibly acting as a resistance inducer. As a result, a fatty acid extract (30 g/L) completely reduced *Botrytis cinerea* mycelial growth and suppressed *Penicillium digitatum* conidia germination by 43% (de Corato et al., 2017). However, many biochemical processes can occur in the complex host/ antimicrobial compound/ pathogen system, each with a different influence on the biological activity of the antimicrobial compounds (Ippolito et al., 2000; Lattanzio, 2003).

Despite *C. peregrina* widespread distribution, there have only been a few investigations on its biomass valorization (Beacham et al., 2019; Rostami et al., 2018). Nonetheless, the high level of K and P (46.96 and 0.67 mg/g, respectively) implies that this seaweed could be a possible biomass feedstock for the agriculture industry as a natural fertilizer or biostimulant, according to the available data (Beacham et al., 2019).

Although brown seaweeds are the most exploited in agriculture, red seaweeds are also worth exploring for their potential as biopesticides due to their high biochemical diversity (Asimakis et al., 2022).

For instance, *G. turuturu* aqueous extracts have previously been identified as an antimicrobial agent with antioxidant action (Ferreira et al., 2021; García-Bueno et al., 2014). However, it was discovered that the bioactivity of *G. turuturu* aqueous extracts varies depending on the harvesting season. For example, García-Bueno et al. (2014) discovered that spring extracts (10 µg/µl) decreased the growth rate of *Vibrio harveyi* by 12%, whereas winter extracts at the same dosage increased the growth rate by 25%. This could be explained by the seasonal change in metabolite production, which has a direct impact on biological activity (García-Bueno et al., 2015; Munier et al., 2013).

Also, a diethyl ether extract of *G. gracilis* collected in the coast of Izmir (Turkey) during the spring, exhibited a high antimicrobial activity against the fungi *Candida* sp. and the bacteria *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Escherichia coli*, exhibiting an inhibition halo higher than 15 mm in the disc-diffusion assays (Tuney et al., 2007).

A study conducted by Soares et al. (2016) with the red seaweed *Chondracanthus teedei* var. *lusitanicus*, an unexploited resource of the Portuguese coast, found that carrageenan extracted from different stages of this seaweed's life cycle caused alterations on the cell wall

of the fungi *Alternaria infectoria* and *Aspergillus fumigatus*. In *A. infectoria*, kappa/iota carrageenan (125 µg/mL) from the gametophyte phase and xi/ theta carrageenan (60 µg/mL) from the tetrasporophyte phase both caused the development of swollen hyphal segments. While *A. fumigatus* hyphae were shortened and branched by kappa/ iota carrageenan application (87.5 µg/mL) (Soares et al., 2016). Hence, it is highlighted the antifungal potential of these algal compounds. However, no research has been conducted to report on the use of *Chondracanthus teedei* var. *lusitanicus* extract as an antimicrobial product, or even its characterization.

Despite that, some compounds isolated from plants have already shown antifungal potential against *B. dothidea*, such as cuminaldehyde (Mesripour et al., 2019; Zhang et al., 2018), nerol (Zhang et al., 2018), geraniol, citral, and α-terpineol (dos Santos Negreiros et al., 2019; Zhang et al., 2018). It is reported that these terpenes change the morphology of the hyphae of *B. dothidea*, reducing its growth (Wei et al., 2021). Bearing this in perspective, although the aqueous extracts had no antifungal impact on *B. dothidea*, a study in which the chemical analysis of *U. pinnatifida* biomass was assessed, it indicated that this seaweed includes beta-cyclocitral, a monoterpenoid formally derived from citral by cyclisation (Balbas et al., 2015; López-Pérez et al., 2017; Pubchem, 2022).

From another perspective, seaweed extracts can be a source of vitamins, carbon, and nitrogen, acting as a promoter of mycelial growth (Kim, 1989). However, the biostimulant potential of algal compounds and extracts, on crop plants, have not yet been thoroughly investigated (Khan et al., 2009).

From another perspective, seaweeds' isolated compounds or extracts have not previously been investigated its potential against *B. dothidea*, being this study a first screening assay to assess its potential.

4. Conclusion

The ever-increasing urgency of climate change and the environmental effects of agriculture has posed the difficulty of rapidly finding new solutions for the sustainable practices for mass crop production. While these issues are complex and may require a full rethinking of how agriculture should be managed globally, the development of seaweed-based biostimulant products has provided a viable interim solution toward agriculture's sustainable future.

In this perspective, the physical-chemical parameters of the seaweed polysaccharide solution (pH and EC), as well as the polysaccharide chemical structure and uronic acid content, revealed to have a significant impact on kale seed germination and development. As a result, the polysaccharide kappa/ iota-carrageenan isolated from the female gametophyte of the red seaweed *C. teedei* var. *lusitanicus* enhanced kale seed germination and seedling aerial part length and weight.

In addition, the brown seaweed *Saccharina latissima* proved to be a good source of not only important minerals like nitrogen, phosphorus, and potassium, but also of sulphated polysaccharides like alginate and fucoidan, as well as other bioactive compounds like phenolic compounds. As an outcome, the results demonstrated that *S. latissima* aqueous extract employed as a foliar spray itself and when combined with the biofertilizer BlueN, can increase the mass of lettuce leaves while also enhancing them nutritionally, particularly in the micronutrients zinc and manganese, important for human diet.

The biopesticide potential of seaweed extracts and isolated compounds as an agricultural tool for integrated pest and disease management has been evaluated in the literature. As a result, their ability to control a variety of phytopathogens has been highlighted. However, as a biopesticide, the algal aqueous extracts and polysaccharides did not present antifungal action against *B. dothidea*.

Still, both algal aqueous extracts and polysaccharides exhibited their effectiveness by promoting crop plant growth and development, even at low concentrations. However, additional biochemical characterization of those seaweed extracts that showed improved outcomes is needed to fully unveil their potential as crop plant biostimulants/ biopesticide. Moreover, agronomic characteristics, such as cultivar selection and biostimulant/ biopesticide management, including concentration and volume of the treatment application, when and how many applications are made, and the administration approach (foliar, drench, seed treatment, nutrient solution), can make or break the crops' and products' full potential and should be carefully taken in consideration.

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