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Seaweed Carrageenan as Promoter of Plant Growth and Elicitor of Natural Defenses Against Magnaporthe oryzae in Rice

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Abstract

Rice (*Oryza sativa* L.) is one of the world's major staple foods. However, its production is severely constrained by rice blast disease, caused by *Magnaporthe oryzae*, which leads to substantial yield losses. Conventional management relies on fungicides and chemical treatments; however, these methods raise concerns regarding the development of pathogen resistance and potential environmental impacts. This study evaluated carrageenan from *Hypnea musciformis*, collected from the coast of Saint Martin (92°19′21.28″E and 20°37′38.12″N), located in the Bay of Bengal, Bangladesh, as a natural plant growth promoter as well asbiocontrol agent. Carrageenan was characterized by high sulfate (19–35%) and galactose (12–18%) contents, with FT-IR confirming characteristic κ-carrageenan functional groups. Application of 15% carrageenan significantly increased germination of seed (27%), seedling vigor (93%), shoot and root lengths (54% and 47%), and biomass compared with untreated controls. Carrageenan markedly suppressed *M. oryzae*, inhibiting mycelial growth (83%), reducing conidiogenesis and conidial germination, and decreasing lesion length in detached leaves and potted plants. Treated rice seedlings exhibited improved soluble sugars, photosynthetic pigments, proline, phenolic and flavonoid contents, and enhanced antioxidant enzyme activities such as CAT (catalase) and POD (peroxidase), while lowering oxidative stress markers such as H_2O_2 and MDA (malondialdehyde). These results demonstrate that carrageenan from *H. musciformis* enhances rice growth and elicits defense responses against rice blast, offering a sustainable and environment friendly alternative to chemical-based fungicides for integrated *M. oryzae* management.

Keywords: rice; *Magnaporthe oryzae*; carrageenan; seaweed extract; antifungal activity antioxidant defense; photosynthetic pigments; oxidative stress

1. Introduction

Rice (*Oryza sativa* L.) ranks among the three main staple foods globally, nourishing over half of the world's population. The farming of rice plays a crucial role in the livelihoods of countless smallholder farmers in various communities, especially in Asia and Africa. Approximately 168 million hectares of land are dedicated to rice cultivation, yielding around 800 million tons (Kou et al., 2026). The demand for rice continues to rise due to increasing population figures and evolving dietary preferences. The Food and Agriculture Organization of the United Nations (FAO) forecasts that the global demand for rice will rise by 33% from 2019 to 2050 (Falcon et al. 2022). Beyond its critical role in food security, rice is also deeply intertwined with economic growth and social stability (Arouna et al. 2021). Given its critical role as a food crop, a decline in rice productivity significantly impacts global food production patterns and food security (Ding et al., 2026). However, rice productivity is significantly constrained by a wide range of diseases, among which rice blast disease, caused by the phytofungal pathogen *Magnaporthe oryzae* (syn. *Pyricularia oryzae*), is the most devastating. Rice blast remains a serious and persistent threat to rice production in both lowland and upland systems across the globe, causing yearly yield reductions of about 10% to 30%, which corresponds to an estimated

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loss of roughly 157 million tons worldwide (Amin et al., 2026). Under favorable conditions, this disease can rapidly destroy entire crops, leading to losses of up to 100% within a short period(Simkhada and Thapa, 2022). Traditional approaches for controlling rice blast disease primarily rely on chemical fungicides, which pose risks of environmental contamination, pathogen resistance, and negative effects on human health. As a result, there is an urgent need for sustainable and eco-friendly alternatives that can deliver effective, broad-spectrum, and durable protection against rice blast.

In recent years, marine-derived polysaccharides have emerged as a rich source of bioactive compounds with significant agricultural potential. Seaweeds contain structurally diverse polysaccharides that exhibit a wide range of biological activities, including plant growth promotion and induction of stress tolerance (Hossain et al., 2024). Among the polysaccharides, carrageenans are the most prominent sulfated linear hydrophilic polysaccharides, which are extracted from red algae by hot alkali separation and consists of 3, 6-anhydrogalactose linked by α-1, 3, galactose and β-1, 4 glycosidic bonds alternately, allowing for the formation of curling helical structures (Kang et al., 2024).

Carrageenans significantly enhance traits associated with the growth of plants, including height of plant, number of pods, branches, and leaves, while promoting earlier flowering and increasing levels of resistance-related metabolites (Hossain et al., 2024). These effects occur through multiple metabolic pathways, such as carbon fixation, chlorophyll metabolism, protein synthesis, photosynthesis, detoxification of reactive oxygen species, and secondary metabolite production. They are thought to be mediated by modulation of plant hormonal balance, improved nutrient uptake, and stimulation of metabolic activities (Bi et al., 2011; Mohamed et al., 2025). Additionally, their growth-promoting properties, carrageenans have been shown to act as elicitors of plant defense responses (Shukla et al., 2016). Due to their structural similarity to pathogen-associated molecular patterns (PAMPs), they can be recognized by plant receptors, thereby triggering immune signaling pathways. Moreover, these compounds may suppress pathogens through direct antimicrobial activity and/or enhance plant resilience by inducing biochemical changes via salicylate (SA), jasmonate (JA), and ethylene (ET) signaling pathways. This leads to increased production of antioxidants, defense-related proteins,and secondary metabolites (Hossain et al., 2024). Furthermore, carrageenan treatments have been reported to strengthen cell walls, thereby enhancing resistance against insects and wave-induced stress (Hossain et al., 2024).

While seaweed-derived polysaccharides such as carrageenan have been explored for their biostimulant and elicitor properties in some crops, their potential role in enhancing rice growth and triggering natural defense mechanisms against blast (*M. oryzae*) remains largely under-investigated. Moreover, there is a limited understanding of the physiological and biochemical mechanisms through which carrageenan may modulate rice immunity and growth under pathogen stress. Addressing this gap, this research was undertaken to evaluate the efficacy of carrageenan extracted from *Hypnea musciformis* as a natural growth promoter and biocontrol agent in rice. The main goal of this study is to develop an eco-friendly and sustainable strategy for integrated management of rice blast disease while simultaneously enhancing rice growth and productivity.

2. Materials and Methods

2.1. Seaweed Collection and Carrageenan Preparation

In June 2022, *H. musciformis* was brought from the coast of Saint Martin (92°19′21.28″E and 20°37′38.12″N), located in the Bay of Bengal, Bangladesh, during its mature stage. The seaweed was washed under running tap water and dried in the sunlight for two days. For extraction, 4 g of sun-dried seaweed was hydrated in 100 mL of deionized water at room temperature for 12 h. After that, the depigmentation was then performed using 100 mL of a methanol–acetone (1:1) mixture to remove organic-soluble components. The depigmented seaweed was subsequently treated with either 3% KOH (alkaline treatment) at ~150 mL per gram of seaweed and heated at 80 °C for 4 h. The extract

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was filtered and washed repeatedly with deionized water to remove residual KOH salts. It was then redissolved in 1 L of deionized water and heated at 90 °C for 4 h, followed by coarse filtration through cotton cloth and fine filtration using a glass microfiber filter (Whatman GF/D). The concentrated extract was precipitated with three volumes of 95% ethanol (1:3, v/v), centrifuged, dried, and milled into a fine powder passing through a 500-µm mesh.

2.2. Estimation of Chemical Composition of Carrageenan

2.2.1. Estimation of Sulfate Content

turbidometric assay was used to quantify the sulfate concentration in carrageenan (Jackson and McCandless 1978). Briefly, the barium–agarose reagent was freshly formulated by mixing 0.02% agarose with 0.5% barium chloride ($BaCl_2$). Subsequently, 1 mL of the carrageenan sample was combined with 1.2 mL of 8% trichloroacetic acid (TCA), and 600 µL of the prepared barium–agarose reagent was added. The mixture was allowed to stand at room temperature for 30 minutes. Absorbance was then recorded at 500 nm using a blank as reference. Sodium sulfate was used as the calibration standard.

2.2.2. Estimation of Silver Content

The silver content of carrageenan was quantified following the procedure outlined by Yaphe (1960). In summary, 2 mL of the carrageenan solution was combined with 10 mL of freshly prepared resorcinol reagent in a boiling tube. The mixture was first cooled in an ice bath for 5 minutes, then incubated at 80 °C for 10 minutes. Subsequently, it was cooled again in an ice bath, after which the absorbance was recorded at 500 nm.

2.2.3. Estimation of Galactose Content

The galactose concentration in carrageenan was quantified through a colorimetric assay employing anthrone reagent, based on a slightly adapted version of the method described by Yaphe (1960). In this procedure, 200 mg of anthrone was dissolved in 100 mL of 83.6% sulfuric acid and kept at 4 °C for storage. Subsequently, 1 mL of the carrageenan solution was combined with 10 mL of freshly prepared anthrone reagent in a boiling tube. The mixture was heated in a boiling water bath for 11 minutes and then immediately cooled in an ice bath. The absorbance was then recorded at 630 nm.

2.3. Fourier Transform Infrared (FT-IR) Spectroscopy Analysis

The freeze-dried extracted carrageenan was analyzed using Fourier Transform Infrared (FT-IR) spectroscopy in the frequency range of 4000–650 cm−1 with a PerkinElmer Spectrum X instrument (USA). The sample spectrum was generated by averaging 128 scans at four different resolutions (Rafiquzzaman et al., 2016).

2.4. Effect of Carrageenan on Seed Germination, Seedling Vigour and Growth Parameter

Seed Germination was observed daily over a period of eight days, following the methods outlined by the Association of Official Seed Analysts (AOSA, 2005). For the germination testing in each experimental treatment, four groups of 100 seeds each were used, and the experimental units were arranged in a randomized complete block design. The rice seeds were placed on Whatman No. 5 filter paper in sterilized 90-mm Petri dishes. Each dish was treated with either 5 mL of liquid extract or distilled water (as a control), along with different concentrations of carrageenan (10%, 15%, and 20%). The plates were then incubated at 25 ± 1°C under a 16-hour light and 8-hour dark regime. Germination was defined as the emergence of a radicle longer than 2 mm. Seven days after imbibition, the germination percentage and seedling vigor index were calculated.

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On the other hand, a hydroponics experiment and a Petri dish experiment were conducted to evaluate the effect of carrageenan on the growth of rice plants. In the hydroponics experiment, twenty-five pregerminated seeds were placed on metal nets floating in a liquid growth medium within a hydroponic system. This system utilized plastic pots filled with MGRL medium, as outlined by Hossain et al. (2007). For the pot experiment, sterilized soil was placed in plastic pots, and the pregerminated seeds were planted in the soil. After one week of growth, diluted carrageenan at concentrations of 10%, 15%, and 20% was added to both media, while the control group received an equal amount of distilled water. After three weeks of growth, plant height, along with the fresh and dry biomass weights of both shoots and roots, was recorded, and the mean values were calculated.

2.5. Effect of Carrageenan on Mycelial Growth Inhibition of Magnaporthe oryzae

PDA media was prepared and supplemented with different concentrations of carrageenan (10%, 15%, and 20%), then plated. A control plate was also prepared without any carrageenan. The pathogen *Magnaporthe oryzae* was inoculated at the center of the agar plates. The radial growth of the pathogen was recorded on both the control and treated plates. The percentage of growth inhibition was calculated using the following equation:

$$\% \text{ Inhibition of growth} = (X - Y)/X \times 100$$

where:

X = Mycelial growth of the pathogen in the absence of carrageenan

Y = Mycelial growth of the pathogen in the presence of carrageenan

2.6. Effect of Carrageenan on Inhibition of Conidial Germination

For conidial germination, 100 µl of a 1×10^5 conidia/ml suspension was mixed with carrageenan (10%, 15%, and 20%) or Nativo WG75 to a final volume of 200 µl in 2 ml tubes. Sterile water served as the negative control. The mixtures were incubated at 25°C in a moisture chamber in darkness and observed at 0, 6, 12, and 24 hours. Conidial germination percentage and morphological changes in germ tubes and appressoria were assessed using the same microscope at 40× magnification. Each treatment was replicated three times and repeated in three independent experiments. Conidial germination (%) was calculated as:

$$CG\%=(C-T)/C\times100$$

where, CG = conidial germination, C = percentage of germinated conidia in control, and T = percentage of germinated conidia in treated sample.

2.7. Effect of Carrageenan on Suppression of Rice Blast in Detached Leaf Assays

Leaves without visible disease symptoms were excised from 18-day-old plants and placed on two layers of moistened filter paper in Petri dishes. Each leaf was gently punctured at the center with a needle to facilitate infection by M. oryzae. Then, 10 µl droplets of carrageenan solution with different treatments were applied to the puncture sites. The leaves were incubated in darkness at 25°C. After 24 hours, the puncture sites were inoculated with 10 µl of M. oryzae spore suspension (1×10^5 spores/ml) and incubated again at 25°C in darkness for 24 hours. The leaves were then transferred to a growth chamber at 25°C under light conditions. Lesion diameters were measured and compared after seven days. Leaves treated with water and Nativo WG75 (10 µg/ml) instead of carrageenan served as negative and positive controls, respectively (Li et al., 2018). Blast lesion length was measured on two leaves per plant for each treatment and concentration.

2.8. Effect of Carrageenan on Suppression of Rice Blast in Pot Assays

The 10%, 15%, and 20% carrageenan concentrations were evaluated for their ability to suppress rice blast in pot assays. Pregerminated rice seeds were sown in pots (22 × 30 cm) containing sterile paddy soil and grown under natural light and temperature in a net house. Carrageenan solutions (10%, 15%, and 20%) were applied to 1-week-old seedlings at 5-day intervals. Seedlings treated with

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sterile distilled water served as the negative control, while those treated with Nativo WG75 served as the positive control. After 21 days, seedlings were transferred to an inoculation chamber. The pathogen inoculum was prepared by culturing the blast fungus on PDA medium at 25°C for 2–3 weeks. Spores were harvested by adding 5–7 ml of sterile water containing 0.5% Tween 20 to the culture plates, followed by filtration through 0.2 µm nylon mesh. The spore suspension was kept on ice to prevent germination and adjusted to a final concentration of 1×10^5 spores/ml. After 24 hours in the chamber, seedlings were spray-inoculated with freshly prepared M. oryzae spore suspension. Plants were then incubated overnight in a humid chamber at 28–30°C before being returned to the net house. Leaf samples were collected from three replicates per treatment, three days post-inoculation, for biochemical analysis, frozen in liquid nitrogen, and stored at −80°C. Disease severity was assessed seven days after inoculation using a 0–6 scoring scale, where 0 = No evidence of disease, 1 = Brown specks smaller than 0.5 mm in diameter, no sporulation, 2 = Brown specks about 0.5 to 1 mm in diameter, no sporulation, 3 = Roundish to elliptical lesions about 1 to 3 mm in diameter, with gray centers surrounded by a brown margin; lesions capable of sporulation, 4 = Typical spindle-shaped blast lesions capable of sporulation, 3 mm or longer, with necrotic gray centers and water-soaked brown margins, 5 = Similar to 4 but with about half of one or two leaf blades killed by coalescing lesions and 6 = One or two leaf blades killed by coalescing lesions (Hayashi and Fukuta, 2009; Khan et al., 2016).

2.9. Quantifying Chlorophylls and Carotenoids

Chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (Chls), and carotenoids were measured from supernatant extracts obtained using 80% (v/v) acetone, with analysis performed via spectrophotometry. The concentrations of Chl a, Chl b, total chlorophyll, and carotenoids were determined based on the equations described by Arnon (1949) and Lichtenthaler and Wellburn (1983).

2.10. Quantifying Malondialdehyde and Hydrogen Peroxide Levels

The levels of hydrogen peroxide and malondialdehyde in rice leaf tissues were accurately determined using a spectrophotometer, following the protocols described by Yu et al. (2003) and Kim et al. (2020), respectively.

2.11. Determination of Enzymatic and Non-Enzymatic Antioxidant Activities

Enzyme extracts were obtained from the leaves of rice seedlings, and the activities of key antioxidant enzymes catalase (CAT) and peroxidase (POD) were determined following the procedure described by Rahman et al. (2019). The concentrations of total phenolics and flavonoids in fresh samples of the third leaf were measured using the method of Das et al. (2022) to evaluate non-enzymatic antioxidant components. Furthermore, the overall antioxidant capacity of the plant material in each sample was assessed using a modified version of the Girennavar (2007) method, based on the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH).

2.12. Determination of Proline and Total Soluble

Proline content was quantified by extracting leaf tissue with 3% sulfosalicylic acid, followed by reaction with an acidic ninhydrin reagent, according to the procedure described by Bates et al. (1973). Total soluble sugars were estimated using the anthrone assay (Somogyi, 1952), with 80% ethanol employed as the extraction buffer.

2.13. Statistical Analysis

The data were analyzed using two-way analysis of variance (ANOVA) in RStudio (version 2025.09.2+418). Differences among the various treatments were considered statistically significant at $p < 0.05$ and were denoted using different letters. All results were based on three biological replicates

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(n = 3), and values are presented as means ± standard errors (SEs) in the corresponding figures and tables.

3. Results

3.1. Chemical Composition of Carrageenan

Carrageenan was enriched with galactose, silver, and sulfate content (Figure 1). The amounts of sulfate, galactose, and silver increased progressively with increasing carrageenan concentration. The highest levels of all components were observed at 20% concentration of carrageenan. Sulfate content ranged from 19.08% to 35.45%, galactose content varied from 12.34% to 18.34%, and silver content ranged from 74.78% to 91.90%. Among these compositions, silver content was consistently the highest across all carrageenan concentrations.

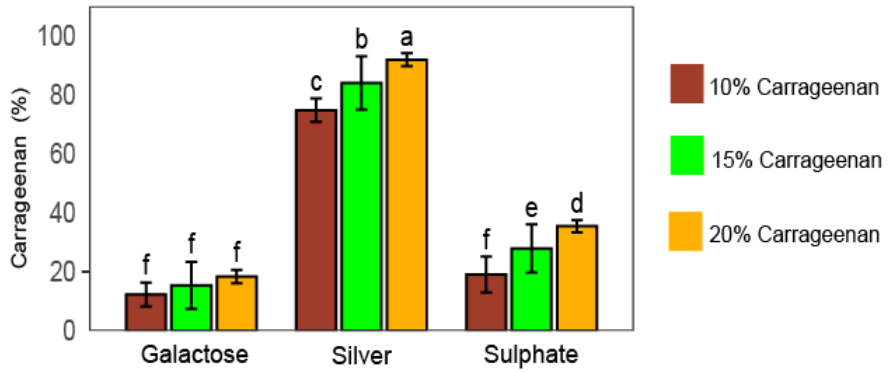

Figure 1. The chemical composition of carrageenan extracted from *H. musciformis* at different concentration levels. Increasing carrageenan concentration (e.g., 10%, 15%, and 20%) resulted in a progressive rise in all measured components. Silver content remained consistently higher than sulfate and galactose across all treatments.

3.2. Fourier Transform Infrared (FT-IR) Spectral Analysis of Carrageenan

Fourier-transform infrared spectroscopy (FT-IR) was performed on alkali-treated carrageenan extracted from *H. musciformis* (Figure 2). The FT-IR spectra displayed absorption bands at 1220–1226 cm^{-1}, corresponding to sulfate esters. Strong bands at 926–930 cm^{-1} indicated the presence of agarose (AG), while a distinct band at 845 cm^{-1} was attributed to D-galactose-4-sulfate (G4S). Peaks within the range of 3000–3600 cm^{-1} represented O–H stretching, whereas lipid content was identified by peaks between 2800–2900 cm^{-1}. Primary amines were confirmed by peaks at 1650 cm^{-1} and 1550 cm^{-1}. Carbohydrate content was indicated by absorption peaks between 1200 cm^{-1} and 950 cm^{-1}.

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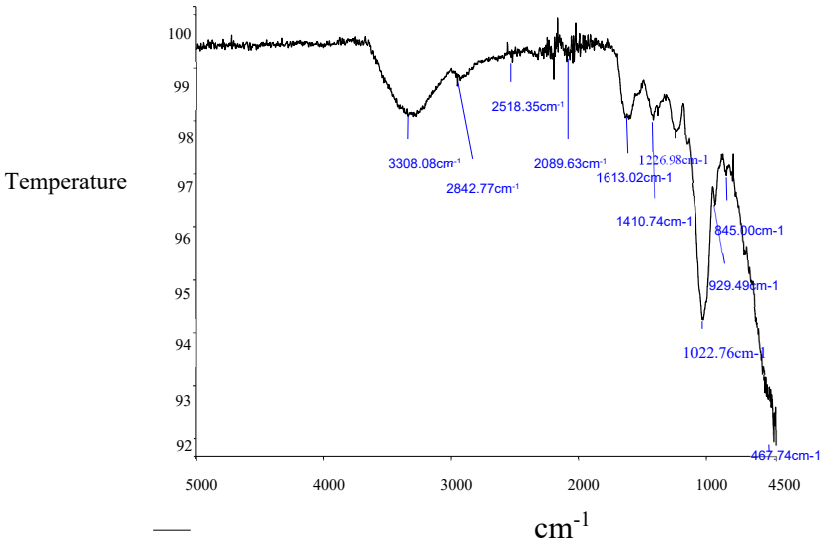

Figure 2. FTIR-based structural characterization of carrageenan isolated from *H. musciformis*. The spectra show characteristic absorption bands associated with key functional groups of carrageenan, including sulfate esters, 3,6-anhydrogalactose, and D-galactose-4-sulfate. Broad absorption in the higher wavenumber region corresponds to O–H stretching vibrations, while peaks in the aliphatic region indicate lipid-associated C–H stretching. Signals attributed to primary amines are also evident, alongside prominent bands within the fingerprint region that confirm the carbohydrate-rich polysaccharide structure.

3.7. Carrageenan Application in Enhancement of Seed Germination, Growth and Morphological Attributes of Rice Plants

The application of carrageenan increased seed germination, seedling vigour, plant height, shoot length, root length, fresh weight and dry weight (Figure 3). Rice plants with 15% carrageenan concentration exhibited the highest germination rate at 93.33%, whereas those treated with 10% carrageenan concentration showed a germination rate of 88.00% (Figure 3A-B). The average seedling height of the untreated control plants was 18.46 cm, while carrageenan-treated seedlings showed markedly enhanced growth, with 15% carrageenan exhibited 28.00 cm (Figure 3C). In addition, the seedling vigor index of the control was 1353.67, but carrageenan application significantly elevated this parameter, with 15% carrageenan attaining the maximum vigor index of 2613.24 (Figure 3D). Moreover, the highest shoot length of 20.00 cm and root length of 8.00 cm were observed in the application of 15% carrageenan (Figure 3E-F). Both fresh and dry biomass were significantly increase in 15% carrageenan concentration, with values of 2.04 g and 0.31 g, respectively (Figure 3G-H).

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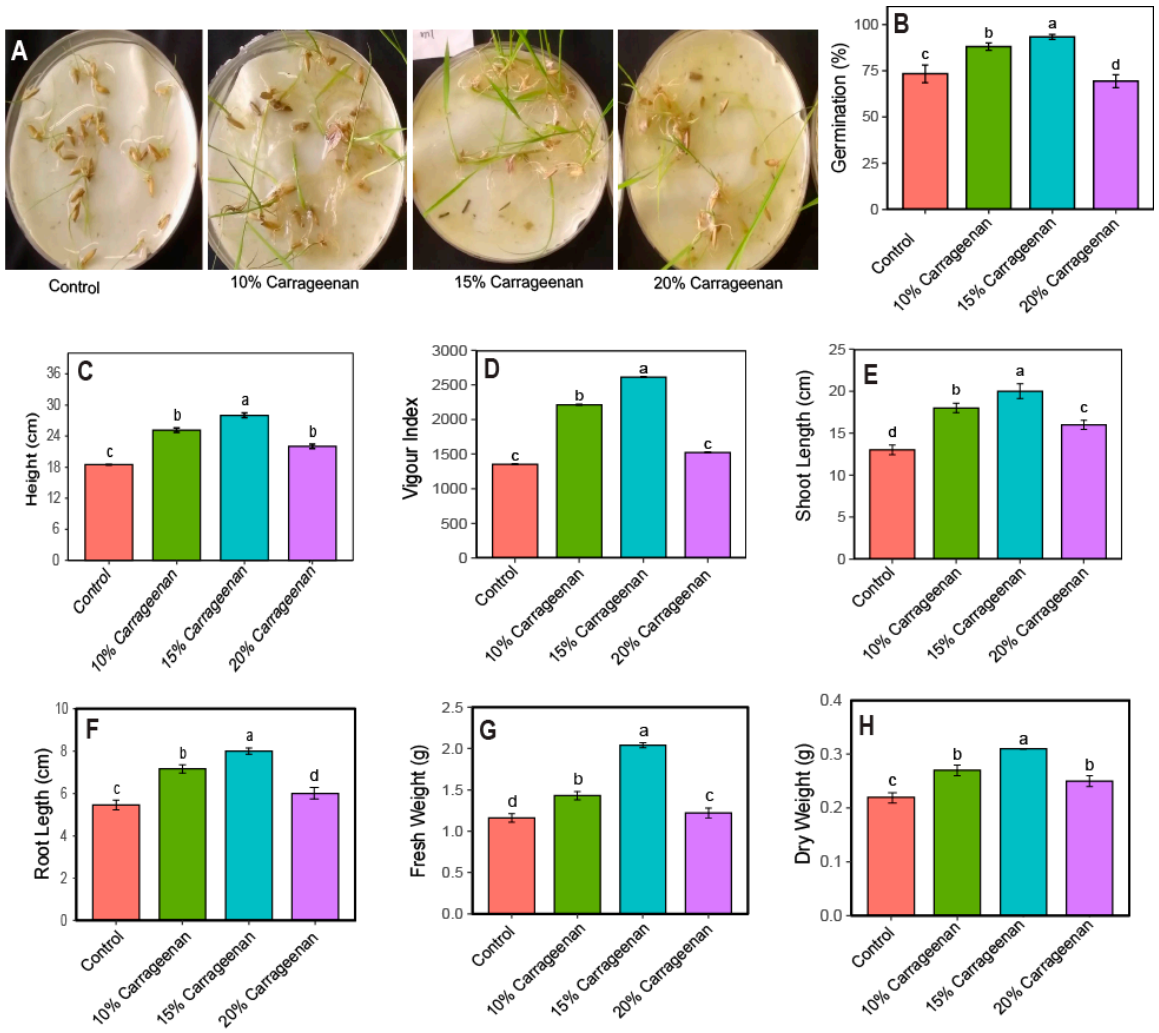

Figure 3. Effect of carrageenan application on seed germination, seedling growth, and biomass parameters of rice plants. (A) Seeds with water-treated (B), Seeds with 10% carrageenan, (C) Seeds with 15% carrageenan, (D) Seeds with 20% carrageenan, (E) Germination %, (F)Plant height, (G) Seedling vigour, (H) Shoot length (I) Root length (J) Fresh weight (K) Dry weight. Error bars represent the standard error of the mean. Different lowercase letters above the bars indicate statistically significant differences among treatments at $p \leq 0.05$ according to Fisher's LSD test.

3.3. Effects of Carrageenan on Mycelial Growth Inhibition of Magnaporthe oryzae

The result showed that all the concentrations of carrageenan and Nativo 75 WG significantly inhibited the hyphal growth of *Magnaporthe oryzae* on PDA plates (Figure 4). Nativo WG75 showed a higher inhibition rate of 90%. The 20% carrageenan concentration showed the inhibition at 83%, followed with 73.22% inhibition at 15% carrageenan concentration, while 100% carrageenan concentration showed only 30%, whereas the control plates exhibited no inhibition. Moreover, the fungal cell wall is a complex structure that plays a key role in determining cell shape. The microscopic data showed that untreated control samples produced polar, cylindrical hyphae that were smooth, hyaline, branching, plump, septate, and unbroken. In contrast, carrageenan and fungicide treatments caused irregular hyphal growth with a higher branching rate per unit length.

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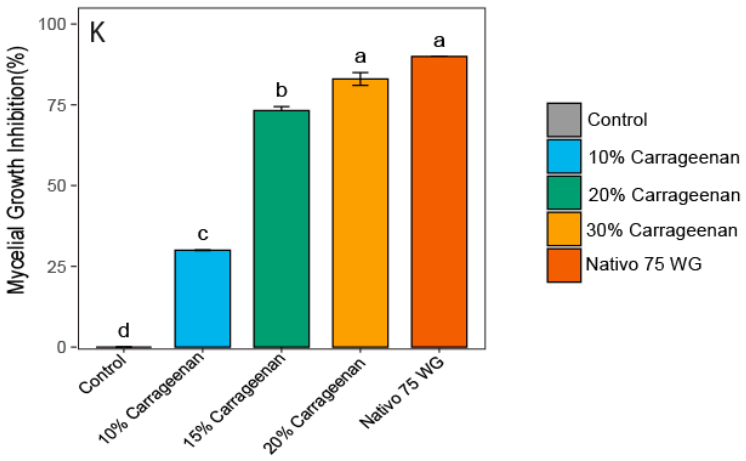

Figure 4. Effect of carrageenan treatments on the inhibition of mycelial growth of *Magnaporthe oryzae*. Bars represent percentage inhibition under different treatments: control, 10%, 15%, 20%, and 30% carrageenan, and the fungicide Nativo 75 WG. Error bars indicate the standard error of the mean. Different lowercase letters above bars denote statistically significant differences among treatments at $p \leq 0.05$ according to Fisher's LSD test.

3.4. Effects of Carrageenan on the Inhibition of Conidial Germination

The germination of conidia by *Magnaporthe oryzae* was significantly reduced by all carrageenan and fungicide concentrations compared to the control (Table 1). The 10%, 15%, and 20% carrageenan concentrations, along with the fungicide Nativo WG75 at a concentration of 10 µg/ml, significantly reduced the conidial germination of *Magnaporthe oryzae*. All treatments demonstrated substantial inhibition after 24 hours compared to the control. No germination occurred in either the carrageenan or fungicide-treated groups after 6 hours, while the control group had a 13.80% germination rate. Similarly, after 12 and 24 hours, the control group exhibited 100% germination. The carrageenan concentrations and fungicide-treated group showed progressively lower germination rates of 32.03%, 38.34%, 69.04%, and 29.08% after 12 hours, respectively. In addition, the 20% carrageenan treatment exhibited the lowest germination at 17.12%, while the fungicide-treated group had 9.11% after 24 hours (Table 1). Microscopic examination also showed the presence of broken hyphal tips and the complete suppression of conidiophore formation in fungal colonies grown on Petri plates treated with these concentrations of carrageenan and fungicide (Figure 5).

Table 1. Effects of carrageenan on conidia germination and their subsequent developmental transitions of inhibition percentage of *Magnaporthe oryzae* in vitro.

Treatment	Time (h)	Effects of compounds on developmental transitions of conidia of rice blast fungus *M. oryzae*	
		Germinated conidia (%)	Major morphological change/developmental transitions in the treated conidia
Control	0	0.0 ± 0.00g	No germination
	6	13.80 ± 0.60e	No germination
	12	100.00 ± 00a	Germinated with a short germ tube and appressoria developed
	24	100.00 ± 00a	Fully developed germ tube.
T1 (10%)	0	0.0 ± 0.00g	No germination
	6	0.0 ± 0.00g	No germination

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	12	69.04 ± 0.50b	Germinated with 28.05%normal germ tube and 40.99% abnormal germ tube formation
	24	52.04 ± 0.30c	Abnormally long hyphae-like germ tube
T2 (15%)	0	0.0 ± 0.00g	No germination
	6	0.0 ± 0.00g	No germination
	12	38.34 ± 0.00d	Germinated with a short germ tube, and abnormal appressoria were formed
	24	31.64 ± 0.80d	19.65% normal germ tube and 12.03%abnormally elongated germ tube and lysed thereafter
T3 (20%)	0	0.0 ± 0.00g	No germination
	6	0.0 ± 0.00g	No germination
	12	32.03 ± 0.60 d	Germinated with an abnormally elongated germ tube
	24	17.12 ± 0.50 e	9.00% normal germ tube, 8.12%abnormally elongated germ tube and some conidia are lysed
Nativo ® WG75	0	0.0 ± 0.0 g	No germination
	6	0.0 ± 0.0 g	No germination
	12	29.08 ± 0.30 de	Germinated with a short germ tube.
	24	09.11 ± 0.70 f	Abnormally elongated germ tube and appressoria formed.

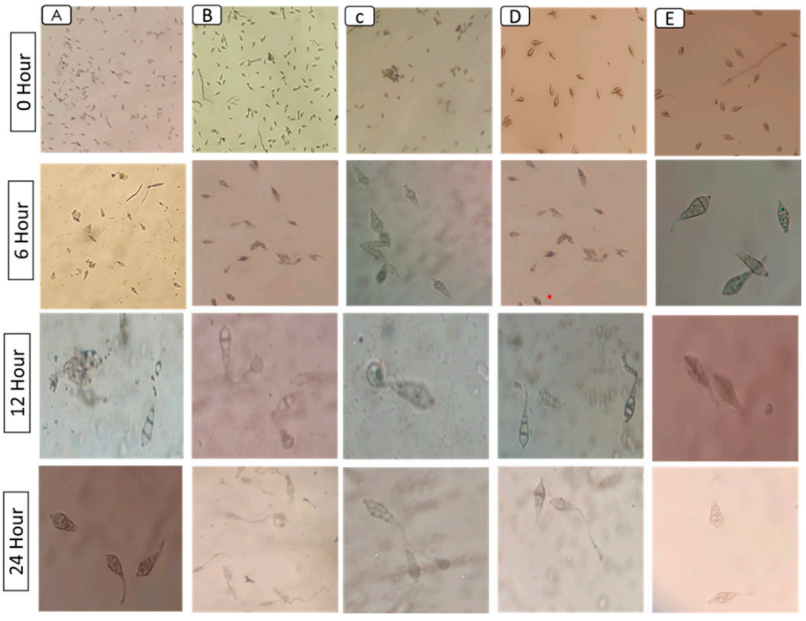

Figure 5. Effects of carrageenan on the inhibition of conidial germination in *Magnaporthe oryzae* at 0-hour, 6-hour, 12-hour, and 24-hour intervals (A) Control water treated, (B) Carrageenan concentration 10%, (C) Carrageenan concentration 15% and, (D) Carrageenan concentration 20% and (E) Fungicide Nativo WG75 at 10 µg/ml.

3.5. Inhibition of Rice Blast Disease in Detached Leaves

The application of 10%, 15% and 20% carrageenan concentrations and 10 µg/mL Nativo 75 WG significantly inhibited rice blast symptoms in detached leaves inoculated with the *Magnaporthe oryzae* (Figure 6A-B). The average lesion lengths in leaves pretreated with carrageenan were 4.17 mm for 10% carrageenan concentration, 2.95 mm for 15% carrageenan concentration, and 2.71 mm for 20% carrageenan concentration. However, water-treated control leaves displayed typical blast lesions

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with an average length of 5.92 mm. Notably, rice leaves treated with Nativo WG75 at 10 µg/mL showed no blast symptoms.

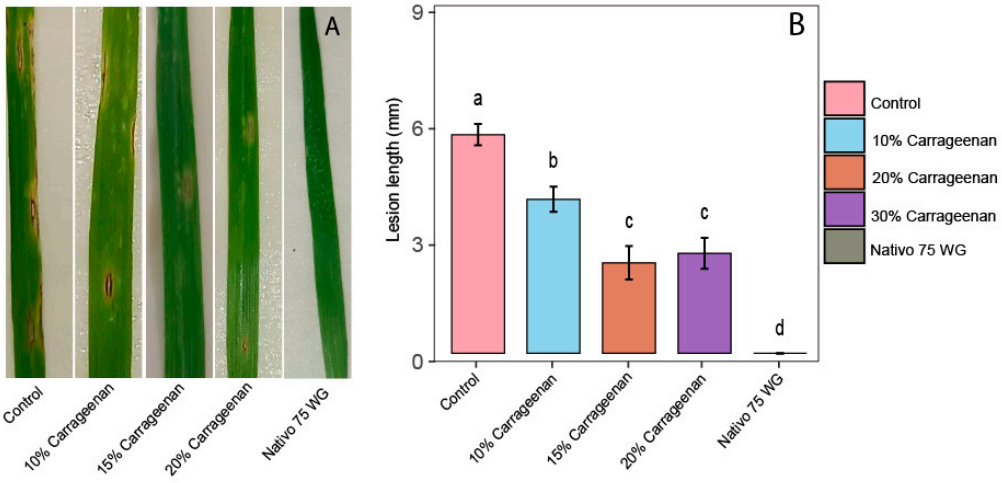

Figure 6. Effect of carrageenan treatments on rice leaf blast symptom development and lesion length caused by *Magnaporthe oryzae* in detached leaf assay. (A) Representative images of rice leaves under different treatments: control, 10% carrageenan, 15% carrageenan, 20% carrageenan, and Nativo 75 WG. (B) Quantitative assessment of average lesion length (mm) in leaves treated with carrageenan (10%, 15% and 20%), Control and Nativo 75 WG. Error bars represent the standard error of the mean. Different lowercase letters above bars indicate significant differences among treatments at $p \leq 0.05$ according to Fisher's LSD test.

3.6. Suppression of Rice Blast Disease in Pot Assay

The study evaluated the potential of carrageenan to control rice blast disease in pot culture (Figure 7). The control plants exhibited the highest disease severity (3.4), indicating substantial infection. However, all treatments significantly reduced disease severity compared to the control. Application of 10% carrageenan resulted in a moderate but significant reduction in disease severity (2.8), suggesting partial protection. Further reductions were observed at higher concentrations, with 15% and 20% carrageenan treatments showing lower disease scores (2.3–2.5), indicating enhanced suppression of disease progression. The fungicide treatment (Nativo 75 WG) showed the strongest effect, with the lowest disease score (1.6), significantly lower than all carrageenan treatments. These results indicate that while 15% and 20% carrageenan performed similarly, they were more effective than 10% carrageenan but less effective than the fungicide.

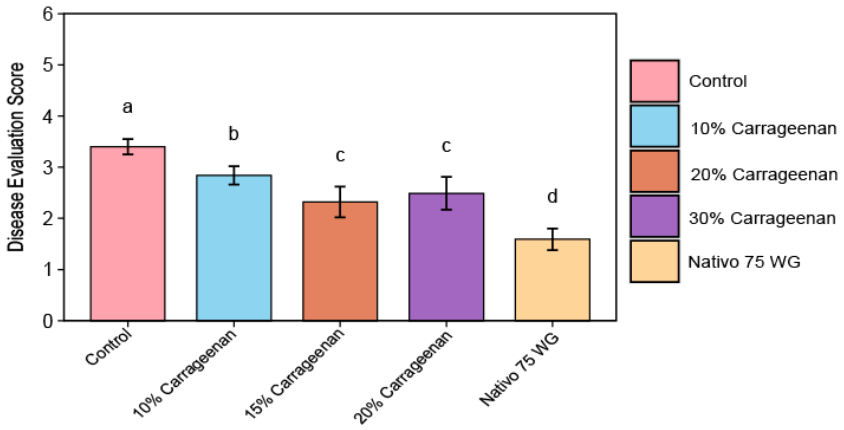

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Figure 7. Effect of carrageenan treatments on rice blast disease severity caused by *Magnaporthe* oryzae in pot assays. Disease evaluation scores were recorded under control, 10%, 15%, and 20% carrageenan treatments, and the fungicide Nativo 75 WG. Error bars represent the standard error of the mean. Different lowercase letters above the bars indicate statistically significant differences among treatments at $p \leq 0.05$ according to Fisher's LSD test.

3.8. Carrageenan Application Improves Photosynthetic Pigment Levels in Rice Leaves Under Rice Blast Disease Conditions

Rice plants inoculated with M. oryzae resulted in the lowest concentrations of chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoids, highlighting the negative effect of the fungal infection on pigment accumulation. Among the treatments, the highest chlorophyll *a* concentration was observed in 10% carrageenan concentration, which exhibited 1.62 mg g^{-1} FW, significantly higher than the control at 0.99 mg g^{-1} FW. Chlorophyll *a* levels were also higher in the fungicide treatment (1.46 mg g^{-1} FW) compared to the control group (Figure 8A). Chlorophyll *b* concentration was lower in the control (0.14 mg g^{-1} FW) but increased to 0.60 mg g^{-1} FW in the fungicide treatment. Among the carrageenan treatments, 15% carrageenan concentration showed the highest chlorophyll *b* concentration (1.11 mg g^{-1} FW), followed by 20% carrageenan concentration at 1.01 mg g^{-1} FW, and 10% carrageenan concentration at 0.42 mg g^{-1} FW (Figure 8B). Furthermore, 15% carrageenan concentration displayed the highest total chlorophyll and carotenoid levels (Figure 8C-D), with 2.87 mg g^{-1} FW and 180.99 mg g^{-1} FW, respectively. These values were notably higher than those of the fungicide treatment, which had 2.15 mg g^{-1} FW for total chlorophyll and 142.40 mg g^{-1} FW for carotenoids.

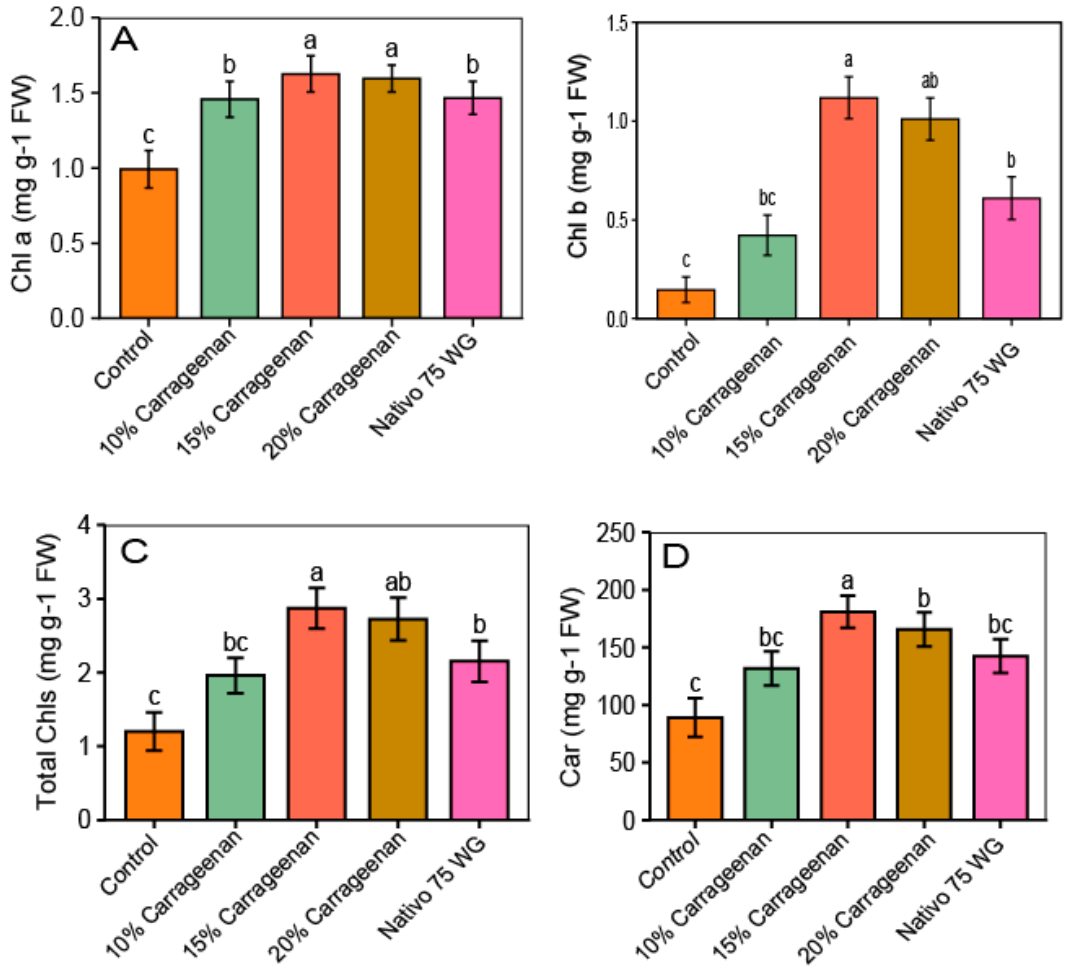

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Figure 8. Effect of carrageenan and Nativo 75 WG treatments on photosynthetic pigment contents in rice plants inoculated with *Magnaporthe oryzae* (A) Chl *a* (Chlorophyll *a*), (B) Chl *b* (Chlorophyll *b*), (C) Total Chls (total chlorophyll), and (D) Car (Carotenoids). Infection in the untreated control resulted in the lowest levels of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids, indicating reduced pigment accumulation due to fungal stress. Error bars represent the standard error of the mean. Different lowercase letters above the bars indicate statistically significant differences among treatments at $p \leq 0.05$ according to Fisher's LSD test.

3.9. Carrageenan Application Reduced Oxidative Damage in Rice Leaves under Rice Blast Disease Conditions

Leaves of rice plants inoculated with M. oryzae showed the highest levels of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) over time (Figure 9). At day 0, all treatments had similar H_2O_2 and MDA levels statistically. In control plants, H_2O_2 and MDA were 874.77 nmol g^{-1} FW and 12.92 µmol g^{-1} FW on day 3, increasing to 889.59 nmol g^{-1} FW and 17.90 µmol g^{-1} FW by day 6, and reaching 951.44 nmol g^{-1} FW and 23.13 µmol g^{-1} FW by day 9, indicating a progressive rise in oxidative stress. In contrast, M. oryzae-inoculated plants treated with 15% carrageenan showed the lowest H_2O_2 and MDA levels. On day 3, values (442.18 nmol g^{-1} FW H_2O_2; 13.97 µmol g^{-1} FW MDA) were comparable to fungicide-treated plants (434.77 nmol g^{-1} FW; 12.92 µmol g^{-1} FW). By day 6, levels declined to 426.25 and 7.88 µmol g^{-1} FW, respectively, similar to fungicide treatment (417.00 and 7.07 µmol g^{-1} FW). By day 9, both treatments showed further reductions, with carrageenan-treated plants at 340.70 nmol g^{-1} FW H_2O_2 and 6.81 µmol g^{-1} FW MDA, and fungicide-treated plants at 330.33 nmol g^{-1} FW and 6.02 µmol g^{-1} FW.

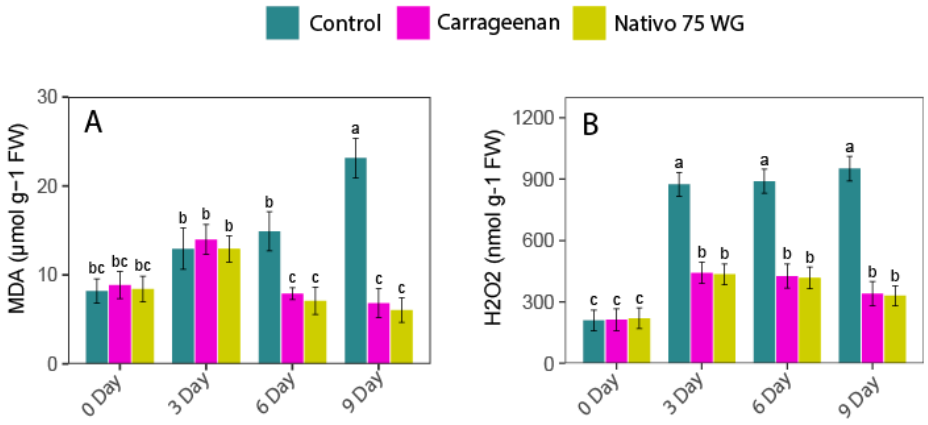

Figure 9. Effect of carrageenan and Nativo 75 WG treatments on oxidative stress markers in rice leaves inoculated with *Magnaporthe oryzae*, measured as hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) over time (A) MDA and (B) H_2O_2. Untreated control plants exhibited a progressive increase in both markers over time, indicating enhanced oxidative stress due to infection. In contrast, carrageenan-treated plants, particularly at the intermediate concentration, showed significantly reduced accumulation of H_2O_2 and MDA, with values comparable to fungicide-treated plants. Error bars represent the standard error of the mean. Different lowercase letters above the bars indicate statistically significant differences among treatments at $p \leq 0.05$ according to Fisher's LSD test.

3.10. Carrageenan Application Improved the Levels of Osmoprotectants in Rice Leaves under Rice Blast Disease Conditions

Proline and soluble sugar levels significantly decreased in rice plants infected with M. oryzae, but this reduction was reversed by carrageenan and fungicide treatments (Figure 10). Among the three sampling intervals, both proline and soluble sugar contents were highest on the 9th day in treated and fungicide-applied plants, and lowest in the control. Before inoculation, proline and soluble sugar contents were statistically similar across the control (4.32 µmol g^{-1} FW; 1.32 mg g^{-1} FW),

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treatment (4.12 µmol g^{-1} FW; 1.13 mg g^{-1} FW), and fungicide (4.19 µmol g^{-1} FW; 1.00 mg g^{-1} FW) groups. By day 3 after inoculation, levels remained statistically comparable among control (6.77 µmol g^{-1} FW; 2.01 mg g^{-1} FW), treatment (9.53 µmol g^{-1} FW; 2.11 mg g^{-1} FW), and fungicide (10.67 µmol g^{-1} FW; 2.47 mg g^{-1} FW). However, by day 6, both parameters increased significantly in carrageenan-treated (14.99 µmol g^{-1} FW; 3.25 mg g^{-1} FW) and fungicide-treated plants (24.11 µmol g^{-1} FW; 4.98 mg g^{-1} FW) compared to the control (2.51 µmol g^{-1} FW; 1.00 mg g^{-1} FW). By day 9, carrageenan-treated (18.96 µmol g^{-1} FW; 5.84 mg g^{-1} FW) and fungicide-treated plants (17.02 µmol g^{-1} FW; 7.58 mg g^{-1} FW) showed the highest levels, while the control remained lowest (2.35 µmol g^{-1} FW; 0.88 mg g^{-1} FW).

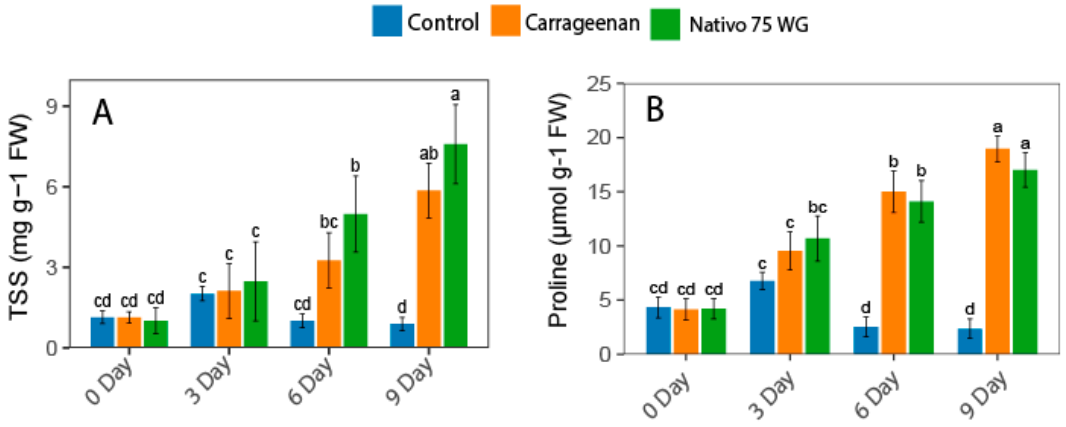

Figure 10. Effect of carrageenan and fungicide treatments on proline and soluble sugar contents in rice plants inoculated with *Magnaporthe oryzae* over time. (A) TSS and (b) Proline. Carrageenan application mitigates the adverse effects of *M. oryzae* infection by promoting osmoprotectant accumulation. Error bars represent the standard error of the mean. Different lowercase letters above the bars indicate statistically significant differences among treatments at $p \leq 0.05$ according to Fisher's LSD test.

3.11. Carrageenan Application Enhanced Antioxidant Defense Responses in Rice Leaves under Rice Blast Disease Conditions

To assess the effect of carrageenan on antioxidant defense regulation, we measured total flavonoids, phenolic compounds, overall antioxidant content, and the activities of key enzymes CAT and POD (Figure 11 A–E). Before inoculation with M. oryzae, phenolic and flavonoid contents were similar across treatments. Control plants contained 0.83 mg g^{-1} FW phenolics and 2.01 mg g^{-1} FW flavonoids, while carrageenan-treated plants (15%) had 0.78 and 2.00 mg g^{-1} FW, respectively. Fungicide-treated plants showed slightly higher values (0.84 and 2.19 mg g^{-1} FW). By day 6 post-inoculation, both carrageenan and fungicide treatments significantly increased phenolic and flavonoid contents compared to the control. Carrageenan-treated plants reached 1.18 and 3.11 mg g^{-1} FW, and fungicide-treated plants 1.76 and 3.01 mg g^{-1} FW, while the control remained lower (0.78 and 1.92 mg g^{-1} FW). By day 9, the highest levels were observed in treated plants: carrageenan (2.19 and 3.38 mg g^{-1} FW) and fungicide (2.12 and 3.36 mg g^{-1} FW), both significantly exceeding the control (0.40 and 1.70 mg g^{-1} FW) (Figure 11A-B).

Catalase and peroxidase showed significantly higher activity in carrageenan- and fungicide-treated plants compared to untreated controls (Figure 11D-E). 15% carrageenan concentration notably enhanced enzyme activity after inoculation. The highest CAT and POD activities were recorded in carrageenan-treated plants (177.32 and 2.89 µmol min^{-1} mg^{-1} protein) and fungicide-treated plants (180.11 and 1.67 µmol min^{-1} mg^{-1} protein), compared to the control (59.78 and 0.41 µmol min^{-1} mg^{-1} protein). Before inoculation, enzyme activities were similar across all groups. Over time, CAT and POD activities increased in treated plants but declined in controls.

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Antioxidant activity was further evaluated using the DPPH assay. A clear relationship was observed between antioxidant concentration and free radical scavenging activity, with higher antioxidant levels corresponding to greater DPPH reduction (Figure 11C). Control plants exhibited significantly higher DPPH levels, indicating lower scavenging activity, than carrageenan- and fungicide-treated plants. By day 9, the lowest DPPH values were observed in carrageenan (48.58 µg/ml FW) and fungicide-treated plants (43.32 µg/ml FW), while the control showed the highest level (148.00 µg/ml FW). Before inoculation, DPPH levels were similar across treatments. Over time, DPPH increased in controls but decreased in treated plants, indicating enhanced scavenging activity in the latter.

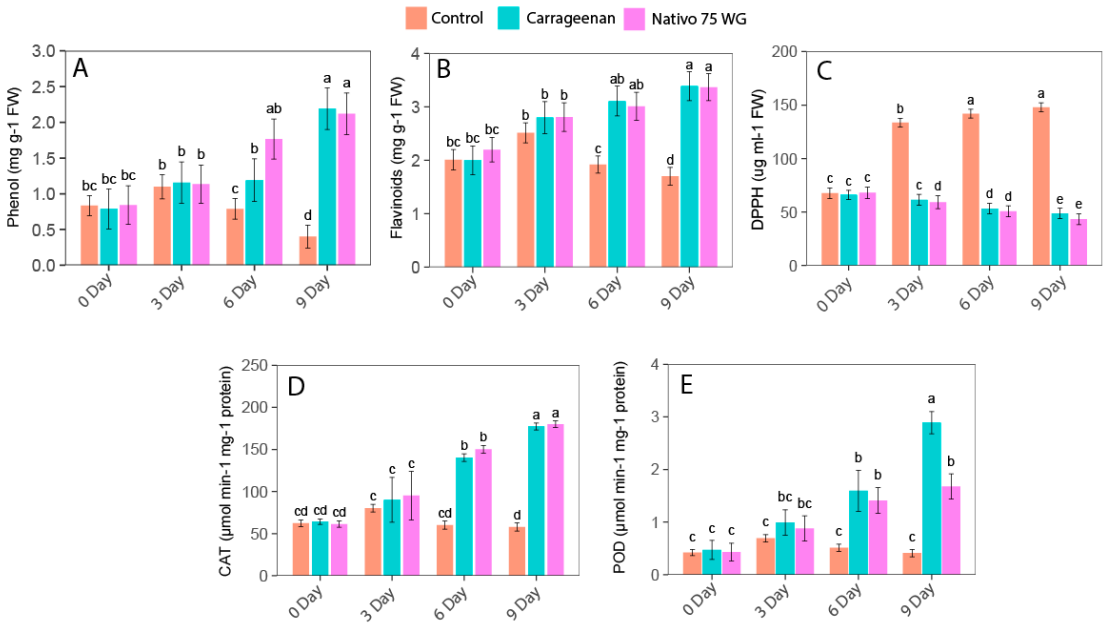

Figure 11. Effect of carrageenan and fungicide treatments on antioxidant defense responses in rice plants inoculated with *Magnaporthe oryzae*, including total phenolic and flavonoid contents, antioxidant activity, and enzyme activities (CAT and POD) (A) Phenol and (B) flavonoids (C) Total Antioxidant activity (DPPH) (D) CAT and (E) POD. Carrageenan-treated plants exhibited enhanced phenolic and flavonoid contents, increased catalase and peroxidase activities, and improved free radical scavenging capacity, as indicated by lower DPPH values compared to the fungicide treatment. Error bars represent the standard error of the mean. Different lowercase letters above the bars indicate statistically significant differences among treatments at $p \leq 0.05$ according to Fisher's LSD test.

4. Discussion

In recent years, phytopathogens have caused substantial yield losses in economically and agriculturally important crops. Consequently, considerable attention has been directed toward identifying new classes of antipathogenic compounds that are environmentally safe yet effective for crop protection (Hossain et al., 2024). However, options for directly controlling phytopathogens using conventional chemical approaches remain limited. In this context, seaweed-derived carrageenans have emerged as promising candidates for sustainable disease management.

Our findings demonstrate that carrageenan possesses a distinctive composition of sugars, silver, and sulfate (Figure 1). The polysaccharide-enriched extracts (PEEs) from *Ulva lactuca* exhibited notably higher concentrations of total sugars, including glucose, maltose, and galactose, whereas PEEs from *Fucus spiralis* contained comparatively lower sugar levels. Overall, this compositional analysis indicates that the extracted compounds predominantly consist of neutral sugars and sulfate groups (Mzibra et al., 2018). Furthermore, FT-IR spectral analysis confirmed the presence of

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characteristic functional groups such as sulfate esters, 3,6-anhydrogalactose, and D-galactose-4-sulfate, which are typical structural features of carrageenan (Figure 2). Specifically, the band at 1220–1226 cm^{-1} corresponds to sulfate esters, while bands at 926–930 cm^{-1} and 845 cm^{-1} are associated with agarose and D-galactose-4-sulfate. Additional peaks at 3000–3600 cm^{-1}, 2800–2900 cm^{-1}, 1650 cm^{-1}, 1550 cm^{-1}, 1200 cm^{-1}, and 950 cm^{-1} correspond to O–H stretching, lipid content, primary amines, and carbohydrates, respectively (Chopin et al., 1999; Ghannam et al., 2013; Ramlov et al., 2019).

Carrageenan significantly enhanced rice growth attributes, including seed germination, seedling vigor, plant height, shoot and root length, and both fresh and dry biomass compared to the control (Figure 3A–H), highlighting its potential as a natural growth promoter. These findings are in agreement with previous studies demonstrating carrageenan-induced growth enhancement across multiple crops. For instance, carrageenan increased chickpea plant height and yield-related traits (Bi et al., 2011), while λ-carrageenan improved growth in infected plants (Sangha et al., 2015). Similarly, extracts of *Ulva lactuca* improved biomass accumulation (Castellanos-Barriga et al., 2017), and combined extracts of *Ulva lactuca* and *Caulerpa scalpelliformis* enhanced germination and seedling growth in green gram (Kavipriya et al., 2011). Collectively, these results support the role of seaweed-derived polysaccharides, particularly carrageenan, as effective natural elicitors of plant growth and development.

Carrageenan exhibited strong antifungal activity, as all tested concentrations inhibited *Magnaporthe oryzae* hyphal growth on PDA plates, with Treatment 3 showing the most pronounced effect (Figure 4). This dose-dependent inhibition is consistent with previous studies reporting the suppressive effects of carrageenan on diverse phytopathogenic fungi under both in vitro and greenhouse conditions (Machado et al., 2019; Paulert et al., 2009). In contrast, laminarin has been reported to enhance mycelial growth relative to untreated controls (Ben Salah et al., 2018). Microscopic observations in the present study revealed distorted, highly branched, and irregular hyphal structures in treated samples, suggesting disruption of fungal cell wall integrity. Such morphological abnormalities are typically associated with antifungal agents that interfere with cell wall synthesis or membrane permeability (Latgé, 2007).

The suppression of conidial germination is particularly critical, as these processes are essential for pathogen dissemination and infection. Carrageenan treatments completely inhibited conidial germination at early time points (6 h) and significantly reduced germination at later stages, with only 17.12% germination observed at 24 h under 20% carrageenan treatment. Additionally, abnormal germ tube development and conidial lysis indicate that carrageenan disrupts cellular differentiation and membrane stability (Figure 5A–E; Table 1). Comparable results have been reported for chitosan–carrageenan nanocomposites, which inhibited 83.1% of *Alternaria solani* germination, comparable to mancozeb (84.6%) and completely suppressed *Sclerotinia sclerotiorum* at 1.0–1.5 ppm (Kumar et al., 2021). Similarly, *Halymenia floresii* extracts inhibited *Pseudocercospora fijiensis*, preventing germination for up to 30 days (Gómez-Hernández et al., 2021).

In planta assays further demonstrated that carrageenan significantly reduced lesion length in detached leaves and disease severity in pot experiments. The reduction in lesion length from 5.92 mm in control plants to 2.71 mm in treated plants confirms its protective role (Figure 6A–B). Although Nativo completely suppressed disease symptoms, carrageenan still exhibited substantial efficacy. Notably, 15% carrageenan performed best under pot conditions (Figure 7), suggesting that moderate concentrations may optimize plant physiological responses. These findings are consistent with previous studies; for example, Sangha et al. (2015) reported that carrageenans reduced Tomato Chlorotic Dwarf Viroid symptoms, with λ-carrageenan being the most effective. Similar reductions in disease severity have been reported using extracts of *Ulva lactuca*, *Sargassum filipendula*, and *Gelidium serrulatum* (Ramkissoon et al., 2017), as well as Kappaphycus and Eucheuma biostimulants against rice blast (Sahana et al., 2022).

The observed increase in photosynthetic pigments—including chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids—indicates enhanced photosynthetic efficiency and overall plant performance (Figure 8A–D). Similar increases in chlorophyll content following seaweed extract

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application have been reported under optimal conditions (Mannan et al., 2023; Yao et al., 2020; Thye et al., 2022).

Pathogen infection typically induces oxidative stress, as reflected by elevated levels of H_2O_2 and malondialdehyde (MDA) in control plants. In contrast, carrageenan-treated plants exhibited significantly reduced levels of these oxidative stress markers, indicating effective mitigation of cellular damage (Figure 9A–B). These findings are consistent with Farahmand and Nasibi (2023), who reported reductions in ion leakage, MDA, and H_2O_2 following carrageenan application. Overall, these results suggest that carrageenan enhances plant resilience by alleviating oxidative stress. Moreover, carrageenan treatment significantly increased proline and soluble sugar accumulation under pathogen stress. These osmoprotectants play a vital role in maintaining cellular integrity and osmotic balance under stress conditions (Cai et al., 2013; Gupta and Huang, 2014). The elevated levels observed at later stages (day 9) suggest that carrageenan induces sustained stress tolerance mechanisms (Figure 10A–B).

The study also revealed a strong induction of antioxidant defense systems in carrageenan-treated plants. Increased levels of phenolics, flavonoids, and antioxidant activity (DPPH assay) indicate enhanced secondary metabolism. Additionally, significant increases in catalase (CAT) and peroxidase (POD) activities confirm the activation of enzymatic antioxidant defenses (Figure 11A–E). These findings align with previous reports linking elevated phenolic and flavonoid content to enhanced antifungal activity (Elansary et al., 2016). Similarly, seaweed-derived compounds have been shown to upregulate defense-related enzymes and genes (Panjehkeh & Abkhoo, 2016; Abouraïcha et al., 2017; Bajpai et al., 2019; Banakar et al., 2022). The strong DPPH scavenging activity observed in carrageenan-treated plants is attributed to its hydrogen-donating capacity and sulfate-rich polysaccharide structure (das Chagas Faustino Alves et al., 2012), as well as contributions from protein-mediated electron donation (Bradford, 1976; Kim et al., 2012). The decline in DPPH levels in treated plants after inoculation further indicates enhanced free radical scavenging, whereas higher levels in control plants reflect increased oxidative stress (Fenglin et al., 2004; Ghaisas et al., 2008).

Overall, these findings demonstrate that carrageenan enhances plant growth, strengthens antioxidant defense systems, and suppresses disease progression. This highlights its significant potential as a sustainable biostimulant for improving crop productivity and resilience against pathogen-induced stress.

5. Conclusions

The study establishes carrageenan derived from the red seaweed *H. musciformis* as a promising bioelicitor for sustainable rice production. Among the tested concentrations, 15% carrageenan exhibited the most pronounced effects, significantly enhancing seed germination, seedling vigor, biomass accumulation, and photosynthetic pigment content while concurrently suppressing *Magnaporthe oryzae* growth, conidiogenesis, and lesion development. The treatment further improved non-enzymatic and enzymatic antioxidant defenses and reduced oxidative stress markers, underscoring its role in both growth promotion and induction of host resistance. These findings highlight the potential of carrageenan as an eco-friendly alternative to synthetic fungicides for the integrated management of rice blast disease. Future investigations under field conditions, alongside studies on formulation stability and application strategies, are warranted to validate its scalability and efficacy in diverse agro-ecological contexts.

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