

Chlorella sorokiniana aqueous extract suppresses Magnaporthe oryzae and rice blast disease

Ramona Mihaela Ciubotaru

InnovPlantProtect - Associação

Miguel Claro

InnovPlantProtect - Associação

Pedro Rosa

InnovPlantProtect - Associação

Rui Figueiras

InnovPlantProtect - Associação

Aghata C.R. Charnobay

InnovPlantProtect - Associação

Catarina Viana

University of Algarve

Samira Andrade

Fertiprado

Beatriz Duarte

InnovPlantProtect - Associação

Luísa Coelho

GreenColab- Associação Oceano Verde

Sandra Correia

InnovPlantProtect - Associação

Cristina Azevedo

cazevedo@iplantprotect.pt

InnovPlantProtect - Associação

Research Article

Keywords: microalgae, Chlorella sp., biological control, biofungicide, phytopathogenic fungi, Rice blast

Posted Date: October 14th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11131364/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Rice blast, caused by *Magnaporthe oryzae*, is among the most destructive rice diseases worldwide and remains a major constraint to sustainable rice production. Its importance extends to Europe, including Portugal, the fourth-largest rice-producing country in the European Union, where rice blast remains a major disease threat. Developing effective biobased alternatives to conventional fungicides is therefore relevant for more sustainable management of this disease.

This study evaluated the biocontrol potential of an aqueous extract derived from *Chlorella sorokiniana* biomass against *M. oryzae* using complementary *in vitro*, *ex vivo*, and *in planta* assays. The extract inhibited mycelial growth *in vitro*, reaching 66–79% inhibition at 0.1 g L⁻¹, whereas inhibition decreased to 38–45% at the highest concentration tested (1.5 g L⁻¹). At 0.2 g L⁻¹, the extract reduced appressorium formation from 75–96%, indicating an effect on a key infection-related process. This concentration also reduced lesion development on detached rice leaves by more than 75% and decreased rice blast severity by 64.4% following preventive foliar application.

Overall, the results show that a *C. sorokiniana*-derived aqueous extract can suppress *M. oryzae* at multiple stages of disease development, with direct effects on fungal growth and appressorium formation associated with reduced disease severity. These findings provide proof of concept for using microalgae biomass-derived products as biobased tools for rice blast management. Further studies should identify the compounds responsible for the observed activity, elucidate their modes of action, and evaluate efficacy and formulation under field conditions.

1. Introduction

Plant diseases caused by fungi and oomycetes are major constraints to agricultural production and are estimated to account for global yield losses of 20–40% (Agrios, 2005; Savary et al., 2019). Among these pathogens, *Magnaporthe oryzae*, the causal agent of rice blast, is one of the most important fungal pathogens affecting food crops (Dean et al., 2012; Kamoun et al., 2014). Rice is the primary dietary staple for more than half of the world's population, and rice blast can cause yield losses of 10–35%, with considerably greater losses reported under severe disease outbreaks (Dean et al., 2012; Asibi et al., 2019). The disease therefore represents an important threat to rice production and food security. Rice blast is also relevant to European rice production, including in Portugal, where rice is cultivated mainly in the major river basins and where *M. oryzae* has been reported across the principal rice-growing regions (Rosa et al., 2026). Disease management relies largely on resistant cultivars and fungicides. However, changes in pathogen populations can reduce the effectiveness of host resistance, and repeated fungicide use has contributed to the selection of resistant *M. oryzae* populations and raised concerns about environmental impacts (Carvalho, 2017; Pathak et al., 2022). The European Green Deal and the Farm to Fork Strategy have consequently placed greater emphasis on reducing the use and risk of chemical pesticides, creating a need for effective alternatives that can be incorporated into disease-management programmes (Jacquet et al., 2022).

Biological control is one such approach, and researchers have extensively investigated bacterial and fungal antagonists for managing plant diseases. Several microbial biocontrol products are already available, although environmental conditions may be inconsistent in the field (Köhl et al., 2019; Palmieri et al., 2022). In addition to living microbial agents, researchers are investigating metabolites and extracts from biological sources for their direct effects on pathogens and their capacity to stimulate plant defence. Microalgae are of particular interest because they contain a diverse range of compounds with antimicrobial and plant-biostimulant properties (Khan et al., 2009). Polysaccharides, fatty acids, phenolic compounds, peptides, and terpenoids in microalgal biomass have been associated with inhibiting pathogen growth and modulating plant defence responses (Vehapi et al., 2020; Righini et al., 2022; Bhardwaj et al., 2025). Microalgae can also be cultivated rapidly on relatively limited agricultural land and, depending on the production system, on non-arable resources, supporting their use as a biomass source for agricultural applications (Spolaore et al., 2006; Colla and Rouphael, 2020).

Among green microalgae, *Chlorella sorokiniana* has received considerable attention because of its rapid growth and diverse biochemical composition. Its biomass contains fatty acids, pigments, and other metabolites that may contribute to biological activity. Lio et al. (2025) used GC-MS to identify several bioactive compounds in *C. sorokiniana* extracts and correlated their presence with antimicrobial activity, reporting fatty acids as major biomass constituents. More recent analyses have identified palmitic, oleic, linoleic, and α -linolenic acids as the major fatty acids in *C. sorokiniana* biomass (Banskota et al., 2024; Papapanagiotou et al., 2025). Some of these compounds have known antimicrobial properties and may affect microorganisms by disrupting membrane integrity or interfering with cellular metabolism; however, the compounds responsible for the antifungal activity of *C. sorokiniana* biomass extracts remain unidentified (Lio et al., 2025). The antifungal activity of *Chlorella* species have been reported against several phytopathogenic fungi, including *Fusarium oxysporum*, *Colletotrichum acutatum*, and *Alternaria alternata* (Ferreira et al., 2021; Schmid et al., 2022), while related *Chlorella* species have also shown disease-suppressive effects against *F. oxysporum* under plant conditions (Viana et al., 2024) and can induce plant defence responses (Toribio et al., 2021; Lee et al., 2020). Similarly, Jokel et al. (2023) reported strong biopesticide activity of a *C. sorokiniana* extract against *Phytophthora cactorum* on detached strawberry leaves. These findings indicate that *C. sorokiniana* contains compounds with potential activity against plant pathogens, but its effects on *M. oryzae* and the rice blast disease process remain poorly understood.

The well-characterised infection process of *M. oryzae* provides several stages at which bioactive compounds can interfere with disease development, enabling antifungal evaluation. After deposition on the rice surface, conidia germinate and differentiate into an appressorium, a specialised infection structure that generates the mechanical force required to penetrate the host cuticle. Successful appressorium formation and maturation are therefore essential for infection and subsequent lesion development (Chakraborty et al., 2021). Assessing both fungal growth and appressorium formation can therefore indicate whether a treatment affects fungal biomass and/or interferes with an infection-related developmental process. In this study, we evaluated the potential biocontrol activity of an aqueous extract derived from *C. sorokiniana* biomass against *M. oryzae* using complementary *in vitro*, *ex vivo*, and *in*

planta assays. We investigated the extract's effects on fungal growth and appressorium formation and determined whether these effects were associated with reduced lesion development on detached rice leaves and reduced rice blast severity after preventive foliar application. This approach assessed whether the extract's direct activity against *M. oryzae* could translate into protection against rice blast and to provide a basis for further investigation of *C. sorokiniana* biomass as a source of biobased products for disease management.

2. Materials and Methods

2.1. Microalgae biomass

C. sorokiniana dry biomass used in this study was supplied by Allmicroalgae Natural Products S.A. (Leiria, Portugal), batch L201910079, and was produced in accordance with the company's established industrial protocols. According to the supplier, after cultivation, the biomass was harvested by membrane filtration and spray-dried to obtain a fine powder, which was stored in sealed bags containing an oxygen absorber until use, with a storage duration of 3 years. The authors did not have access to detailed cultivation conditions, spray-drying parameters, or particle-size distribution. All *in vitro* and *in planta* preparations used biomass from the same *C. sorokiniana* production batch; however, the authors prepared extract stocks separately for each experiment rather than using a single common stock across all assays. We prepared extracts for each assay according to the preparation and application methods detailed in the respective experimental sections below.

2.1.1. Preparation of *Chlorella sorokiniana* aqueous extracts for *in vitro* assays

Aqueous extracts of *C. sorokiniana* biomass were prepared following an extraction workflow adapted from Schmid et al. (2022). Briefly, we prepared stock suspensions (10 g L⁻¹, w/v) by dispersing dried algal biomass in distilled or Milli-Q water in Erlenmeyer flasks or centrifuge tubes, depending on the experimental setup, and kept them under continuous agitation at 350 rpm for 12 h in the dark. After extraction, suspensions underwent two consecutive centrifugation steps (905–950 RCF) to remove particulate material. The recovered supernatants were clarified by sequential filtration through filters of progressively smaller pore sizes: 20, 6–10, and 4–7 µm (150 mm diameter; LabKit Prat Dumas A015107, A015613, and A015110, respectively).

For the *in vitro* inhibition assays against *M. oryzae*, the clarified aqueous algal extracts were sterilised under a laminar-flow hood by filtration through a sterile 0.2 µm membrane filter. To ensure reproducibility across the study, each extract was characterised by measuring pH and quantifying total protein and phenolic content. Approximately 500 µL of each sample was used for this characterisation, while the remaining extract was aliquoted into 96-well deep-well plates and stored at – 20°C until further use.

To determine the total protein content of the *C. sorokiniana* extracts, the Thermo Scientific kit: Pierce™ BCA Protein Assay Kit was used (He, 2011). Phenolic compound content was determined using the Folin-Ciocalteu method (Velioglu et al., 1998). The average of both protein content and phenolic compounds was determined by preparing three independent extracts as described above.

2.1.2. Preparation of *Chlorella sorokiniana* aqueous extracts for *in planta* assays

C. sorokiniana aqueous extract stocks were prepared for *in planta* assays by dispersing dried algal biomass in water and agitating at 350 rpm for 12 h in the dark. Extractions were performed in centrifuge tubes, with two glass beads in each tube to promote mechanical disruption of the algal biomass during agitation; no additional mechanical or chemical cell-disruption treatment was applied. Following extraction, the biomass was allowed to settle, and the supernatant was collected. No further centrifugation or filtration was performed before plant application.

The treatment concentrations were chosen based on the activity observed in the initial *in vitro* assay. Stock suspensions were prepared at 10 g L⁻¹ and used at a working concentration of 0.2 g L⁻¹ for the plant-associated assays.

2.2. *In vitro* *Magnaporthe oryzae* growth inhibition assays

Supplementary Table 1 summarises the main experimental conditions, treatment concentrations, incubation parameters, and evaluation criteria used in the *in vitro* assays.

2.2.1. *Magnaporthe oryzae* strains

Two *M. oryzae* Portuguese isolates were transformed to express the green fluorescent protein GFP, PR0009 (Gladieux et al., 2018) and M22.7 (Rosa et al., 2026). Briefly, PR0009-GFP was generated through *Agrobacterium tumefaciens*-mediated transformation (Rho et al., 2001) using the AGL-1 strain containing pCAMBgfp vector (Sesma and Osbourn, 2004), which expresses synthetic green fluorescent protein (sGFP), a GFP variant that contains a serine-to-threonine substitution at amino acid 65. Stable, GFP-expressing clones were selected in the presence of 250 µg/mL hygromycin B, and fluorescence was observed under ultraviolet light. We selected independent transformants with indistinguishable morphology and growth rates very similar to their wild types (WTs) (data not shown). The PR0009-GFP strain (Supplementary Fig. 1) was used for the *in vitro* fluorescence and microscopy assays, whereas the wild-type strain M22.7 (Rosa et al., 2026) was used for the detached leaf assay and the wild-type strain Guy11 was used for the whole-plant infection assays (*in planta*). The isolates were routinely maintained on complete medium (CM) (Talbot et al., 1993) or rice flour (RF) (Le Naour-Vernet et al., 2023), consisting of 20 g L⁻¹ organic rice flour, 2.5 g L⁻¹ yeast extract, and 1.5% agar. Cultures were incubated at 24–25°C for seven to ten days under a continuous illumination source consisting of 2 white and 2 purple fluorescent lamps with an intensity of 80–90 µmol m⁻² s⁻¹ to promote vegetative growth and conidiation. Conidia were harvested from sporulating cultures using sterile distilled water containing

0.02% (v/v) Tween-20, filtered to remove mycelial fragments, and adjusted to the appropriate concentration for each assay (Supplementary Table 1).

2.2.2. High-throughput microplate fluorescence assay for *Magnaporthe oryzae* growth inhibition

The antifungal activity of the *C. sorokiniana* aqueous extracts was assessed using a high-throughput GFP-based fluorescence assay, in which fluorescence served as a proxy for conidial germination and mycelial growth. Conidia from 7-day-old PR0009-GFP cultures were harvested in sterile distilled water containing 0.02% (v/v) Tween-20 and adjusted to 1×10^3 conidia mL⁻¹. Assays were performed in black flat-bottom 96-well microplates (Thermo Scientific Nunc F96 Microwell) containing conidial suspensions and *C. sorokiniana* aqueous extracts at 0.1 and 1.5 g L⁻¹, in a final volume of 150 µL. Sterile distilled water served as the negative control, and non-inoculated wells were included to correct for background. Each treatment had four technical replicates, repeated in 3 independent experiments (experiments 1 to 3). Green fluorescent protein (GFP) fluorescence (excitation 479 nm, emission 520 nm) was recorded every 30 min for 72–96 h at 25°C using an Agilent BioTek Synergy H1 microplate reader under continuous orbital shaking (282 rpm).

Subsequent assay concentrations were based on the response observed during this initial *in vitro* screening, which evaluated *C. sorokiniana* aqueous extract at 0.1 and 1.5 g L⁻¹. Significantly greater inhibitory activity was observed at 0.1 g L⁻¹ rather than at 1.5 g L⁻¹, and subsequent *in vitro* mechanistic assays assessing conidial germination and appressorium formation focused on the lower concentration range, 0.2 g L⁻¹. This concentration represented a modest increase from 0.1 g L⁻¹ that was selected to support subsequent *in planta* evaluation, given the greater biological complexity and variability of plant-based assays. Because we observed direct inhibitory effects on conidial germination and appressorium formation at 0.2 g L⁻¹, we evaluated this concentration in *in planta* assays.

2.2.3. Assessment of conidial germination and appressorium formation through fluorescence microscopy

To determine whether *C. sorokiniana* aqueous extracts affected the pathogen's early infection-related development, conidial germination and appressorium formation were assessed and quantified by fluorescence microscopy using the PR0009-GFP strain. Conidial suspensions were prepared as described above and adjusted to 5×10^4 conidia mL⁻¹. The suspensions were mixed with aqueous extracts of *C. sorokiniana* at a final concentration of 0.2 g L⁻¹ in the presence of 100 µM 1,16-hexadecanediol, a cutin analogue that stimulates germination and synchronises appressorial development *in vitro*. Control suspensions were prepared identically without the algae extract. Aliquots (15 µL) were placed on borosilicate coverslips (Epredia®, 20 x 20 mm) and incubated in sealed humid chambers for 24 h before evaluation of conidial germination and appressorium formation. Before microscopy, we stained samples with propidium iodide (1 µg mL⁻¹) to assess membrane integrity. Images were acquired at 20× magnification using identical acquisition settings for all treatments. We

performed four independent experiments (biological replicates) and observed at least 50 conidia per treatment.

2.3. Evaluation of *Chorella sorokiniana* aqueous extract treatment on *Magnaporthe oryzae*-infected rice

2.3.1 Rice detached leaves

To determine the efficacy of *C. sorokiniana* aqueous extract in controlling rice blast infection, detached-leaf assays were performed using rice (*Oryza sativa* L. cv. Presto), which is susceptible to the *M. oryzae* isolate M22.7 (Rosa et al., 2026). Rice plants were grown from seed in a commercial substrate (SIRO® Relva) under controlled-environment conditions (22–24°C, approximately 80% relative humidity, 16 h light/8 h dark photoperiod) until they reached 3–4 weeks of age. Fully expanded leaves were cut, surface-sterilised with 70% ethanol, rinsed with sterile distilled water, and immersed in the *C. sorokiniana* aqueous treatment. Excess solution was removed using sterile filter paper before the leaves were placed on a Petri dish containing 0.6% agar (w/v) supplemented with 2 ppm 6-benzylaminopurine (6-BAP) to delay senescence, as previously described (Zhu et al., 2020). Detached-leaf treatments were maintained under the same 6-BAP conditions. Five technical replicates were prepared for each treatment that were incubated overnight in the dark. The *M. oryzae* conidial suspension inoculum (1×10^5 conidia mL⁻¹) was prepared as described in section 2.3.1. in sterile distilled water supplemented with 0.02% (v/v) Tween-20 and 0.25% (w/v) gelatine. Leaves were infected the following day by applying a 4 µL droplet of an *M. oryzae* conidial suspension (1×10^5 conidia mL⁻¹) to the centre of the leaf blade, avoiding any mechanical wounding. After inoculation, the Petri dishes containing the infected leaves were incubated overnight in the dark and then maintained under a 16 h light/8 h dark photoperiod for up to 14 days. Disease development was assessed by measuring lesion area at 7- and 14-days post-inoculation (dpi) using ImageJ software (version 1.54d, Java 1.8.0_345(64-bit)).

2.3.2. Whole rice plants

2.3.2.1. Experimental design

To further corroborate the efficacy of *C. sorokiniana* aqueous extracts in controlling rice blast infection, we performed whole-plant experiments under growth-chamber conditions. Supplementary Table 2 summarises the principal experimental conditions. All experiments included untreated non-infected and infected controls. Experiments followed a completely randomised design, with pots randomly distributed and periodically repositioned throughout the experimental period to minimise positional effects. We determined disease severity at the final assessment at 7 dpi. Treatment efficacy was calculated relative to the untreated infected control using the following equation: Treatment efficacy (%) = (Disease severity in control - Disease severity in treatment) / Disease severity in control × 100. The main experimental conditions are summarised in Supplementary Table 2.

2.3.2.2. Treatments evaluation

The preventative efficacy of *C. sorokiniana* aqueous extract against rice blast was evaluated using rice (*Oryza sativa* L. cv. Bomba) grown in 1 L pots containing commercial substrate (SIRO® Relva) under controlled growth chamber conditions (22–24°C, approximately 80% relative humidity, 16 h light/8 h dark photoperiod). Treatments were applied preventively, 24 h before pathogen inoculation, as a foliar spray at the vegetative stage, 2–3 weeks after sowing. We applied approximately 4 mL of aqueous *C. sorokiniana* extract per pot using an airbrush sprayer (Timbertech® ABPST05) fitted with a 0.5 mm nozzle. Each treatment consisted of three pots, each containing four rice plants. All plants within each pot received the same treatment and inoculation. Non-infected and infected control plants were sprayed with sterile distilled water. For the infection, *M. oryzae* Guy11 (Leung et al, 1988) conidial suspension was prepared at 1×10^5 conidia mL⁻¹ in sterile distilled water supplemented with 0.02% (v/v) Tween-20 and 0.25% (w/v) gelatine. Twenty-four hours after treatment application, plants were spray-infected with approximately 2 mL of conidial suspension per pot under the same spraying conditions. Infected plants were incubated at 25°C under saturated humidity (dew) in darkness for 24 h and transferred to a growth chamber maintained at 25°C, approximately 95% relative humidity, and a 16 h light/8 h dark photoperiod for 6–7 days to allow symptom development. Disease symptoms were evaluated 7 days after infection using a modified disease assessment framework adapted from Hensawang et al. (2017) and IRRI (2002). Unlike the original disease assessment, which combines lesion morphology and disease severity into a single ordinal scale, the framework separates these components into two independent assessment scales, each applied to every evaluated leaf. Scale 1 - Lesion morphology classified lesion development according to the diameter ($\varnothing$) of the spot using six ordinal classes: 0 (no lesions); 1 (0–1 mm); 3 (1–2 mm); 5 (2–4 mm); 7 (4–7 mm); and 9 (> 7 mm). Scale 2 - Disease severity was classified by the percentage of leaf area exhibiting blast symptoms into six ordinal classes: 0 (0%); 1 (1–5%); 3 (5–25%); 5 (25–50%); 7 (50–75%); and 9 (75–100%). The relationship between lesion morphology and disease severity was also evaluated across five independent experiments using Spearman's rank correlation (Supplementary Fig. 2); these experiments were conducted during the development of the assessment framework, before refinement to the final six-class scale described.

Representative photos were used to standardise scoring for both scales (Supplementary Fig. 2). The dual-scale framework enables quantification of disease progression using complementary descriptors of lesion morphology and disease severity, allowing disease index calculations based on the ordinal scores of both parameters. For quantitative statistical analyses, we used only the disease severity scale because its classes represent predefined disease severity intervals. Following Del Ponte (2023), Willocquet (2023), and Chiang (2022), we converted the ordinal disease severity scores to the midpoints of their corresponding percentage intervals before statistical analysis. We averaged the converted values across all assessed leaves to obtain a disease severity value for each plant. We then used plant-level values for quantitative statistical comparisons and graphical representation. This framework therefore extends conventional rice blast assessment by enabling both disease index calculations from complementary symptom descriptors and statistically robust disease severity analyses based on interval-derived percentage leaf area values.

3. Statistical analysis

All statistical analyses were performed in R (version 4.3.3). Data were pooled from independent experiments before statistical analysis for all *in vitro*, *ex vivo*, and *in planta* *M. oryzae* assays. Results are presented as mean $\pm$ standard deviation (SD). Significant statistical differences were considered at $p < 0.05$.

3.1. High-throughput microplate fluorescence assay for *Magnaporthe oryzae* growth inhibition

For the high-throughput fluorescence-based *M. oryzae* growth inhibition microplate assay, blank-corrected fluorescence data were analysed using a linear mixed-effects model, with treatment as a fixed effect and experiments as random effects. Differences among treatment conditions were assessed using post-hoc Tukey tests.

3.2 Assessment of conidial germination and appressorium formation through fluorescence microscopy

Appressorium formation data were analysed using generalised linear models (GLMs) with a binomial error distribution and a logit link function, using raw count data. Treatment effects were assessed within each experiment.

3.3. Rice detached-leaves

For detached-leaf assays, disease inhibition was calculated relative to the mean lesion area corresponding to the untreated infected control for each experimental group. The statistical significance of the treatment was assessed using a one-sample Student's *t*-test, testing whether the mean disease inhibition differed significantly from 0% inhibition.

3.4. Whole rice plants

We independently scored disease symptoms in the *M. oryzae*–rice pathosystem using two ordinal scales describing lesion morphology (spot diameter) and disease severity (percentage of diseased leaf area). The dual-scale framework provided complementary descriptors of disease development and allowed us to calculate disease indices from both parameters. For quantitative statistical analyses of disease severity, we used only the percentage-based severity scale, as its classes corresponded to predefined intervals of diseased leaf area. Following Willocquet et al. (2023), ordinal severity scores were converted to the midpoints of their corresponding percentage intervals before analysis. We evaluated the association between lesion morphology and disease severity scores using Spearman's rank correlation coefficient (ρ). We assessed normality with the Shapiro–Wilk test and homogeneity of variance with Levene's test. When assumptions were met, we analysed treatment effects using one-way ANOVA followed by Tukey's HSD test; otherwise, we used the Kruskal–Wallis test followed by Dunn's post hoc test, where appropriate.

4. Results

4.1. *In vitro* *Magnaporthe oryzae* growth inhibition by *Chlorella sorokiniana* aqueous extract

The inhibitory effect of *C. sorokiniana* aqueous extract on *M. oryzae* growth was evaluated *in vitro* using a fluorescence-based 96-well microtiter plate assay with the GFP-expressing strain PR0009-GFP. First, to validate the assay, we determined that *C. sorokiniana* aqueous extract did not affect GFP signal detection (masking effects due to quenching or autofluorescence) in *E. coli* or in PR0009-GFP spores or cultures immediately after treatment (Supplementary Fig. 4). In this assay, increased fluorescence over time reflects spore germination and hyphal development. Treatment with *C. sorokiniana* aqueous extract at 0.1 g L⁻¹ significantly reduced fungal biomass accumulation by 66–79% relative to the untreated control ($p < 0.001$, Tukey post-hoc) across three independent experiments, whereas the treatment at 1.5 g L⁻¹ resulted in 38–45% inhibition ($p < 0.001$, Tukey post-hoc; Fig. 1). Inhibition at 0.1 g L⁻¹ was therefore greater than at 1.5 g L⁻¹. Thus, we focused subsequent mechanistic assays on the lower concentration.

To determine whether the extract interfered with the initial events required for host infection, we next investigated its impact on conidial germination and appressorium formation. Untreated conidia germinated and developed healthy appressoria with short germination tubes. However, conidia treated with the *C. sorokiniana* aqueous extract at 0.2 g L⁻¹ showed abnormal fungal development, including elongated germ tubes and deformities in the germination tubes/appressoria (indicated by arrows for germination tubes and triangles for appressoria; Fig. 2A). Moreover, propidium iodide staining indicated loss of membrane integrity and appressorium viability, a critical structure for host infection. To evaluate appressorium formation, *M. oryzae* spores were treated with 0.2 g L⁻¹ of *C. sorokiniana* aqueous extract (Fig. 2B). The treatment significantly reduced appressorium formation by 75–96% in 4 independent experiments compared with the untreated control ($p < 0.001$, binomial GLM; Fig. 2C). Although variability was observed among biological replicates, the reduction in appressorium formation was consistent across independent experiments.

Regarding extract characterisation, the aqueous extracts contained, on average, 64 mg of total protein (+/- 3.2) and 14.8 µg of phenolic compounds (+/- 0.2) per gram of *C. sorokiniana* biomass, with a pH of 6.86 (+/- 0.015).

4.2. Protective activity of *Chlorella sorokiniana* aqueous extract in the Rice-*Magnaporthe oryzae* pathosystem

We then evaluated the extract *in planta* to determine whether its biological activity translated into disease suppression under plant conditions. This efficacy was assessed in rice using a stepwise approach. We first tested the extract on detached rice leaves to determine whether it could suppress disease development under controlled conditions. Leaves treated with 0.2 g L⁻¹ extract before inoculation with *M. oryzae* developed significantly smaller lesions than the untreated infected controls

(Fig. 3A). Lesion development was reduced by more than 75%, indicating significant inhibition of disease development ($p < 0.001$, Student's t -test; Fig. 3B).

To evaluate whether the protective effects observed *ex vivo* translated to whole plants under a more biologically complex infection system resembling natural infection, preventive foliar application of *C. sorokiniana* aqueous extract was assessed in the *M. oryzae*–rice pathosystem (Fig. 3C–F). Preventive application of the extract at 0.2 g L^{-1} significantly reduced rice blast severity at the final assessment (7 dpi). This protective effect was also evident in the representative plant photos, with untreated infected plants showing more pronounced disease symptoms and reduced vigour than both the uninfected control and infected plants treated with *C. sorokiniana* aqueous extract (Fig. 3C). Representative individual leaves further illustrate reduced blast symptoms following extract treatment (Fig. 3D).

To assess consistency between the two disease-assessment parameters, we evaluated the relationship between lesion morphology (spot diameter) and disease severity (percentage of diseased leaf area) across five independent *M. oryzae*–rice experiments. The two parameters were consistently and strongly positively correlated (Spearman's $\rho = 0.939\text{--}0.995$, $p < 0.0001$ across all experiments, Supplementary Fig. 2).

Using the percentage-based disease severity scale, quantitative analysis confirmed that the preventive treatment significantly reduced rice blast severity when compared with the infected untreated control ($p < 0.0001$, Kruskal-Wallis test followed by Dunn's post hoc; Fig. 3E). Across the independent experiments, mean disease severity decreased from $17.06 \pm 8.20\%$ diseased leaf area in the infected control to $6.07 \pm 4.88\%$ in plants treated with 0.2 g L^{-1} *C. sorokiniana* extract. Relative to the infected control (100%), disease severity in treated plants was approximately 35.6%, corresponding to a treatment efficacy of approximately 64.4% (Fig. 3F). Lesion development was consistently reduced across independent experiments, demonstrating that the inhibitory effects observed on fungal growth and appressorium formation *in vitro* translated into substantial suppression of rice blast in the whole plant.

5 Discussion

This study shows that an aqueous extract of *C. sorokiniana* can suppress rice blast, with direct activity against *M. oryzae* observed at different infection stages. The extract inhibited fungal growth *in vitro*, impaired appressorium formation, and significantly reduced disease development in detached leaves and whole plants following preventive foliar application. The consistency of these effects across complementary experimental systems suggests that direct pathogen inhibition contributed to the observed disease suppression. However, because molecular plant responses were not directly investigated, we cannot exclude the possibility that the extract also enhances host defence mechanisms.

Among the observed effects, inhibition of appressorium formation likely plays a particularly important role. Appressoria are specialised infection structures that generate the turgor pressure required to penetrate the rice cuticle and initiate host colonisation (Chakraborty et al., 2021). Consequently,

disruption of appressorium differentiation is expected to substantially reduce fungal infectivity. The 75–96% reduction in appressorium formation observed in this study therefore provides a plausible mechanistic contribution to the reduced lesion development observed in detached leaves and whole plants. The inhibition of both fungal growth and appressorium differentiation extends previous reports showing that algae extracts can impair early developmental processes such as spore germination and infection establishment in phytopathogenic fungi, suggesting that microalgal metabolites may reduce pathogenicity through multiple complementary mechanisms (Bibi et al., 2026; Esserti et al., 2017).

Interestingly, the direct antifungal activity against *M. oryzae* differed between the two concentrations evaluated. Significantly greater inhibition was observed at 0.1 g L⁻¹ (66–79%) than at 1.5 g L⁻¹ (38–45%). Thus, increasing the extract concentration from 0.1 g L⁻¹ to 1.5 g L⁻¹ did not increase inhibitory activity under the conditions tested; however, because only two concentrations were compared, these results are insufficient to establish a concentration-response relationship. Non-monotonic responses to algae extracts have been reported in phytopathogenic fungi, with biological activity varying with extract concentration. Concentration-dependent variation in biological activity has previously been reported for algal extracts against phytopathogenic fungi, with different extract dilutions producing either growth-inhibitory or growth-promoting responses (Barreto et al., 2002). More broadly, the antimicrobial activity of complex natural extracts may be influenced by interactions among multiple bioactive compounds, including synergistic, additive, or antagonistic effects (Vaou et al., 2022). Increasing extract concentration could therefore alter interactions among its constituents, potentially introducing antagonistic effects that partially neutralise inhibitory compounds. Alternatively, threshold or saturation effects may occur, whereby relatively low concentrations are sufficient to induce substantial antifungal activity without a proportional increase at higher concentrations. These possibilities require further investigation because the chemical composition of the aqueous extract and interactions among its constituents remain uncharacterised. A broader concentration range and formal concentration-response analysis are needed to determine whether the observed pattern reflects a non-monotonic response or another concentration-related effect, and to assess potential threshold, saturation, inhibitory, or phytotoxic effects.

The detached-leaf assay further supports the biological relevance of the *in vitro* observations. Because infection of intact detached leaves depends on successful spore germination, appressorium differentiation, and host penetration, the substantial reduction in lesion development is consistent with the inhibition of these early infection processes observed microscopically. Likewise, the high level of protection achieved after a single preventive foliar immersion application indicates that the treatment retained sufficient biological activity to interfere with disease establishment during the initial stages of infection. However, because the detached-leaf infection and whole-plant assays used different application methods (leaf immersion and foliar spraying, respectively), and the amount of extract retained on the leaf surface following immersion was not quantified, extract exposure cannot be directly compared between the two experimental systems. Taken together, the consistent inhibition observed across fungal growth, appressorium formation, detached-leaf infection, and whole-plant disease development supports a role for direct antifungal activity in rice blast suppression. Nevertheless, plant-

mediated contributions, such as defence priming or activation, cannot be excluded, as reported for several algae-derived biostimulants and elicitors. Future studies should therefore experimentally distinguish pathogen-directed activity from host-mediated responses by analysing defence-related gene expression and other markers of induced resistance.

Although the bioactive compounds responsible for these effects remain largely unknown, *Chlorella* species produce a wide range of metabolites, including phenolic compounds, fatty acids, glycolipids, peptides, terpenoids, and sulphated polysaccharides, several of which exhibit antifungal properties and/or elicit plant defence responses (Vehapi et al., 2020; Righini et al., 2022). Given the reported composition of *C. sorokiniana*, fatty acids, sterol derivatives, and terpenoid-related compounds warrant particular investigation as potential contributors to the observed activity. Metabolomic profiling and testing of extract fractions could help identify the compounds responsible for antifungal activity and determine whether they act individually or in combination. Future studies should also determine extraction yield and identify chemical markers associated with biological activity to enable extract standardisation and assessment of batch-to-batch variability.

Microalgal extracts are increasingly recognised as effective biostimulants capable of improving root architecture, nutrient uptake, and overall plant vigour through their diverse repertoire of amino acids, polysaccharides, phytohormone-like compounds, phenolics, and carotenoids (Barone et al., 2018; Rachidi et al., 2020; Parmar et al., 2023; Ramakrishnan et al., 2023). Plant-associated responses triggered by the extract may therefore also have contributed to the observed disease suppression *in planta*. Enhanced root growth and plant vigour could increase tolerance to infection by helping plants maintain physiological function; however, this would represent increased disease tolerance rather than disease resistance. In parallel, numerous studies have shown that algal extracts can activate plant defence signalling pathways, stimulate defence-related enzymes, and induce systemic resistance against a range of pathogens (Vera et al., 2011; Prasanna et al., 2013; Roberti et al., 2015; Righini et al., 2022). The present study did not directly assess growth promotion or defence activation, so their contribution to the observed protection remains uncertain. Future studies should investigate plant-associated responses by measuring defence-related enzymes such as phenylalanine ammonia-lyase, peroxidase, and superoxide dismutase, alongside the expression of pathogenesis-related genes. Growth-related responses could be evaluated through root biomass, root length, and plant nutrient status, including non-infected plants treated with *C. sorokiniana* to distinguish treatment-induced effects from those associated with pathogen infection. Combining these host-focused analyses with pathogen-focused assays will be essential for resolving the mechanisms underlying the observed protection.

From an applied perspective, a simple aqueous extract's ability to suppress *M. oryzae*-induced disease has strong economic interest for all rice-producing countries. Unlike purified natural products, crude aqueous extracts require minimal processing, potentially reducing production costs and facilitating large-scale agricultural applications. However, practical development will depend on establishing reproducible composition, efficacy, stability, and safety under conditions that more closely reflect

agricultural use. These findings provide a foundation for further development of *C. sorokiniana* aqueous extract as a promising candidate for rice blast management.

Declarations

Funding: This work was supported by “Pacto da Bioeconomia Azul” (Project No. 16, No. C644915664-00000026) within the WP5 Algae Vertical, funded by Next Generation EU European Fund and the Portuguese Recovery and Resilience Plan (PRR), under the scope of the incentive line “Agendas for Business Innovation” through the funding scheme C5 - Capitalization and Business Innovation, by 01/C05-i02/2022.P242 to InnovPlantProtect, GreenCoLab and Fertiprado. PRR Missão Interface and FCT - Fundação para a Ciência e a Tecnologia, I.P. and Green-it Bioresources for Sustainability R&D Unit (UID/04551/2025, DOI: 10.54499/UID/04551/2025; UID/PRR/04551/2025, DOI: 10.54499/UID/PRR/04551/2025; UID/PRR2/04551/2025, DOI:10.54499/UID/PRR2/04551/2025) to InnovPlantProtect. CV's work is supported by the Portuguese Foundation for Science and Technology (Fellowship 2024.01477). BDANA (<https://doi.org/10.54499/2024.01477.BDANA>). LC's work is supported by Algarve 2030, Portugal 2030, and the European Union under the scope of AlgaeBoost—Support for the Boost and Development of GreenCoLab's Institutional Capacity in the Area of Sustainable Blue Biotechnology, ALGARVE-FSE+-01493100. CV, LC, and FG were also supported by the R&D unit MED—Mediterranean Institute for Agriculture, Environment and Development (<https://doi.org/10.54499/UID/05183/2025>) and the Associate Laboratory CHANGE—Global Change and Sustainability Institute (<https://doi.org/10.54499/LA/P/0121/2020>).

Competing interests: No competing interests

Availability of data and material – Most data supporting the findings of this study are included in this paper and its Supplementary Information files or can be made available upon request to the corresponding author.

Code availability – not applicable

Authors' contributions: RMC, SC and CA coordinated the studies; RMS analysed and interpreted the data. PR, RF, ACRC, and RMC developed the *M. oryzae*–rice pathosystem, including the GFP-expressing strain, optimisation of infection and disease assessment protocol, disease evaluation, and the statistical framework. MC and PR performed the *in vitro* assays for *M. oryzae*, pathogen inoculum preparation, *C. sorokiniana* mode-of-action studies, and data analysis. LC coordinated research activities at GCL, and CA and SC at InPP. CA also contributed to data interpretation. All authors contributed to manuscript writing and approved the final manuscript.

Acknowledgments: The authors gratefully acknowledge Professor Nick Talbot and his laboratory (The Sainsbury Lab, Norwich, UK) for providing the Guy11 laboratory strain of *Magnaporthe oryzae*, Anne Sesma (University of East Anglia, Norwich, UK) for the pCAMBgfp vector, Pierre Gladieux and Didier Tharreau (Plant Health Institute, Montpellier, France) for providing PR0009 isolate, and Allmicroalgae

Natural Products S.A. (Leiria, Portugal) and Necton (Olhão, Portugal) for providing *C. sorokiniana* biomass.

References

1. Agrios, G. (2005). Plant Pathol. 5th Edition, Elsevier Academic Press, Amsterdam, 26–27, 398–401.
2. Asibi, A. E., Chai, Q., Coulter, J. A. (2019). Rice Blast: A Disease with Implications for Global Food Security. *Agronomy*, 9(8), 451. <https://doi.org/10.3390/agronomy9080451>
3. Banskota, A. H., Stefanova R., Hui, J.P.M Bermarija, T., Stemmler, H., McGinn, P.J. and O’Leary, S. J. B. (2024). Comprehensive Analysis of Biomass from *Chlorella sorokiniana* Cultivated with Industrial Flue Gas as the Carbon Source. *Molecules* 29(14), 3368. <https://doi.org/10.3390/molecules29143368>
4. Barreto, M., Critchley, A.T., Straker, C.J. (2002). Extracts from seaweeds can promote fungal growth. *J Basic Microbiol.*, 42(5), 302–10. [https://doi.org/10.1002/1521-4028\(200210\)42:5](https://doi.org/10.1002/1521-4028(200210)42:5)
5. Barone, V., Baglieri A., Stevanato P., Broccanello C., Bertoldo G., Bertaggia M., Cagnin, M., Pizzeghelo, D., Moliterni, V.M.C., Mandolino, G., Fornasier, F., Squartini, A., Nardi, S., Concheri, G. (2018). Root morphological and molecular responses induced by microalgae extracts in sugar beet (*Beta vulgaris* L.). *J. Appl. Phycol.*, 30, 1061–1072. <https://doi.org/10.1007/s10811-017-1283-3>
6. Bhardwaj, R., Yadav, A., Sahoo, A., Kumari, P., Singh, L.A., Swapnil, P., Meena, M., Kumar S. (2025). Microalgal-based sustainable bio-fungicides: A promising solution to enhance crop yield. *Discov. Sustain.*, 6, 39. <https://doi.org/10.1007/s43621-025-00795-9>
7. Bibi, S., Jaoua, S., Al-Ghouti, M. A., Zouari, N., Abu-Dieyeh M.H. (2026). Exploring the antifungal potential of the brown macroalga *Hormophysa triquetra*: Morphological disruptions and structural changes in plant pathogens. *Environ. Microbiol. Rep.*, 18(3), e70304. <https://doi.org/10.1111/1758-2229.70304>
8. Carvalho, F. P. (2017). Pesticides, environment, and food safety. *Food Energy Secur.*, 6(2), 48–60. <https://doi.org/10.1002/fes3.108>
9. Chakraborty, M., Mahmud, N. U., Ullah, C., Rahman, M., Islam, T. (2021). Biological and biorational management of blast diseases in cereals caused by *Magnaporthe oryzae*. *Crit. Rev. Biotechnol.* <https://doi.org/10.1080/07388551.2021.1898325>
10. Chiang, K. S., Bock, C. H. (2022). Understanding the ramifications of quantitative ordinal scales on accuracy of estimates of disease severity and data analysis in plant pathology. *Trop. Plant Pathol.*, 47(1), 58–73. <https://doi.org/10.1007/s40858-021-00446-0>
11. Colla, G., Rouphael, Y. (2020). Microalgae: New Source of Plant Biostimulants. *Agronomy*, 10(9), 1240. <https://doi.org/10.3390/agronomy10091240>
12. Dean, R., Van Kan, J.A.L., Pretorius, Z.A., Hammond-Kosack, K.E., Di Pietro A., Rudd, J.J., Dickman, M., Kahmann R., Ellis, J., Foster, G.D. (2012). The Top 10 fungal pathogens in molecular plant pathology. *Mol. Plant Pathol.*, 13, 414–430. <https://doi.org/10.1111/j.1364-3703.2011.00783.x>

13. Del Ponte, E. M. (2023). R for Plant Disease Epidemiology (R4PDE). <https://r4pde.net>
14. Esserti, S., Smaili, A., Rifai, L. A., Koussa, T., Makroum, K., Belfaiza, M., Kabil, E.M., Faize, L., Burgos, L., Albuquerque, N., Faize, M. (2017). Protective effect of three brown seaweed extracts against fungal and bacterial diseases of tomato. *J. Appl. Phycol.*, 29, 1081–1093. <https://doi.org/10.1007/s10811-016-0996-z>
15. Ferreira, A., Melkonyan, L., Carapinha, S., Ribeiro, B., Figueiredo, D., Avetisova, G., Gouveia, L. (2021). Biostimulant and biopesticide potential of microalgae growing in piggery wastewater. *Environ. Adv.*, 4, 100062. <https://doi.org/10.1016/j.envadv.2021.100062>
16. Gladioux, P., Ravel, S., Rieux, A., Cros-Arteil, S., Adreit, H., Milazzo, J., Thierry, M., Fournier E., Terauchi, R., Tharreau, D. (2018). Coexistence of multiple endemic and pandemic lineages of the rice blast pathogen. *mBio*, 9(2), e01806-17. <https://doi.org/10.1128/mBio.01806-17>
17. Hensawang, S., Wangwongchai, A., Humphries, U., Bunsri, T. (2017). Simulation of severity of rice blast disease in Prachin Buri using plant disease epidemiological model: simulation of rice blast disease. *Agric. Food. Sci.*
18. IRRI. (2002). Standard Evaluation System for Rice (SES). International Rice Research Institute, Philippines.
19. Jacquet, F., Jeuffroy, M.H., Jouan, J. Le Cadre, E., Litrico, I., Malausa, T., Reboud, X., Huyghe, C. (2022). Pesticide-free agriculture as a new paradigm for research. *Agron. Sustain. Dev.*, 42, 8. <https://doi.org/10.1007/s13593-021-00742-8>
20. Jokel, M., Salazar, J., Chovancek, E., Sirin, S., Allahverdiyeva, Y. (2023). Screening of several microalgae revealed biopesticide properties of *Chlorella sorokiniana* against the strawberry pathogen *Phytophthora cactorum*. *J. Appl. Phycol.*, 35, 2675–2687. <https://doi.org/10.1007/s10811-023-03015-x>
21. Kamoun, S., Furzer, O., Jones, J. D. G., Judelson, H.S., Ali, G.S., Dalio, R.J.D., Roy, S.G., Schena, L., Zambounis, A. (2014). The top 10 oomycete pathogens in molecular plant pathology. *Mol. Plant Pathol.*, 16(4), 413–434. <https://doi.org/10.1111/mpp.12190>
22. Khan, W., Rayirath, U. P., Subramanian, S. Jithesh, M.N., Rayorath, P., Hodges, D.M., Critchley, A.T., Craigie, J.S., Norrie, J., Prithiviraj, B. (2009). Seaweed extracts as biostimulants of plant growth and development. *J. Plant Growth Regul.*, 28, 386–399. <https://doi.org/10.1007/s00344-009-9103-x>
23. Köhl, J., Kolnaar, R., and Ravensberg, W. J. (2019). Mode of action of microbial biological control agents against plant diseases: Relevance beyond efficacy. *Front. Plant Sci.*, 10, 845. <https://doi.org/10.3389/fpls.2019.00845>
24. Köhl, J., Medeiros, Flavio H.V., Lombaers-van der Plas., C, Groenenboom-de Haas., L, van den Bosch., T. (2020). Efficacies of bacterial and fungal isolates in biocontrol of *Botrytis cinerea* and *Pseudomonas syringae* pv. *tomato* and growth promotion in tomato do not correlate. *Biol. Control*, 150, <https://doi.org/10.1016/j.biocontrol.2020.104375>
25. Le Naour-Vernet M., Charriat F., Gracy J., Cros-Arteil S., Ravel, S., Veillet, F., Meusnier, I., Padilha, A., Kroj, T., Cesari, S., Gladioux, P. (2023). Adaptive evolution in virulence effectors of the rice blast

- fungus *Pyricularia oryzae*. PLoS Pathog., 19(9), e1011294.
<https://doi.org/10.1371/journal.ppat.1011294>
26. Lee, S.M., Kim, S.K., Lee, N., Ahn, C.Y., Ryu, C.M. (2020). d-Lactic acid secreted by *Chlorella fusca* primes pattern-triggered immunity against *Pseudomonas syringae* in *Arabidopsis*. Plant J., 102(4), 761–778. <https://doi.org/10.1111/tpj.14661>
 27. Leung, H., Borromeo, E. S., Bernardo, M. A., Notteghem, J.L. (1988). Genetic analysis of virulence in the rice blast fungus *Magnaporthe grisea*. Phytopathology, 78(9), 1227–1233.
 28. Lio, E.; Esposito, C.; Paini, J.; Gandolfi, S.; Secundo, F.; Ottolina, G. (2025). Valorizing Agro-Industrial By-Products for Sustainable Cultivation of *Chlorella sorokiniana*: Enhancing Biomass, Lipid Accumulation, Metabolites, and Antimicrobial Potential. Metabolites, 15, 212.
<https://doi.org/10.3390/metabo15030212>
 29. Palmieri, D., Ianiri, G., Del Grosso, C., Barone, G., De Curtis, F., Castoria, R., Lima, G. (2022). Advances and Perspectives in the Use of Biocontrol Agents against Fungal Plant Diseases. Horticulturae, 8(7), 577. <https://doi.org/10.3390/horticulturae8070577>
 30. Papapanagiotou, G., Charisis, A., Samara, C., Kalogianni, E. P., and Chatzidoukas. C. (2024). Linking Cultivation Conditions to the Fatty Acid Profile and Nutritional Value of *Chlorella sorokiniana* Lipids. Processes 12, 12, 2770. <https://doi.org/10.3390/pr12122770>
 31. Parmar P., Kumar R., Neha Y., Srivatsan V. (2023). Microalgae as next generation plant growth additives: Functions, applications, challenges and circular bioeconomy based solutions. Front. Plant Sci., 30(14), 1073546. <https://doi.org/10.3389/fpls.2023.1073546>
 32. Pathak V.M., Verma V.K., Rawat B.S., Kaur, B., Babu, N., Sharma, A., Dewali S., Yadav, M., Kumari, R., Singh, S., Mohapatra, A., Pandey, V., Rana, N., Cunill, J.M. (2022). Current status of pesticide effects on environment, human health and it's eco-friendly management as bioremediation: A comprehensive review. Front. Microbiol., 13, 962619. <https://doi.org/10.3389/fmicb.2022.962619>
 33. Prasanna, R., Kumar, A., Babu, S., Chawla, G., Chaudhary, V., Singh, S., Gupta, V., Nain, L., Saxena, A.K. (2013). Deciphering the biochemical spectrum of novel cyanobacterium-based biofilms for use as inoculants. Biol. Agric. Hortic., 29, 145–158. <https://doi.org/10.1080/01448765.2013.790303>
 34. Rachidi, F., Benhima, R., Sbabou, L., El Arroussi, H. (2020). Microalgae polysaccharides bio-stimulating effect on tomato plants: Growth and metabolic distribution. Biotechnol Rep (Amst)., 31(25), e00426. <https://doi.org/10.1016/j.btre.2020.e00426>
 35. Ramakrishnan, B., Maddela, N. R., Venkateswarlu, K., Megharaj, M. (2023). Potential of microalgae and cyanobacteria to improve soil health and agricultural productivity: a critical view. Environ. Sci., 2(4), 586–611. <https://doi.org/10.1039/d2va00158f>
 36. Rho, H. S., Kang, S., Lee, Y. H. (2001). *Agrobacterium tumefaciens*-mediated transformation of the plant pathogenic fungus, *Magnaporthe grisea*. Mol. Cells, 12(3), 407–411.
[https://doi.org/10.1016/S1016-8478\(23\)17116-0](https://doi.org/10.1016/S1016-8478(23)17116-0)
 37. Righini, H., Francioso, O., Martel Quintana, A., Roberti, R. (2022). Cyanobacteria: A Natural Source for Controlling Agricultural Plant Diseases Caused by Fungi and Oomycetes and Improving Plant

- Growth. *Horticulturae*, 8(1), 58. <https://doi.org/10.3390/horticulturae8010058>
38. Roberti, R., Galletti, S., Burzi, P.L., Righini, H., Cetrullo, S., Perez, C. (2015). Induction of defence responses in zucchini (*Cucurbita pepo*) by *Anabaena* sp. water extract. *Biol. Control*, 82, 61–68. <https://doi.org/10.1016/j.biocontrol.2014.12.006>
 39. Rosa, P., Bilro, J., Ramiro, R. S., Azevedo, C. (2026). High quality complete genomes of two virulent field isolates of *Pyricularia oryzae* from Portugal. *bioRxiv*, 2026–06. <https://doi.org/10.64898/2026.06.12.731836>
 40. Savary S., Willocquet L., Pethybridge S.J., Esker, P., McRoberts, N., Nelson, A. (2019). The global burden of pathogens and pests on major food crops. *Nat. Ecol. Evol.*, 3 (3), 430–439. <https://doi.org/10.1038/s41559-018-0793-y>
 41. Schmid, B., Coelho, L., Schulze, P. S. C., Pereira, H., Santos, T., Maia, I.B., Reis, M., Varela, J. (2022). Antifungal properties of aqueous microalgal extracts. *Bioresour. Technol. Rep.*, 18, 101096. <https://doi.org/10.1016/j.biteb.2022.101096>
 42. Sesma, A., Osbourn, A. E. (2004). The rice leaf blast pathogen undergoes developmental processes typical of root-infecting fungi. *Nature*, 431(7008), 582–586. <https://doi.org/10.1038/nature02880>
 43. Spolaore, P., Joannis-Cassan, C., Duran, E., Isambert, A. (2006). Commercial applications of microalgae. *J. Biosci. Bioeng.*, 101(2), 87–96. <https://doi.org/10.1263/jbb.101.87>
 44. Talbot, N. J., Ebbole, D. J., Hamer, J.E. (1993). Identification and characterization of MPG1, a gene involved in pathogenicity from the rice blast fungus *Magnaporthe grisea*. *Plant Cell*, 5(11), 1575–1590. <https://doi.org/10.1105/tpc.5.11.1575>
 45. Toribio, A.J., Jurado, M.M., Suárez-Estrella, F. López-González, J.A., Martínez-Gallardo, M.R, López, M.J. (2021). Application of sonicated extracts of cyanobacteria and microalgae for the mitigation of bacterial canker in tomato seedlings. *J. Appl. Phycol.*, 33, 3817–382. <https://doi.org/10.1007/s10811-021-02599-6>
 46. Vaou, N., Stavropoulou, E., Voidarou, C.C., Tsakris, Z., Rozos, G., Tsigalou, C., Bezirtzoglou, E. (2022). Interactions between medical plant-derived bioactive compounds: Focus on antimicrobial combination effects. *Antibiotics*, 11(8), 1014. <https://doi.org/10.3390/antibiotics11081014>
 47. Vehapi, M., Koçer, A. T., Yılmaz, A., Özçimen, D. (2020). Investigation of the antifungal effects of algal extracts on apple-infecting fungi. *Arch. Microbiol.*, 202(3), 455–471. <https://doi.org/10.1007/s00203-019-01760-7>
 48. Vera, J., Castro, J., Gonzalez, A., Moenne, A. (2011). Seaweed polysaccharides and derived oligosaccharides stimulate defense responses and protection against pathogens in plants. *Mar. Drugs*, 9, 2514–2525. <https://doi.org/10.3390/md9122514>
 49. Viana, C., Genevace, M., Gama, F., Coelho, L., Pereira, H., Varela, J., Reis, M. (2024). *Chlorella vulgaris* and *Tetrademus obliquus* protect spinach (*Spinacia oleracea* L.) against *Fusarium oxysporum*. *Plants*, 13(12), 1697. <https://doi.org/10.3390/plants13121697>
 50. Willocquet, L., Savary, S., Singh, K.P. (2023). Revisiting the use of disease index and of disease scores in plant pathology. *Indian Phytopathol.*, 76, 909–914. <https://doi.org/10.1007/s42360-023->

51. Zhu, Z., Yin, J., Chern, M., Zhu, X., Yang, C., He, K., Liu, Y., He, M., Wang, J., Song, L., Wang, L., Wei, Y., Wang, J., Liu, J., Qing, H., Bi, Y., Li, M., Hu, K., Qi, T., Hou, Q., Chen, X., Li, W. (2020). New insights into *bsr-d1*-mediated broad-spectrum resistance to rice blast. *Mol. Plant Pathol.*, 21, 951–960. <https://doi.org/10.1111/mpp.12941>
52. Velioglu, Y. S., Mazza, G., Gao, L., & Oomah, B. D. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *Journal of Agricultural and Food Chemistry*, 46(10), 4113–4117. <https://doi.org/10.1021/jf9801973>
53. He, F. (2011). Bradford protein assay. *Bio-101*, e45. <https://doi.org/10.21769/BioProtoc.45>

Figures

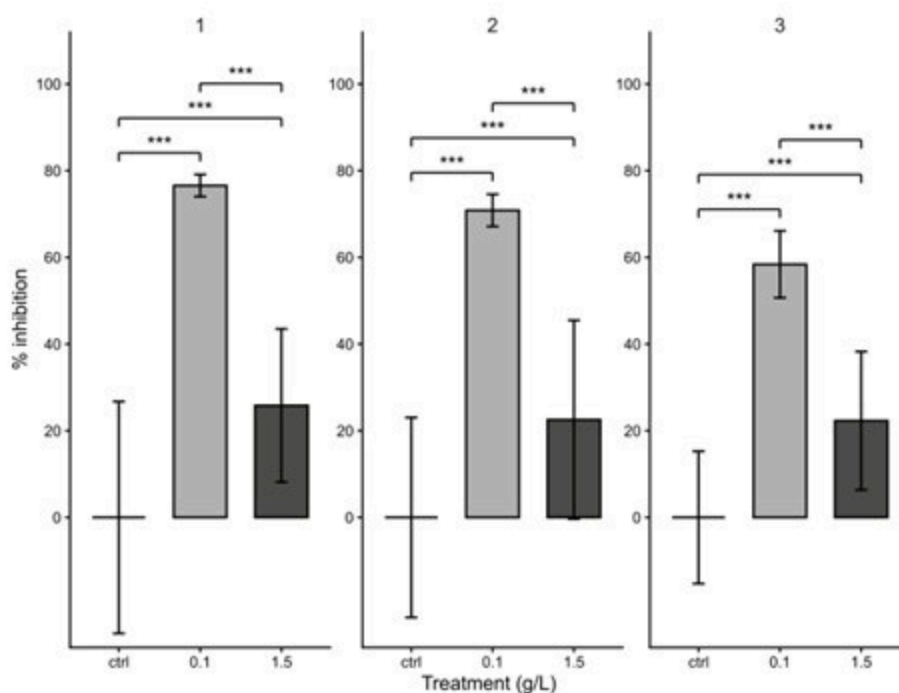


Fig. 1

Figure 1

High-throughput fluorescence-based *Magnaporthe oryzae* growth inhibition microplate assay to quantify the in vitro effect of *Chlorella sorokiniana* aqueous extract on *M. oryzae* growth inhibition (%).

Comparison is made relative to the untreated control at 0.1 and 1.5 g L⁻¹. Data are presented as mean ± SD and reflect pooled results from three independent experiments (biological replicates, n = 3), with four technical replicates per experiment. The statistical analyses were performed using a linear mixed-effects model followed by Tukey-adjusted pairwise comparisons. Asterisks indicate significant differences from the untreated control and between the 0.1 g L⁻¹ and 1.5 g L⁻¹ conditions. (* p < 0.05; *** p < 0.001).

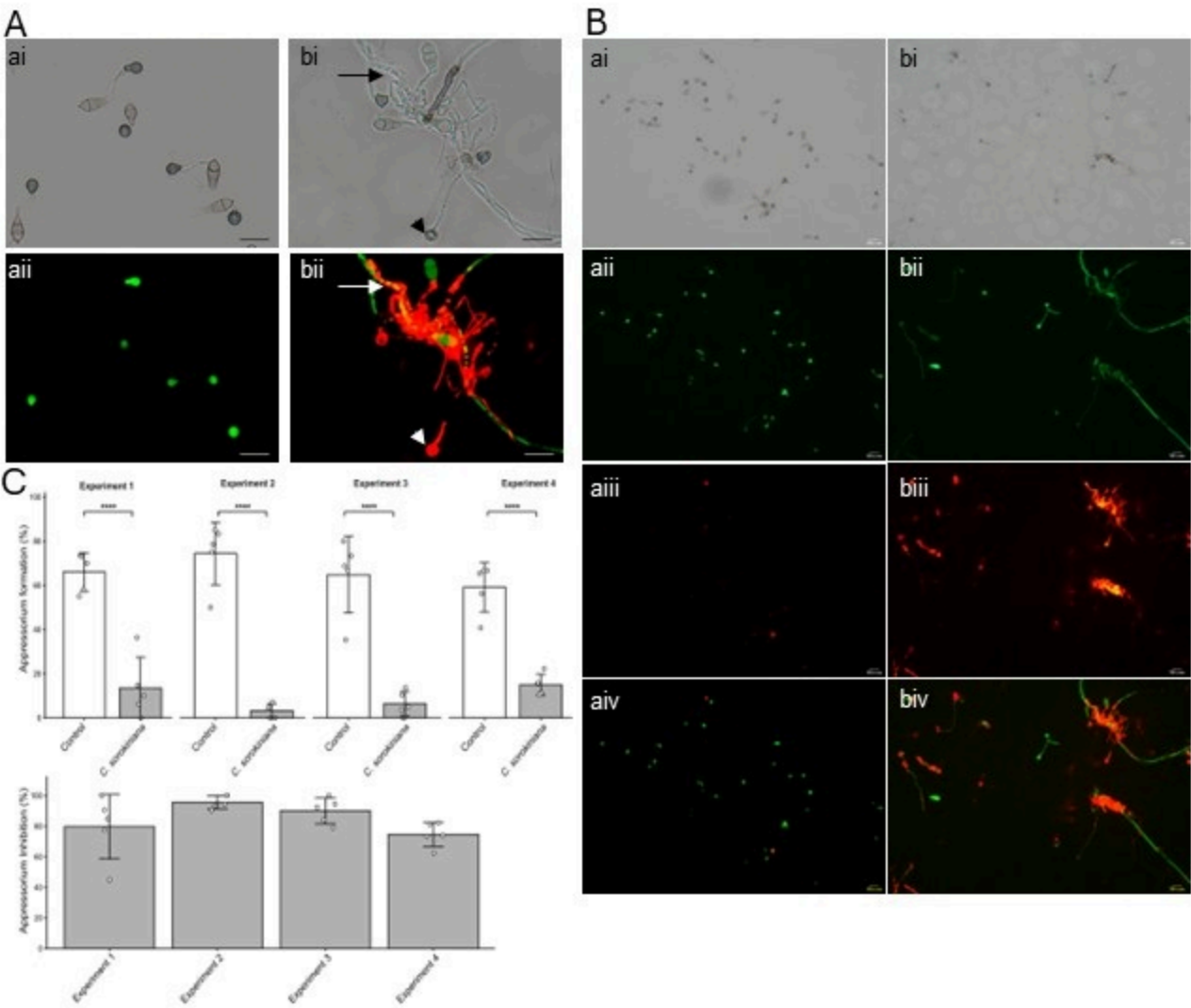


Fig. 2

Figure 2

In vitro assessment of the effect of *Chlorella sorokiniana* aqueous extract on *Magnaporthe oryzae* (PR9-GFP) spore germination and appressorium formation. In panels A and B, “a” represent the untreated conidia control, and “b” the conidia treated with *C. sorokiniana* aqueous extract (0.2 g L⁻¹). The spore

solutions have been stained with propidium iodide to observe cell death (red channel). A) Highlighting the effect of extract on *M. oryzae* conidia germination on a hydrophobic surface (arrows indicate deformities in germ tubes and triangles indicate deformities in appressoria, and both arrows and triangles indicate cell death) by cropping images represented in panel B. Brightfield images represented with ai and bi. Darkfield images merged with Green and Red channels (aii and bii). B) *M. oryzae* appressorium formation in brightfield (ai and bi) and darkfield (all other panels) on different channels: Green (aii and bii), Red (aiii and biii), and merged Green and Red channels (aiv and biv). C) Quantification of the percentage of appressorium formation and percentage inhibition of appressorium formation following treatment. Data represent results of four independent pooled experiments (minimum of 50 spores were counted per treatment; $n = 4$) and bars are presented as the mean $\pm$ SD while points represent individual observations. Statistical comparisons were performed against the untreated control using GLM with a binomial error distribution and logit link function ($***p < 0.001$). Scale 20 μm .

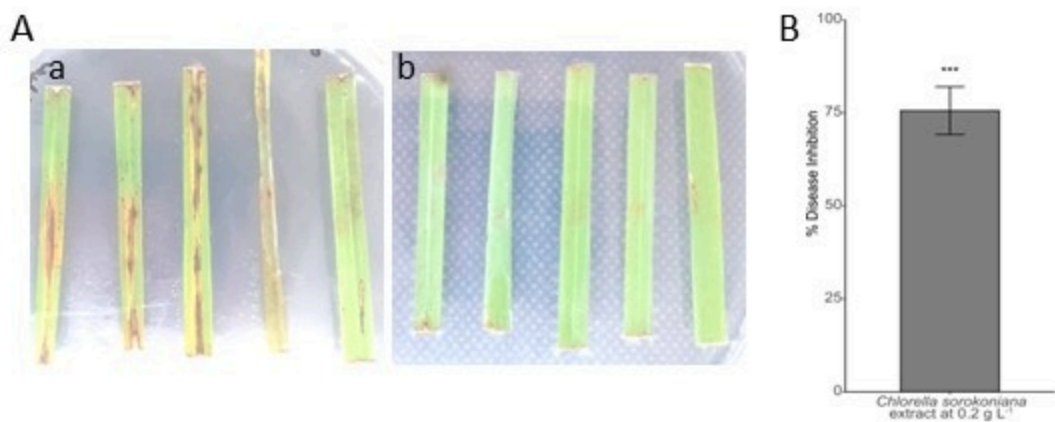


Fig. 3

Figure 3

Preventive activity of *Chlorella sorokiniana* aqueous extract against *Magnaporthe oryzae* in rice detached leaves. A) Representative images of detached rice leaves corresponding to (a) untreated infected control and (b) infected leaves treated with *C. sorokiniana* extract (0.2 g L⁻¹). B) Quantification of the disease inhibition in the detached leaf assay, expressed relative to the untreated infected control. Data represent pooled results from two independent experiments, with five detached leaves per treatment in each experiment ($n = 10$) and are presented as mean $\pm$ SD. Disease inhibition was analysed using a one-

sample Student's *t*-test against a theoretical value of 0% inhibition. Asterisks indicate significant differences from the untreated infected control ($*** p < 0.001$).

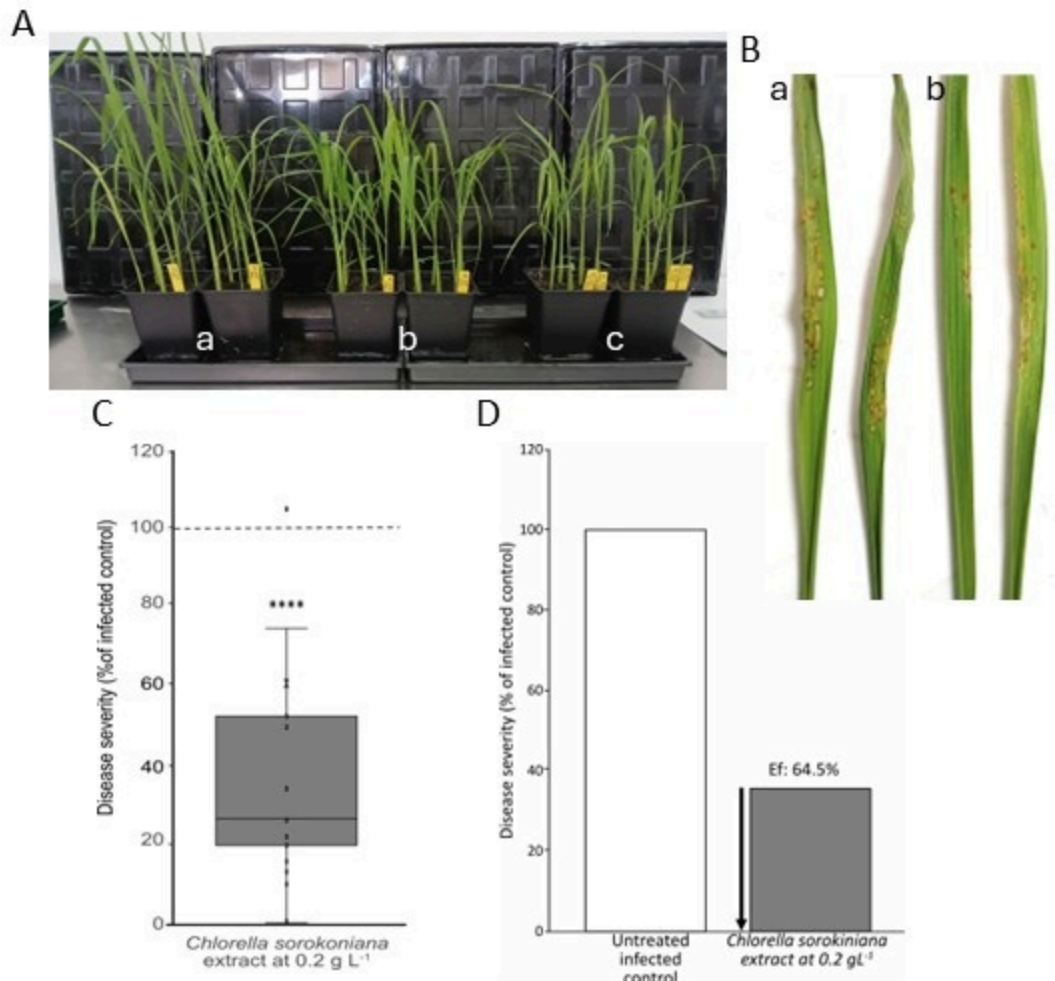


Fig. 4

Figure 4

Preventive activity of *Chlorella sorokiniana* aqueous extract against *Magnaporthe oryzae* in whole rice plants. A) Representative rice plants at 7 days post-inoculation (dpi): (a) non-inoculated untreated control, (b) untreated infected control, and (c) plants treated with *C. sorokiniana* extract 24 h before inoculation with *M. oryzae*. B) Representative images of individual rice leaves from the experiment shown in panel C, corresponding to (a) untreated infected control and (b) infected plants treated with *C. sorokiniana* extract (0.2 g L⁻¹). C) Disease severity in whole rice plants following foliar application of the extract 24 h before inoculation with *M. oryzae*, expressed relative to the untreated infected control (100%). Box-and-whisker plots summarise plant-level values; the dashed line denotes the untreated

infected control (100%), and the $\times$ indicates the mean. Absolute disease severity across the independent experiments was $17.06 \pm 8.20\%$ diseased leaf area for the infected control and $6.07 \pm 4.88\%$ for the *C. sorokiniana*-treated plants. D) Treatment efficacy (Ef), expressed as the percentage reduction in disease severity relative to the untreated infected control. Data in E and F represent pooled results from two independent experiments, each comprising three pots with four plants per treatment. Leaf-level disease severity values were averaged within each plant, resulting in 24 plant-level observations per treatment across the two experiments ($n = 24$), which were used for statistical analysis and are presented as mean $\pm$ SD. Statistical comparisons against the untreated infected control were performed using Kruskal–Wallis test followed by Dunn’s post hoc test. Asterisks indicate significant differences from the untreated infected control (**** $p < 0.0001$).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [STables.docx](#)
- [SFigs.pdf](#)