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Ecology Biology

Antifungal activity of microalgae in phytopathogenic fungi: A systematic review

Atividade antifúngica de microalgas em fungos fitopatógenos: Revisão sistemática

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ABSTRACT

Phytopathogenic fungi are a group of organisms that cause diseases in plants through disturbances in their cellular metabolism. They have major impacts on agriculture, accounting for 8 – 40% of the losses in world agricultural production. Meanwhile, microalgae synthesize a wide variety of allelopathic chemicals and can be used for different biocidal purposes, including antifungal. The objective of this study was to review the antifungal activity of microalgal extracts and their compounds against phytopathogenic fungi. This systematic review of the literature was conducted using SciELO, PubMed, and Periódicos Capes (Scopus). Following the search, 25 articles published in English and Portuguese were selected. Several publications will be recorded from 2015 to 2022. Eight microalgal phyla (Bacillariophyta, Chlorophyta, Cyanobacteria, Haptophyta, Miozoa, Ochrophyta, Prasinodermatophyta, and Rhodophyta) were detected, with Chlorophyta and Cyanobacteria having the highest number of registered publications. The most tested species of phytopathogenic fungi were *Aspergillus niger* and *Botrytis cinerea.* Regarding the categorized inhibition classification for the assays, high inhibition was observed in 31.26% of the trials. Studies performed with cyanobacterial species showed a higher proportion of high inhibition (41.36%) of phytopathogens. Given the high degree of biodiversity of microalgae and their wide range of associated bioactive molecules, this is a vast field to explore for novel biopesticides with antifungal potential.

Keywords: Agriculture; Antifungal activity; Phytopathogens; Fungi; Microalgae

RESUMO

Os fungos fitopatogênicos são um grupo de organismos que podem ocasionar doenças em plantas por meio de distúrbios no seu metabolismo celular. Estes causam grandes impactos na agricultura, sendo responsáveis por 8 a 40% das perdas na produção agrícola mundial. As microalgas sintetizam uma ampla variedade de produtos químicos alelopáticos, podendo ser empregados em diferentes finalidades biocidas, incluindo a antifúngica. O objetivo deste trabalho foi realizar uma revisão acerca da atividade antifúngica de extratos e compostos de microalgas em fungos fitopatógenos. Trata-se de uma revisão sistemática da literatura, realizada nas bases de dados SciELO, PubMed e Periódicos Capes (Scopus). Após a busca, foram selecionados 25 artigos publicados nos idiomas inglês e português. Um maior número de publicações foi registrado no período de 2015 a 2022. Foram detectados oito filos de microalgas (Bacillariophyta, Chlorophyta, Cyanobacteria, Haptophyta, Miozoa, Ochrophyta, Prasinodermatophyta e Rhodophyta), com Chlorophyta e Cyanobacteria os com maior número de publicações registradas. As espécies de fungos fitopatógenos mais testadas foram *Aspergillus niger* e *Botrytis cinerea.* Em relação à classificação de inibição categorizada para os ensaios, inibição alta foi observada em 31.26% dos ensaios. Ensaios realizados com espécies de Cyanobacteria apresentaram maior proporção de inibição alta (41.36%) frente aos fitopatógenos. Tendo em vista a grande biodiversidade de microalgas e a ampla gama de moléculas bioativas associadas, esse é um vasto campo a ser explorado na busca por novos biopesticidas com potencial antifúngico.

Palavras-chave: Agricultura; Atividade antifúngica; Fitopatógenos; Fungos; Microalgas.

1 INTRODUCTION

Phytopathogenic fungi are microorganisms that inhabit the interior of plant tissues (Leannec-Rialland et al. 2022). Under biotic and abiotic stresses, they can cause diseases in plants through disturbances in cellular metabolism (Chandrasekaran et al. 2016). Phytopathogens affect agricultural productivity and food security worldwide, resulting in major economic losses (Omran and Baek 2022). They are estimated to account for 8 – 40% of the losses in global agricultural production (Khan et al. 2021).

The main method of controlling phytopathogenic fungi is the use of fungicides such as benzimidazole, carboxylin, anilinopyrimidine, strobilurin and morpholine (Brauer et al. 2019). However, the use of these synthetic compounds can cause problems to human health and the environment, such as water and soil pollution (Ons et al. 2020) and the selection of azole-resistant fungi for clinical use (Brauer et al. 2019; Verweij et al. 2022). As an alternative to these commercial fungicides, microalgae extracts stand out, which, in addition to their registered biocidal properties, are also a more sustainable option (Mishra and Arora 2018).

Microalgae are photosynthetic organisms that, under different environmental conditions, produce a wide variety of bioactive compounds (Costa et al. 2022). Currently, they are extensively used in biotechnological applications and are directed toward agriculture. Several applications have been associated with them, such as biofertilizer properties (atmospheric nitrogen fixation capacity and phosphorus solubilization), aiding in the cycling of soil nutrients (promoting plant growth), producing bioactive substances (such as phytohormones), removing heavy metals from the soil, treating agricultural wastewater, and protecting against pathogens and pests (Costa et al. 2019; Alvarez et al. 2021). They can protect against pathogens and pests due to their ability to produce allelopathic chemical compounds, which are secondary metabolites that affect individuals other than those who produce theim and can be used for algicidal, herbicidal, insecticidal, nematicidal, and fungicidal purposes (Alvarez et al. 2021).

Although there are articles that have reviewed the antifungal activity of macroalgae (Vicente et al. 2021) and cyanobacteria (Righini et al. 2022) against phytopathogenic fungi, nothing has been directed exclusively toward microalgae, especially eukaryotes. Given the chemical diversity of bioactive compounds isolated from microalgae and their previously recorded antimicrobial properties, these organisms are considered promising agents for the production of novel antifungal compounds for agriculture (Costa et al. 2019). This study is a systematic review of the literature on the antifungal activities of extracts and microalgal compounds against phytopathogenic fungi.

2 METHODOLOGY

A systematic literature review was conducted on the antifungal activities of microalgal extracts and compounds against phytopathogenic fungi. Searches were performed using the SciELO, PubMed, and Capes (Scopus) databases. The search strategy was the same for all databases. The keywords used in Portuguese, English, and Spanish were Microalga/Microalgae/Microalgas AND Antifúngico/Antifungal/

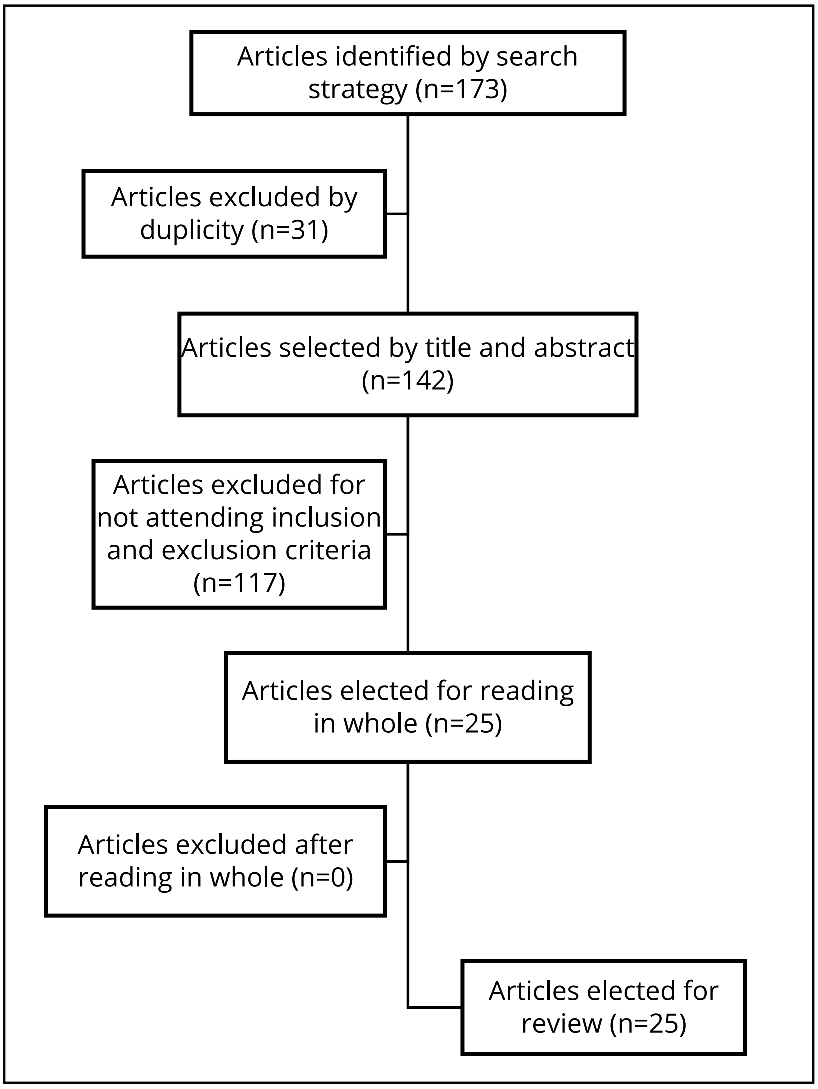
Antifúngico, respectively. No time cut-off was applied.

The selection of articles to compose this systematic review was performed by two researchers, in a double-blind manner, with the aid of the *online* platform Rayyan. Conflict mediation was conducted with the help of a third researcher. The following inclusion criteria were used to select articles: (I) original scientific articles published in national and international journals; (II) articles in Portuguese, English, and Spanish; (III) articles that tested the antifungal action of microalgae on phytopathogenic fungi; and (IV) *in vivo* or *in vitro* experimental studies. The following studies were excluded: (I) letters, editorials, news, comments, and case studies; (II) articles not available in full; (III) articles published in other languages; and (IV) reviews and book chapters. The flowchart in figure 1 presents the search and selection of the articles (Figure 1).

The following information was extracted from the selected articles: bibliometric data, microalgal collection sites, microalgal species studied, extraction methodologies and solvents, detection methodologies, biomolecules present in microalgal extracts, antifungal assay methodologies, tested fungal species, and the presence of inhibitory activity. The antifungal activity of the microalgae was classified according to the following criteria: high (minimum inhibition concentration ≤ 15 mg mL^{-1}, inhibition halo ≥ 5 mm and percentage of inhibition ≥ 60%), low (minimum inhibition concentration > 15 mg mL^{-1}, inhibition halo < 5 mm and percentage of inhibition < 60%), and absent (total absence of inhibition).

The taxonomic classification of the microalgae was verified using the AlgaeBase database (https://www.algaebase.org). Data were tabulated and analyzed using Excel and RStudio (tidyverse and psych packages). The Shapiro-Wilk normality test was performed to evaluate the distribution of numerical data and descriptive statistics (mean and standard deviation) were then determined.

Figure 1 – Flowchart of the search process and selection of articles



Source: Authors (2023)

3 RESULTS AND DISCUSSION

After the search, 25 articles in English and Portuguese were used to evaluate the antifungal activity of microalgae against phytopathogenic fungi. Among the studies in the articles selected for the search, 19 performed assays on eukaryotic microalgae and 13 performed assays on prokaryotic species (Table 1).

Table 1 – Articles included in this review: bibliometric data, collection site and taxonomic classification of the microalgae studied

(continue)

Reference	Collection locations	Country of origin	Microalgae phylum	Microalgae species
Carneiro et al. (2021)	Microalgae bank	Hungary	Chlorophyta	*Chlorella vulgaris* *Scenedesmus acutus*
Davoodbasha et al. (2018)	Microalgae bank	India	Chlorophyta	*Scenedesmus intermedius*
El Semary et al. (2013)	Nile River (Maadi area) and Wastewater Canal (Hellwan Province)	Egypt	Cyanobacteria	*Aphanizomenon* sp. *Synechocystis salina*
			Ochrophyta	*Poterioochromonas malhamensis*
Herrero et al. (2006)	Microalgae bank	Israel	Chlorophyta	*Dunaliella salina*
Kashif et al. (2018)	Microalgae bank	South Coreia	Chlorophyta	*Tetraselmis* sp.1 *Tetraselmis* sp.2
Martínez et al. (2019)	Microalgae bank	United States	Miozoa	*Amphidinium carterae*
Montalvão et al. (2016)	Aegean Sea, Turkey	Turkey	Bacillariophyta	*Amphora capitellata* *Cylindrotheca closterium* *Nanofrustulum* sp. *Nitzschia* sp. *N. communis* *N. thermalis* *Phaeodactylum tricornutum*
			Cyanobacteria	*Calothrix crustacea* *Geitlerinema* sp. *Halospirulina* sp. *Oscillatoria rosea* *Oscillatoria* sp. *Phormidium* sp. *Pseudoscillatoria* sp.
			Chlorophyta	*Chlorella* sp. *Dunaliella* sp. *D. salina* *Picochlorum* sp.
			Haptophyta	*Ochrosphaera* sp.
			Ochrophyta	*Chrysoreinhardia* sp. *Nannochloropsis* sp.
			Prasinodermatophyta	*Prasinococcus* sp.

Table 1 – Articles included in this review: bibliometric data, collection site and taxonomic classification of the microalgae studied

(continue)

Reference	Collection locations	Country of origin	Microalgae phylum	Microalgae species
Morales-Jiménez et al. (2020)	Microalgae Bank	Portugal	Cyanobacteria	Nostoc *sp.*
				Spirulina maxima
				Synechocystis sp.
Neto et al. (2015)	Santa Catarina Coast	Brazil	Chlorophyta	*Chlorella* sp.
				Haematococcus pluvialis
			Cyanobacteria	*Cylindrospermopsis raciborskii*
				Limnothrix sp.
			Rhodophyta	*Porphyridium cruentum*
Patil and Kaliwal (2019)	Microalgae Bank	India	Chlorophyta	*Scenedesmus bajacalifornicus*
Pawar and Puranik (2008)	Salt soils, fresh waters, rice paddies and alkaline lake located in northern Maharashtra	India	Cyanobacteria	*Oscillatoria limosa*
				Oscillatoria ornata
				Phormidium tenue
				Synechococcus elongatus
				Trichodesmium hildebrantii
Peraman and Nachimuthu (2019)	Microalgae Bank	India	Bacillariophyta	*Chaetoceros calcitrans*
				C. gracilis
				Navicula sp.
				Thalassiosira sp.
			Chlorophyta	*Dunaliella salina*
				Tetraselmis gracilis
				T. trahele
			Haptophyta	*Dicrateria inornata*
				Isochrysis galbana
				Pavlova lutheri
Ranglová et al. (2021)	Microalgae Bank	Hungary	Chlorophyta	*Chlorella vulgaris*
Righini et al. (2020)	Microalgae Bank	Spain	Cyanobacteria	*Arthrospira platenses*
Righini et al. (2021)	Microalgae Bank	Spain	Cyanobacteria	*Anabaena minutíssima*
Rodríguez-Meizoso et al. (2008)	Microalgae Bank	Spain	Cyanobacteria	*Phormidium* sp.
Santoyo et al. (2009)	Microalgae Bank	Spain	Chlorophyta	*Haematococcus pluvialis*
Scaglioni et al. (2019 a)	Microalgae Bank	Brazil	Cyanobacteria	*Spirulina* sp.
			Ochrophyta	*Nannochloropsis* sp.
Scaglioni et al. (2019 b)	Microalgae Bank	Brazil	Cyanobacteria	*Spirulina* sp.
			Ochrophyta	*Nannochloropsis* sp.

Table 1 – Articles included in this review: bibliometric data, collection site and taxonomic classification of the microalgae studied

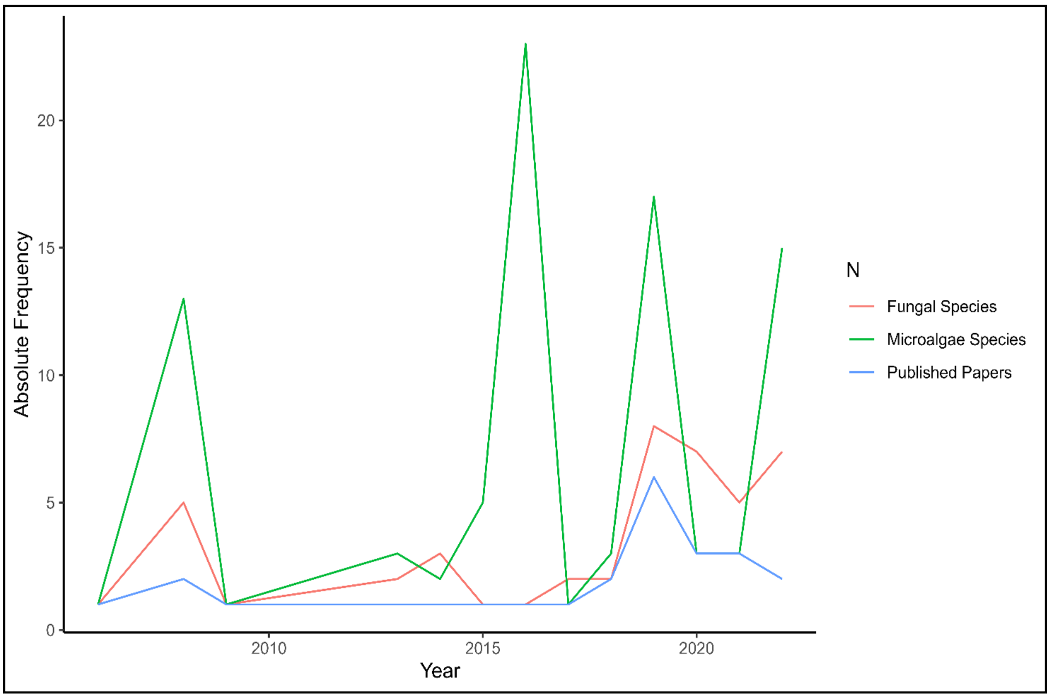
(conclusion)

Reference	Collection locations	Country of origin	Microalgae phylum	Microalgae species
Schmid et al. (2022)	Microalgae Bank	Portugal	Bacillariophyta	*Phaeodactylum tricornutum*
			Chlorophyta	*Chlorella vulgaris*
				Scenedesmus obliquus
			Cyanobacteria	*Spirulina* sp.
			Ochrophyta	*Nannochloropsis* sp.
Senousy et al. (2022)	Water drainage station in Bahr Hadus and rice and wheat field soils in El--Sharkia province	Egypt	Chlorophyta	*Chlorella sorokiniana*
				Dunaliella sp.
			Cyanobacteria	*Anabaena sp.*
				Aphanizomenon gracile
				Dolichospermum circinale
				D. crassum
				D. spiroides
				Oscillatoria nigroviridis
				O. sancta
				Wollea saccata
Sun et al. (2017)	Microalgae Bank	China	Chlorophyta	*Chlamydomonas* sp.
Suresh et al. (2014)	Manjalar dam in Tamil Nadu	India	Cyanobacteria	*Nostoc spongiforme*
				Oscillatoria tenius
Vehapi et al. (2019)	Microalgae Bank	Turkey	Chlorophyta	*Chlorella minutíssima*
				C. protothecoides
				C. vulgaris
Zielinski et al. (2020)	Microalgae Bank	United States	Chlorophyta	*Chlorella vulgaris*

Source: Authors (2023)

The first studies were published in the 2000s and focused on Chlorophyceae and Cyanobacteria (Herrero et al. 2006; Pawar and Puranik 2008; Rodríguez-Meizoso et al. 2008; Santoyo et al. 2009). A greater number of published articles were concentrated from 2015 to 2022, as were the number of species (fungi and microalgae) studied (Figure 2). This is consistent with the fact that this period had greater biotechnological advancement and increased interest in bioprospecting for novel biopesticides, such as antifungal compounds from microalgae (Kumar et al. 2021; Murata et al. 2021).

Figure 2 – Absolute frequency of published articles, species of microalgae studied and species of phytopathogenic fungi submitted to antifungal assays, grouped by year of publication

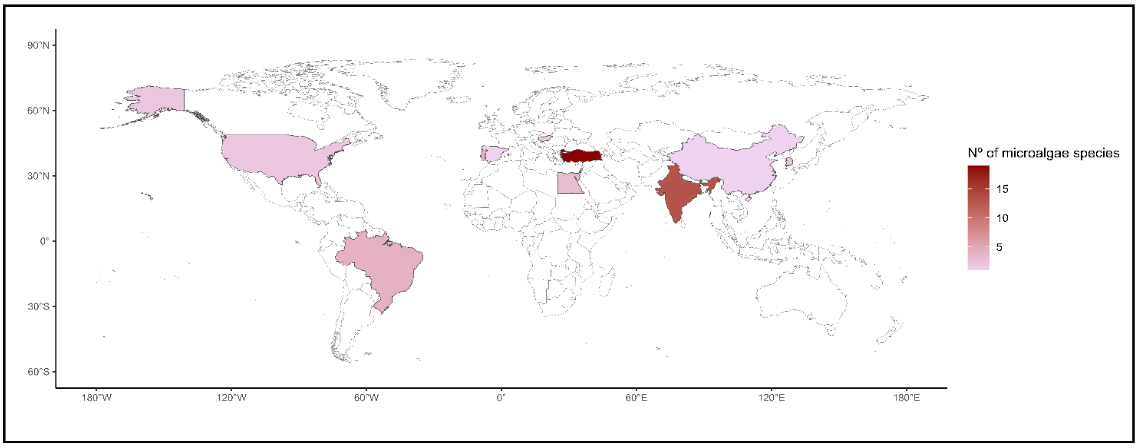


Source: Authors (2023)

The microalgal species studied were from four continents and distributed across 11 countries (Figure 3). It can be observed that a greater number of species (n > 10) was studied in Turkey and India, which are highlighted on the map (Figure 3). Other countries, such as the United States, Brazil, Spain, Egypt, Hungary, Portugal, China, South Korea, and Israel, had fewer studied species (n < 5).

Although the largest number of publications and species of microalgae studied are concentrated, respectively, in India (5), Spain (4), and Brazil (3) and in India (27), Turkey (26), and Egypt (13) (Table 1; Figure 3), the number of microalgal patents linked to agriculture is most concentrated in the United States (23.2%), China (13.6%), Canada (7.2%), and Mexico (6.4%) (Murata et al. 2021).

Figure 3 – Absolute frequency of articles distributed by country of origin of microalgal collection



Source: Authors (2023)

In the selected articles, eight phyla of microalgae (Bacillariophyta, Chlorophyta, Cyanobacteria, Haptophyta, Miozoa, Ochrophyta, Prasinodermatophyta and Rhodophyta) were tested. Cyanobacteria and Chlorophyta had the largest number of microalgal species studied, accounting for 37 and 20 species, respectively (Figure 4). Next were Bacillariophyta (11), Haptophyta (4), and Ochrophyta (3) (Figure 4). The other phyla (Miozoa, Prasinodermatophyta, and Rhodophyta) had only one species evaluated for antifungal potential (Figure 4).

The most studied genera of eukaryotic microalgae were *Chlorella*, *Chaetoceros*, *Dunaliella*, *Nannochloropsis*, *Scenedesmus* and *Tetraselmis* (Table 1). The most studied species were *C. vulgaris*, *D. salina*, *H. pluvialis* and *P. tricornutum* (Table 1). Among the prokaryotic genera, the predominant were *Anabaena*, *Dolichospermum*, *Oscilatoria, Phormidium, Spirulina* and *Synechocystis* (Table 1), with equal frequencies among the species studied.

Microalgae are a diverse group from phylogenetic and evolutionary perspectives, encompassing prokaryotic cyanobacteria and eukaryotic microalgae (Stirk and Staden 2022). Approximately 41,000 species of microalgae, distributed in 38 classes, have been described and elucidated to date (Sexton and Lomas 2018). Given the great biodiversity of this group, the 48 species and eight phyla detected in this literature review (Figure 4) reflect the sparse exploration of the antifungal potential of microalgae. Thus, there is a promising gap to be filled by new prospective antifungal studies that encompass different species of microalgae.

The phyla Chlorophyta and Cyanobacteria were the most explored in relation to the number of microalgal species prospected and the frequency of antifungal assays performed (Figures 4 and 5). These results can be attributed to the fact that these taxa are more commonly found in laboratory collections and microalgal banks. Considering that these are groups with wide biodiversity Chlorophyta, with approximately 8,000 described species (Bowles et al. 2022), and Cyanobacteria, with approximately 3,000 (Nabout et al. 2013), many other species can be used to detect antifungal biomolecules.

Among the eukaryotic microalgae, Chlorophyta is the phylum with the greatest diversity of biologically active compounds (Baudelet et al. 2017). Several biological properties have been associated with this phylum, including antibacterial, antiviral, antiprotozoal, and antifungal (Saeed et al. 2022). Regarding the antifungal potential, despite the wide range of human, animal and plant fungi already explored, few species of eukaryotic microalgae have been contemplated so far, thus leading to the underestimation of the great biodiversity that this group presents (Falaise et al. 2016; Lage et al. 2022).

The phylum Cyanobacteria are popularly called “blue-green algae”, although they are prokaryotic organisms and inserted in the domain of bacteria (Righini et al. 2022). Owing to their high rate of adaptation to fluctuating environmental conditions and the pressure of competing organisms, they produce several defense mechanisms, including metabolites with essential biotechnological applications (Zahra et al. 2020). Among the biological activities already reported, the ones that stand out are antioxidant, antitumor, antibacterial, antiviral, antiprotozoal, and antifungal (Saeed et al. 2022). Cyanobacteria have been investigated for their antifungal activities against a wide range of pharmaceutical and agricultural fungi (Lage et al. 2022; Righini et al. 2022).

The “diatoms”, included in the phylum Bacillariophyta, are among the most diverse groups of microalgae, with an estimated 100,000 species (Archibaldi et al. 2017). Despite their great biodiversity, only 11 species have been investigated against phytopathogens. Antiviral (Fábregas et al. 1999), antifungal (Qasem et al. 2016), and antibacterial (Torres-Bayona et al. 2023) activities have been reported in species of this phylum.

The phylum Haptophyta is composed of “golden-brown algae” which are a well-defined group divided into two classes, Pavlovophyceae and Coccolithophyceae (Prymnesiophyceae), with 330 described species (Archibaldi et al. 2017). Four species of microalgae have been studied for activity against phytopathogenic fungi (Montalvão et al. 2016; Peraman and Nachimuthu 2019), however, previous work has found antiviral (Fábregas et al. 1999), antibacterial (Bashir et al. 2018; Torres-Bayona et al. 2023), and antifungal (Garcia-Mosaica and Rio-Garati 2022) activities in extracts and compounds of other species.

Ochrophyta is a phylum of “brown algae” that encompasses approximately 300 genera (Silberfeld et al. 2014). Among these, three species have been studied for their activity against phytopathogenic fungi (El Semary et al. 2013; Montalvão et al. 2016). Other studies have reported antiviral (Yanuhar et al. 2011), antifungal (Najdenski et al. 2013; Qasem et al. 2016), and antibacterial (Cepas et al. 2021) activities of this phylum.

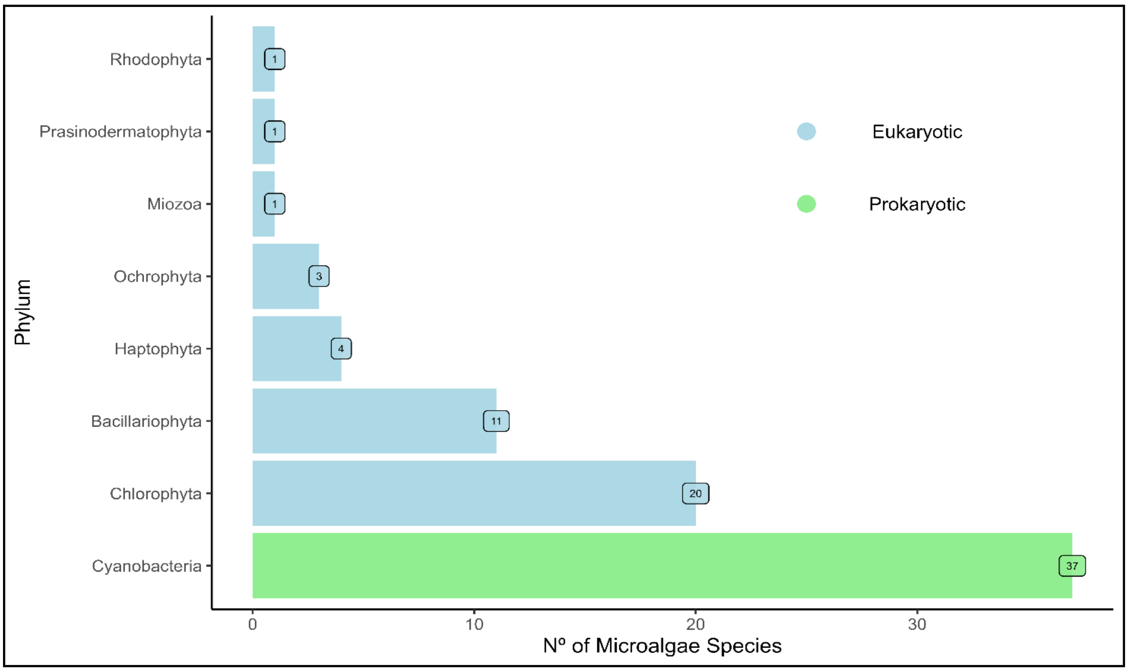
The phylum Miozoa encompasses the so-called “dinoflagellates”, which contains approximately 4,500 species and concentrates most of the natural products isolated from microalgae (Gallardo-Rodrigues et al. 2012; Archibaldi et al. 2017). Only one compound isolated from the species *A. carterae* has been tested against phytopathogenic fungi (Martínez et al. 2019), even though antiviral (Yim et al. 2004; Hermawan et al. 2019), antiprotozoal (Washida et al. 2006), antibacterial (Washida et al. 2006; Torres-Bayona et al. 2023) and antifungal (Washida et al. 2006; Satake et al. 2017) activities have already been detected in other species.

Prasinodermatophyta is a recently described phylum of “green algae” that is associated with the origin of green plants (Viridiplantae), along with the taxa

Chlorophyta and Streptophyta (Bowles et al. 2022). In this study, only one species of Prasinodermatophyta was investigated for its antifungal potential against phytopathogens (Montalvão et al. 2016). No other studies have reported the biological activity of microalgal species in this group.

The phylum Rhodophyta contains only one species of microalgae (*P. cruentum)* investigated for its antifungal potential (Neto et al. 2015). However, the "red algae" group has more than 7,100 described species (Archibaldi et al. 2017), including macro and microalgae, and relevant biological activities have already been recorded, such as antiviral (Fábregas et al. 1999), antibacterial (Najdenski et al. 2013; Bashir et al. 2018), and antifungal (Najdenski et al. 2013) activities.

Figure 4 – Number of microalgal species distributed by taxonomic classification (phylum). Color scale: level of cellular organization

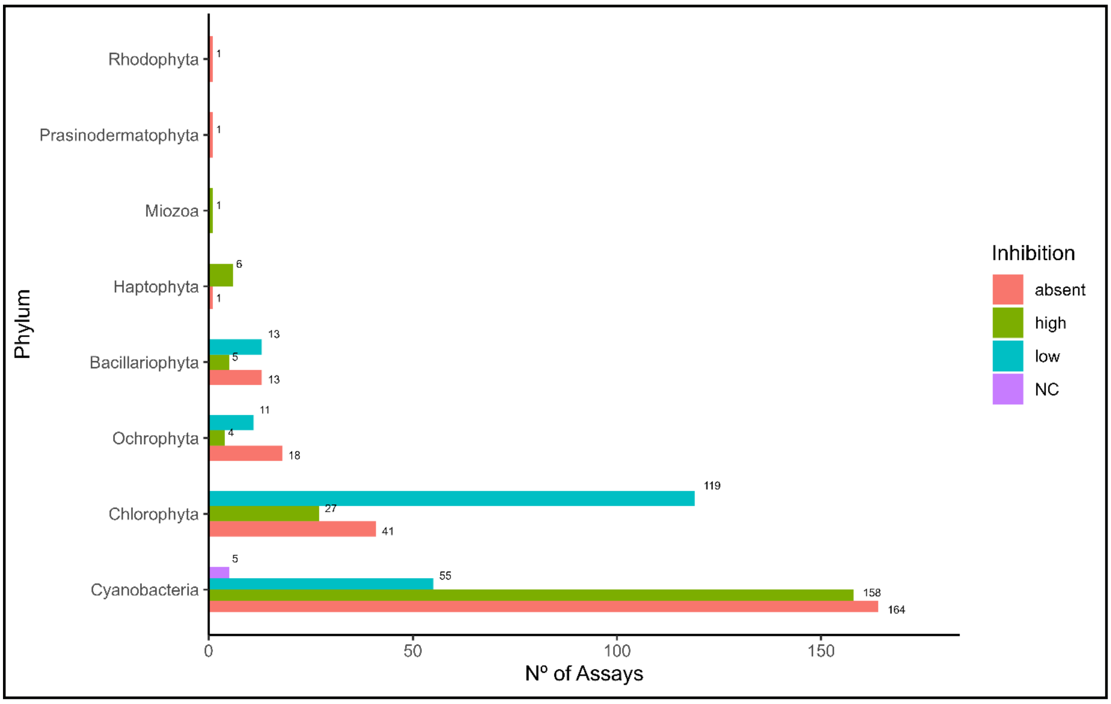


Source: Authors (2023)

A total of 643 antifungal assays were performed using microalgal extracts and compounds against phytopathogenic fungi. Considering the frequency of antifungal assays performed per microalgae phylum (Figure 5), the taxa with the highest number of assays performed were Cyanobacteria (59.41%) and Chlorophyta (29.08%), both with more than 150 experiments, in contrast to the other phyla that had fewer than 50 assays performed each.

Regarding the categorized inhibition classification for the assays, high inhibition was observed in 31.26%, low inhibition in 30.79%, and absent in 37.17%. Considering the phyla with more than 30 experiments performed, we can observe that the assays with Cyanobacteria species showed the highest proportion of high inhibition (41.36%), followed by Bacillariophyta (16.13%), Chlorophyta (14.44%), and Ochrophyta (12.12%) (Figure 5). The lowest inhibition occurred with the highest proportion in the phylum Chlorophyta (63.63%), followed by Bacillariophyta (41.93%), Ochrophyta (33.33%), and Cyanobacteria (14.40%) (Figure 5). Ochrophyta presented the highest proportion of assays without inhibition (54.54%), followed by Cyanobacteria (42.93%), Bacillariophyta (41.93%), and Chlorophyta (21.93%) (Figure 5).

Figure 5 – Classification of inhibition of antifungal assays performed by taxon (phylum) of microalgae. Color scale: Classification categories of the inhibition found in the assays. NC: Not classified

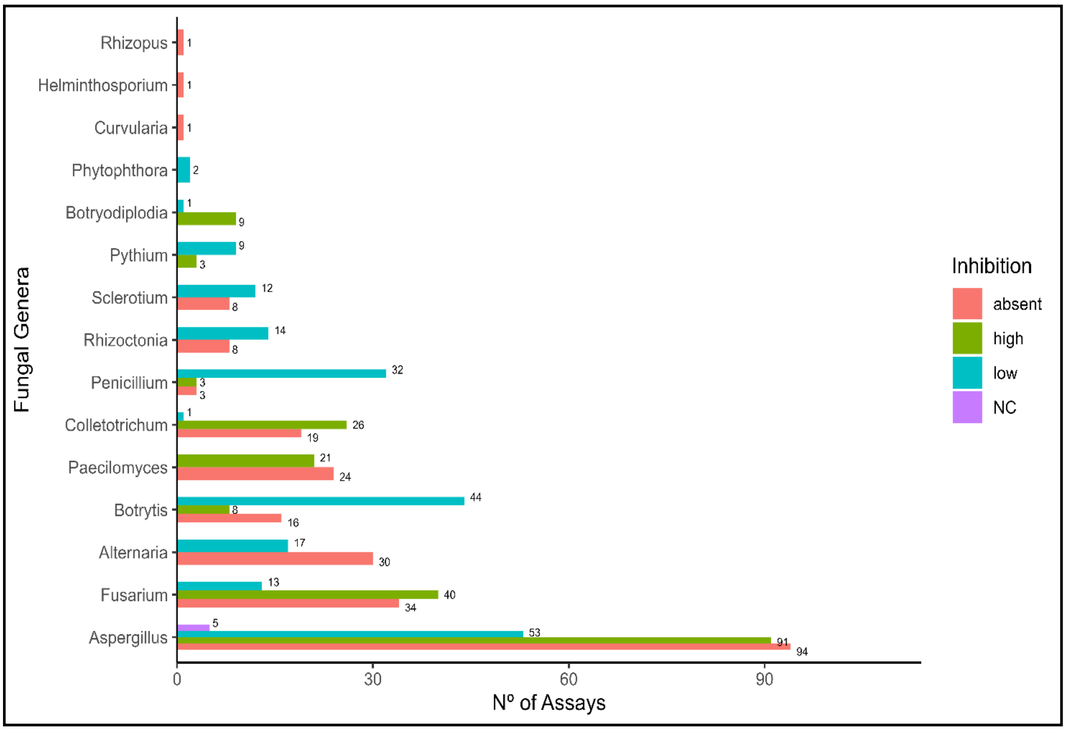


Source: Authors (2023)

The most tested plant pathogen species were *Aspergillus flavus, A. niger, A. fumigatus, Botrytis cinerea*, *Fusarium oxysporum, Pythium ultimum,* and *Rhizoctonia solani* (Figure 6), which is consistent with the fact that these microorganisms are frequently identified as infecting plants and generate major impacts on agriculture (Perrone et al. 2007; Anees et al. 2010; Dean et al. 2012). Among these fungi, *B. cinerea* and *F. oxysporum* were cited in the list of 10 phytopathogenic fungi with the greatest scientific and agronomic importance (Dean et al. 2012; Kaur 2019), in a study conducted by the Journal of Molecular and Plant Biology based on votes from the international scientific community.

Regarding the categorized inhibition classification for the assays, most of the fungal genera evaluated showed low inhibition or absence of susceptibility to the extracts or compounds of the microalgae tested (Figure 6). Among the fungal genera for with the number of tests performed equaled or exceeded 30, the highest proportion of assays with high inhibition was exhibited by *Colletotrichum* (56.53%), followed by *Paecilomyces* (46.66%), *Fusarium* (45.98%), *Aspergillus* (37.45%), *Botrytis* (11.76%), and *Penicillium* (7.89%). In contrast, the genus *Alternaria* presented the highest proportion of assays without inhibition (63.83%), followed by *Paecilomyces* (53.33%), *Colletotrichum* (41.30%), *Fusarium* (39.08%), *Aspergillus* (38.68%), *Botrytis* (23.53%), and *Penicillium* (7.89%).

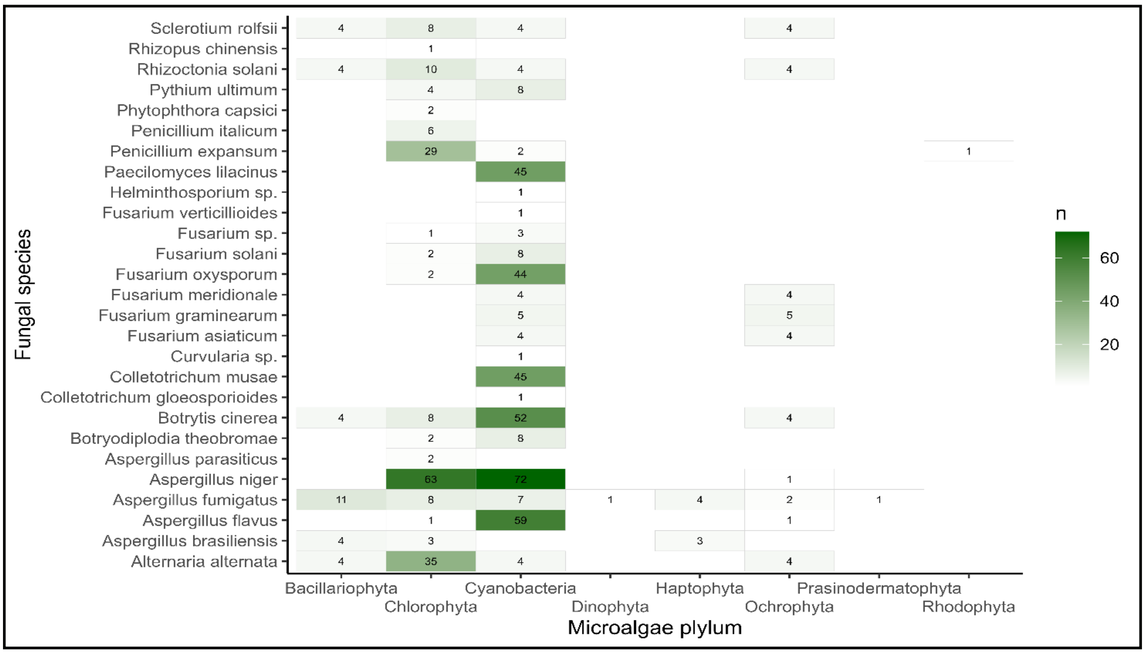
Figure 6 – Absolute frequency of antifungal assays performed distributed by genus of phytopathogenic fungi. NC: Not classified



Source: Authors (2023)

The antifungal assays performed with extracts or compounds of microalgae were concentrated in two phyla and eight species of fungi, as shown in Figure 7, by the heat map that illustrates the absolute frequency of experiments carried out by phyla of microalgae and species of phytopathogenic fungus. Species of the phylum Cyanobacteria were further tested against fungi A. *niger, A. flavus, B. cinerea, P. lilacinus, C. musea*, and *F. oxysporum* (Figure 7). Species belonging to the phylum Chlorophyta were further tested against *A. niger, A. alternata*, and *P. expansum* (Figure 7). The low diversity of microalgae and phytopathogenic fungal species reveals a field open to the bioprospection of sustainable fungicidal pesticides.

Figure 7 – Heat map derived from the absolute frequency of tests performed against species of phytopathogenic fungi by phylum of microalgae



Source: Authors (2023)

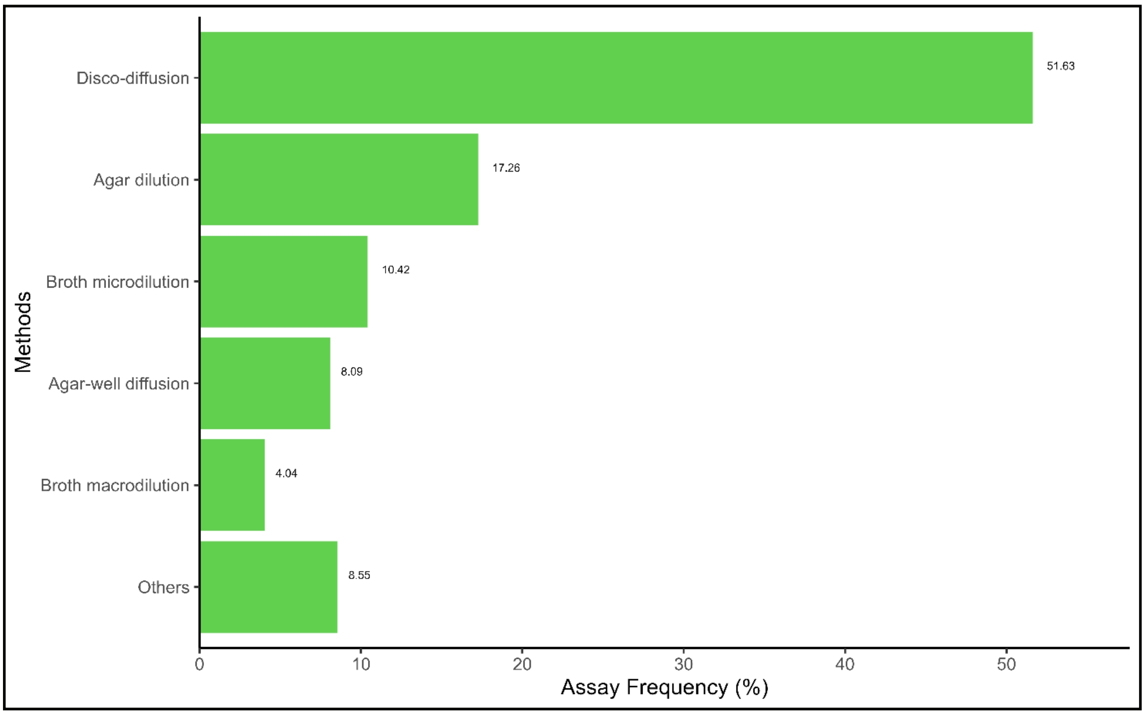
The methodologies for the evaluation of the antifungal potential were used in the articles individually or together, the most frequent being disc-diffusion (51.63%), agar dilution (17.26%), broth microdilution (10.42%), agar perforation (8.09%) and broth macrodilution (4.04%) (Figure 8). The other methodologies used totaled the frequency of 8.55% (Figure 8).

Analyzing the results obtained, it is observed that the methods of diffusion (disc-diffusion, dilution in agar and perforation in agar) and dilution (micro and macrodilution in broth) were the most used according to this review, which is consistent with the fact that these are the most known and easy methods to apply, thus being more commonly used (Balouiri et al. 2016).

A wide variety of laboratory methods can be used to evaluate or track antimicrobial activity of an extract or compound. Other relevant methodologies for the evaluation of antimicrobial activity, such as cytofluorometric method, ATP bioluminescence assay, time-kill test, thin layer chromatography (TLC) and antimicrobial gradient method (Etest) were little performed or absent in this review, which shows a gap to be filled in the area.

Among the solvents used to obtain the extracts or microalgae biocompounds, water and methanol stand out, used in 50.23% and 14.86% of the assays, respectively. Among the published studies, 24% (6/25) did not perform chemical analysis of the extracts. Gas Chromatography coupled to Mass Spectrometry (GC-MS) or Flame Ionization (CG-FID) detectors were the most used chemical analysis methodologies in the studies (28%), followed by High Performance Liquid Chromatography (HPLC) coupled to diode (DAD), ultraviolet (UV) and mass (MS) detectors (20%) and Fourier Transform Infrared Spectroscopy (FTIR) analysis (20%).

Figure 8 – Frequency in percentage of methodologies tested



Source: Authors (2023)

Considering the size of the halo generated and the concentrations tested using the agar diffusion methodology, the extracts of the microalgae *Nannochloropsis* sp. and *Spirulina* sp. exhibited antifungal activity at the lowest concentration tested (40 µg/mL) against the phytopathogenic fungi *Fusarium asiaticum*, F. *graminearum*, and *F. meridionale* (Scaglioni et al. 2019a; Scaglioni et al. 2019b). Comparing the MIC and

MFC values obtained by the broth microdilution methodology, the isolated compound "Amphidinol 22" from the microalgae *Amphidinium carterae* had a high antifungal performance against the phytopathogen *Aspergillus fumigatus*, with a MIC of 64 μg/mL (Martínez et al. 2019). In the broth macrodilution methodology, the lowest MIC value obtained was 58.88 μg/mL, referring to the cyanobacterium *P. tenue* against the phytopathogen *A. niger* (Pawar and Puranik 2008). The lack of standardization and great variability of methodologies used in the articles prevented statistical analyses and robust comparisons between the data obtained.

In the present review, eight phyla of microalgae were detected in the articles searched, but it is known that this group of microalgae, despite not having a well-defined taxonomic classification, encompasses many other taxa, such as the phyla Charophyta, Chrysophyta, Euglenophyta, Raphidophyta, Xanthophyta and Zygnematophyta, which did not have their extracts or isolated compounds tested against phytopathogenic fungi. Given the great biodiversity of microalgae, this result reinforces the fact that many species have not yet been explored for their inhibitory activity against phytopathogenic fungi.

4 CONCLUSIONS

Despite the great diversity and rich composition of biomolecules, there are limited studies on the antifungal activity of microalgae against phytopathogenic fungi, and only eight phyla have been considered (Bacillariophyta, Chlorophyta, Cyanobacteria, Haptophyta, Miozoa, Ochrophyta, Prasinodermatophyta, and Rhodophyta). Among them, Chlorophyta and Cyanobacteria had the largest number of registered publications, microalgal species studied, antifungal assays performed and were also the groups with the largest number of species of phytopathogenic fungi tested. The phylum that presented extracts or biocompounds of microalgal species with the highest proportion of high inhibitory activity against phytopathogens was Cyanobacteria.

The most tested species of phytopathogenic fungi were *Aspergillus niger* and

Botrytis cinerea. A higher number of publications was recorded from 2015 to 2022, and a greater number of publications was associated with India, Turkey, and Spain. The lack of standardization of antifungal tests makes it difficult to perform a more robust comparative analysis of the results as well as statistical analyses. Considering the need for novel biopesticides to control phytopathogenic fungi, bioactive compounds from microalgae can be exploited.

Considering that only approximately 0.12% of microalgal biodiversity has been contemplated in studies on phytopathogenic fungi, there are still a large number of secondary metabolites to be explored. In addition, no studies have evaluated the additive effects of microalgal extracts together with pesticides commonly used in the control of agricultural pests, a gap in the literature to be filled.

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