In vitro STUDIES OF THE ANTAGONISTIC EFFECT OF SELECTED FUNGI ON *Bipolaris sorokiniana* (Sacc.) Shoem.

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ABSTRACT

Natural protection of plants against diseases, pests and environmental stresses is the only acceptable alternative to the progressive application of chemicals in plant production. Amidst evolving climatic patterns, various diseases pose significant threats to crop plant production. Among these concerns, a prominent menace across multiple regions of the world is seedling blight, incited by the pathogenic agent *Bipolaris sorokiniana Sacc*. The antagonistic effect may occur in/on the host itself or in its vicinity in the case of saprotrophic organisms. *B. sorokiniana* attacks many species of crop plants, especially in warmer growing areas and causes significant losses of field emergence and yield. This study aimed to assess the inhibitory impact of selected microscopic fungi on the growth and development of *B. sorokiniana* through dual-culture experiments. The study also aimed to identify potential fungal candidates for the biocontrol of seedling blight caused by this pathogen. The outcomes demonstrated that only several of the fungi subjected to testing had a noteworthy influence on the growth of *B. sorokiniana*. The presence of a few fungi species, such as *Trichoderma viride*, *Fusarium graminearum*, and *Botrytis cinerea* led to a decrease in *B. sorokiniana* growth by a minimum of 50%. In the instance of other fungi such as *Sordaria fimicola*, *Epicoccum nigrum*, *Fusarium sporotrichioides*, *F. culmorum*, and *Nigrospora oryzae*, the reduction amounted to at least 40%. The vast majority (75%) of the fungal species used in the test limited the growth of *Bipolaris* colonies by up to 39%.

Keywords: antagonistic effect of fungi, *Bipolaris sorokiniana*, biological control

INTRODUCTION

The fungus *Bipolaris sorokiniana* is a known pathogen that causes common root rot, leaf spot disease, seedling blight and black point. This pathogen occurs on seeds of many species of crop plants, including barley and wheat, as well as vegetables, e.g. carrots, parsley, and peas [Al-Sadi 2021, Dutbayev et al. 2022]. Plants are susceptible to infection at any stage of their growth, especially seedlings, and the decrease in field emergence and yield can reach over 30% [Iftikhar et al. 2009, Vaish et al. 2011, Ghazvini and Tekauz 2012]. The source of infection may be either seed or soil. In the case of infected seeds, infection occurs through the mycelium located between the chaff and the fruit seed cover and the surface of the seeds is contaminated through conidia [Kumar et al. 2001]. The frequency of seed infection by *B. sorokiniana* depends on the amount of infectious material in the plant close environment during flowering and seed formation. Most often, this involves conidia carried by air currents from infected leaves. High humidity favors the assimilation apparatus of the leaves, sporulation and seed infection [Couture and Sutton 1978].

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Diseased seedlings grow out of the infected seed, and the fungus forms conidia, causing secondary infections on the sheaths and leaf blades.

This fungus is a warm climate zone pathogen, and its presence in a cooler climate should not cause serious damage to crops [Kumar et al. 2002]. However, due to the dynamic climate changes taking place in Europe, there has been an increasing loss in yield caused by *B. sorokiniana*, so it was decided that this species should be dealt with more widely. The pathogen survives as mycelium in infected seeds or as a saprotroph on dead plant tissues [Barba et al. 2002]. In cocci, mycelium may remain viable for up to four years [Christensen 1963]. Additionally, *B. sorokiniana* is an optional parasite that can live saprophytically in soil, which was confirmed by studies in which its propagules were detected or isolated from soil samples [Malaker et al. 2007, Zhang et al. 2022]. It survives as thick-walled spores, which are the main causative agent of root rot [Clark and Wallen 1969]. The important issue is the pathogenic specialization of this species, which was first described by Christensen [1922]. It is well known that the populations of this pathogen are characterized by a high variability of virulence.

The occurrence and harmfulness of this pathogen depend on many factors, especially on the growing area, weather conditions, susceptibility of the cultivars, and pathogens occurring in this region and their virulence. In the course of the pathogenic process, plants, on the other hand, are influenced by the interactions between microorganisms within the rhizosphere and phyllosphere [Knudsen et al. 1995, Dutbayev et al. 2022]. The antagonistic interaction may occur in/ on the host itself or in its vicinity in the case of saprotrophic organisms. Biological control of plant pathogens can be effective, especially with the potential of antagonists on pathogenic fungi [Darshan et al. 2020]. This is possible because fungi have a comparatively high reproductive rate (sexually as well as asexually), a short generation time, and are target-specific [Thambugala et al. 2020]. In the absence of the host organism, they can survive in the environment, shifting their mode of parasitism to saprotrophism thus maintaining sustainability. Many fungal species have been described as having mechanisms that allow them to efficiently protect plants from diseases caused by plant pathogenic fungi [Baker 1987, Howell 2003,

Heydari and Pessarakli 2010]. Currently, along with emerging care for the environment, biological control is widely commercialized, and several microbial antagonists have been patented and introduced to the market [Thambugala et al. 2020].

The use of antagonism is a promising strategy for combating *B. sorokiniana*. Some potential approaches include utilizing antagonistic fungal species, developing biopesticides, modifying microbial flora in the soil, implementing agricultural practices or stimulating plant immunity. Antagonistic fungal species such as *Trichoderma viride* or *Bacillus amyloliquefaciens* strains can be used as biological agents against *B. sorokiniana*. These beneficial species can be introduced into the cultivation environment to suppress the growth and development of the pathogen [Alippi et al. 2000, Al-Sadi 2021]. However, pathogenic species such as *Fusarium graminearum* and *Botrytis cinerea* can also have an antagonistic impact on *B. sorokiniana* and inhibit its growth and development. Certain antagonistic fungal species can induce defensive responses in plants, making them more resilient to pathogen attacks, including those by *B. sorokiniana* [Sarkar et al. 2019]. Biopesticides based on antagonistic fungal species can be developed as an alternative to traditional chemical agents. These formulations can contribute to the reduction of negative environmental impacts. Modifying soil microbiota through the introduction of antagonistic fungi can provide natural defense mechanisms against pathogens, including *B. sorokiniana* [Al-Sadi 2021].

In the authors' previous studies, during seed health analysis, varied growth of *B. sorokiniana* was observed despite identical conditions and incubation time (unpublished data, fig. 1). The only difference regarding the growth conditions of this pathogen was the presence of other microscopic fungi colonizing the tested sowing material. This prompted the undertaking of research on the impact of the most commonly isolated fungi from seeds on the growth and development of *B. sorokiniana*. Moreover, the rapidly progressing climate changes may cause an increase in the occurrence and harmfulness of this species, and the search for alternative methods of crop protection for chemical protection also inspired the presented research.

The research hypothesis assumes that the presence of various fungi, encompassing both pathogenic and
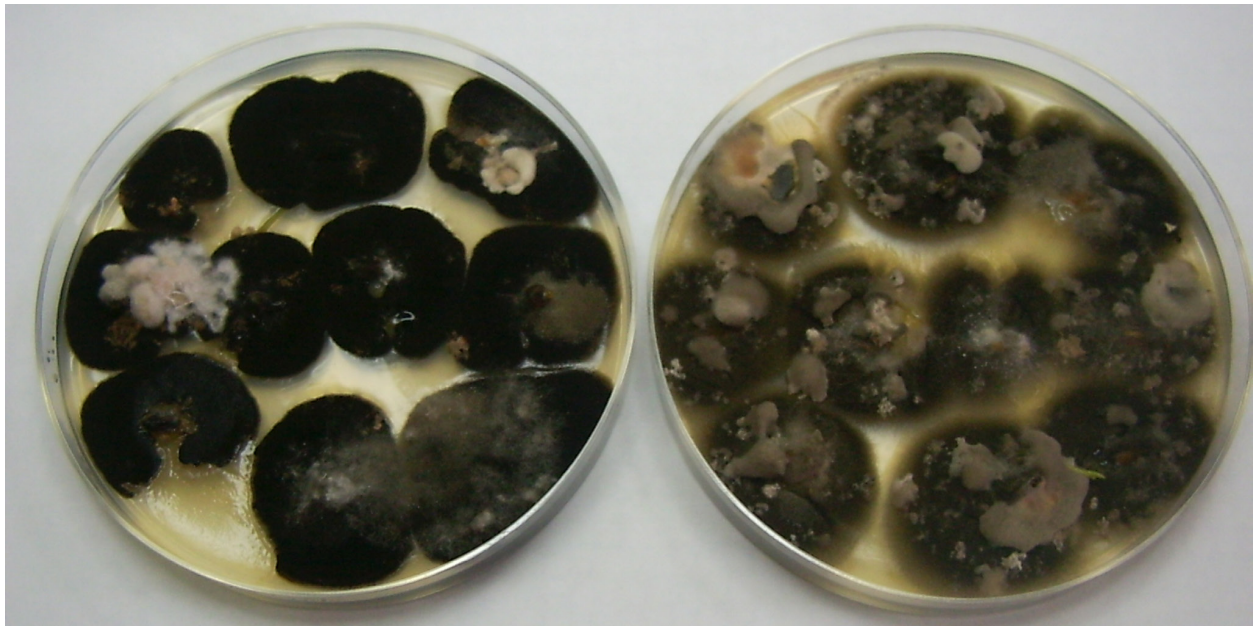
Fig. 1. Appearance of colonies of different isolates of *B. sorokiniana*

saprotrophic species, alongside *B. sorokiniana*, exerts inhibitory effects on the growth of *B. sorokiniana*. These inhibitory interactions, mostly in non-pathogenic species to plants, hold promise for their potential application in the realm of biological protection strategies.

MATERIALS AND METHODS

Preparation of the inoculum

The pathogenicity of two isolates (BS1Rap, BS2Rap) from parsley and three isolates (BS3Ra, BS4Ra, BS5Ra) from barley were tested. The isolate BS2Rap with the most pathogenic ability to seeds (51% ungerminated seeds and 32% infected seedlings) was used for further research.

The inoculum was prepared from a monospore culture of *B. sorokiniana*, BS2Rap isolate which was incubated for 14 days at 24°C and alternating 360 nm NUV lighting and darkness (12 h / 12 h). The pathogen was identified according to the morphological characters and sequences of the internal transcribed spacer (ITS) regions of fungal ribosomal DNA (rDNA) amplified by specific primer pair ITS1 and ITS4 [White et al. 1990].

Both *B. sorokiniana* species and other fungi used in the experiment were isolated from seed samples (500 parsley seeds and 500 barley seeds), which were disinfected with 1% NaOCl for 10 min and then washed with sterile water three times. Disinfected seeds were placed on a potato–dextrose–agar medium (PDA) with 0.003% streptomycin sulphate. Fungal colonies were grown at 20°C in alternating cycles of 12 h NUV light (360 nm) and 12 h darkness. Developed colonies were transferred to potato-dextrose-agar plates and incubated in the above-mentioned conditions to stimulate sporulation. Fungi were identified after 15–20 days of incubation according to the descriptions by Chidambaram et al. [1972], Kwaśna et al. [1991] and Malone and Muskett [1997]. Fungal isolates (pure cultures) were maintained on potato-dextrose-agar (PDA) slants respectively at 4°C until used for further study. In case of uncertainty, the identification of some fungi (*B. sorokiniana*, *Trichoderma viride* and some *Fusarium* species) was confirmed by rDNA sequence analysis [White et al. 1990].

Further research involved pure cultures of *B. sorokiniana*, *Alternaria alternata*, *Aspergillus* sp., *Botrytis cinerea*, *Phoma* sp. *Cladosporium herbarum*, *Epicoccum nigrum*, *Fusarium avenaceum*, *F. culmorum*,

F. oxysporum, *F. equiseti*, *Stemphylium botryosum*, *S. consortiale*, isolated from parsley seed and *Acremonium* sp., *Chaetomium globosum*, *Colletotrichum* sp., *Drechslera dematioidea*, *D. teres*, *F. graminearum*, *F. heterosporium*, *F. fujikuroi*, *F. poae*, *F. semitectum*, *F. solani*, *F. sporotrichioides*, *F. tricinctum*, *Microdochium nivale*, *Nigrospora oryzae*, *Papularia arundinis*, *Papulaspora* sp., *Sordaria fimicola* and *Trichoderma viride* isolated from barley.

Antagonistic assay

The antimicrobial capacity of selected fungi was evaluated using a dual solid culture assay in Petri dishes. Dual cultures were created from *B. sorokiniana,* and one of the previously isolated species inoculated on PDA plates at a distance of 2 cm from each other. Three replicates of each pathogen-potential antagonist interaction were prepared. After six days of incubation, the effects of interaction were assessed by measuring the mycelial growth (i.e. colony diameter) of these fungi and comparing them with the diameter of a pure culture grown under the same conditions. Two diameters perpendicular to each pathogenic colony were measured for all plates. The percent inhibition of radial growth (PIRG) of *B. sorokiniana* was calculated according to the formula:

$$PIRG = (A - B)/A \cdot 100 \, [\%],$$

where A – mycelial growth of the pathogen (*B. sorokiniana*) on the control plate, B – mycelial growth of the pathogen on the dual culture plate of tested fungal species [Sundar et al. 1995]. Experimental research complied with relevant institutional guidelines. Obtained values were further analyzed using the Kruscal-Wallis test, and the Tukey test was then conducted to find the differences among dual cultures used in the experiment.

RESULTS

The results derived from the investigations into the intricate interactions among the fungi under scrutiny unveil a notable and impactful observation: every single one of them exerted an influence on the growth of *B. sorokiniana*. Among these fungal interactions, a particular group emerged as a strong contender in in-

hibiting the growth of *B. sorokiniana*. The presence of *Trichoderma viride*, *Fusarium graminearum*, *Botrytis cinerea*, *Sordaria fimicola*, *Epicoccum purpurascens*, *Fusarium sporotrichioides*, *F. culmorum*, *Nigrospora oryzae*, *Papularia arundinis*, *Phoma sp.*, *Drechslera dematioidea*, and *Alternaria alternata* induced the most notable suppression of *B. sorokiniana* growth. In effect, these aforementioned fungi prompted the most substantial percentage reductions in the size of these fungus colonies when compared to their growth in pure culture conditions. For instance, *Trichoderma viride* brought about a remarkable reduction of 41.6% (PIRG 58.4%), while *Fusarium graminearum* induced a significant decrease of 49.2% (PIRG 50.8%), and *Botrytis cinerea* exhibited an equally impressive reduction of 50.9% (PIRG 49.1%) (fig. 2). However, two of these species (*F. graminearum* and *B. cinerea*) are pathogens and, despite their positive effect on limiting the growth of *B. sorokiniana*, cannot be used in biological protection.

What is particularly noteworthy is that the majority of the examined fungi, comprising 18 species (equivalent to 58.1% of the total), demonstrated notably elevated PIRG values, surpassing the 30% threshold. This suggests a substantial inhibitory effect on *Bipolaris* growth due to competition. Conversely, the remaining assessed fungal species exhibited varying degrees of impact on *Bipolaris* growth: three species showed only minor limitations (with PIRG values below 10%), while six species imposed moderate constraints (with PIRG values ranging from 10% to 30%). However, it is worth noting that not all tested fungi displayed the same antagonistic prowess against *B. sorokiniana*. Fungi such as *F. oxysporum*, *F. tricinctum*, and *Acremonium* sp. did not exhibit discernible antagonistic effects against *B. sorokiniana* in this context. The remaining fungal species demonstrated a comparatively milder inhibitory impact on the growth of the examined fungus. These findings underscore the complexity of competitive interactions among these fungi and their potential implications for ecological dynamics.

Below, a visual representation is provided of the diverse array of reactions exhibited by *B. sorokiniana* in response to the presence of other fungal species, each distinctively influencing the size and morphology of the colonies. Differences were observed in the
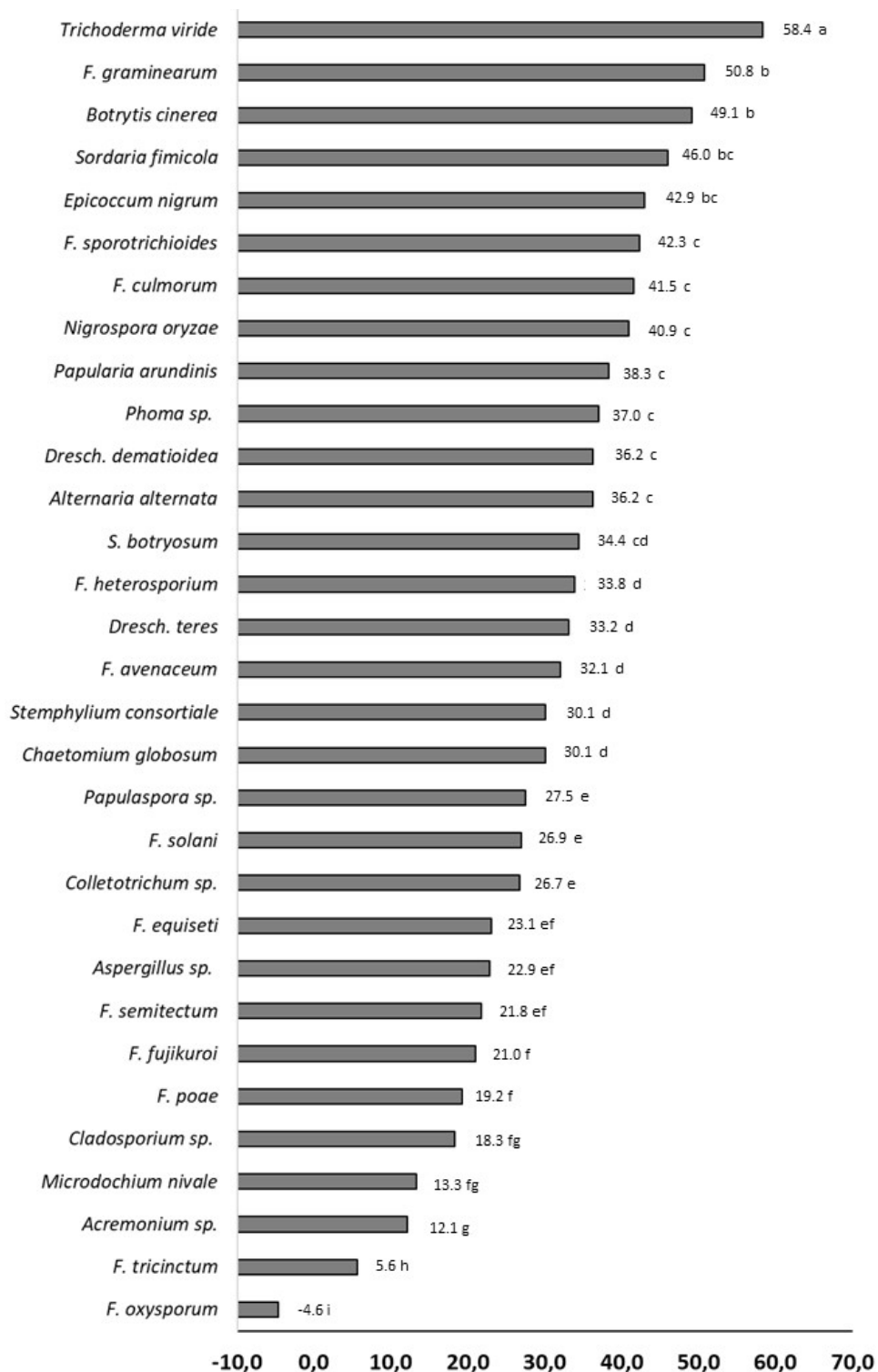
Fig. 2. The percentage inhibition of radial growth (PIRG) of *Bipolaris sorokiniana* in the presence of a given fungus colony

size of the *B. sorokiniana* colony, as well as in its color and the number of conidial spores produced. The accompanying images aptly illustrate these interactions, offering valuable insights into the intricate dynamics of the growth (fig. 3).

In certain instances, the visual documentation unmistakably captures a distinct demarcation between the two fungi, highlighting a clear boundary that separates their respective domains (fig. 3e and 3i). Conversely, other images portray a scenario wherein the studied species is seemingly overwhelmed by the presence of another fungus, indicating an overgrowth phenomenon (fig. 3a, 3d, and 3h). Furthermore, specific observations showcase an intriguing phenomenon where a discernible gap exists between the two coexisting species, indicating an inhibition zone (fig. 3f and 3g).

DISCUSSION

Cereals have evolved various natural defense mechanisms to counter fungal pathogens like *Bipolaris sorokiniana*. These defense mechanisms help plants recognize and respond to pathogen attacks, limiting the spread of infection and minimizing damage. Some of these mechanisms include physical barriers, cell wall fortification, antimicrobial compounds, induced
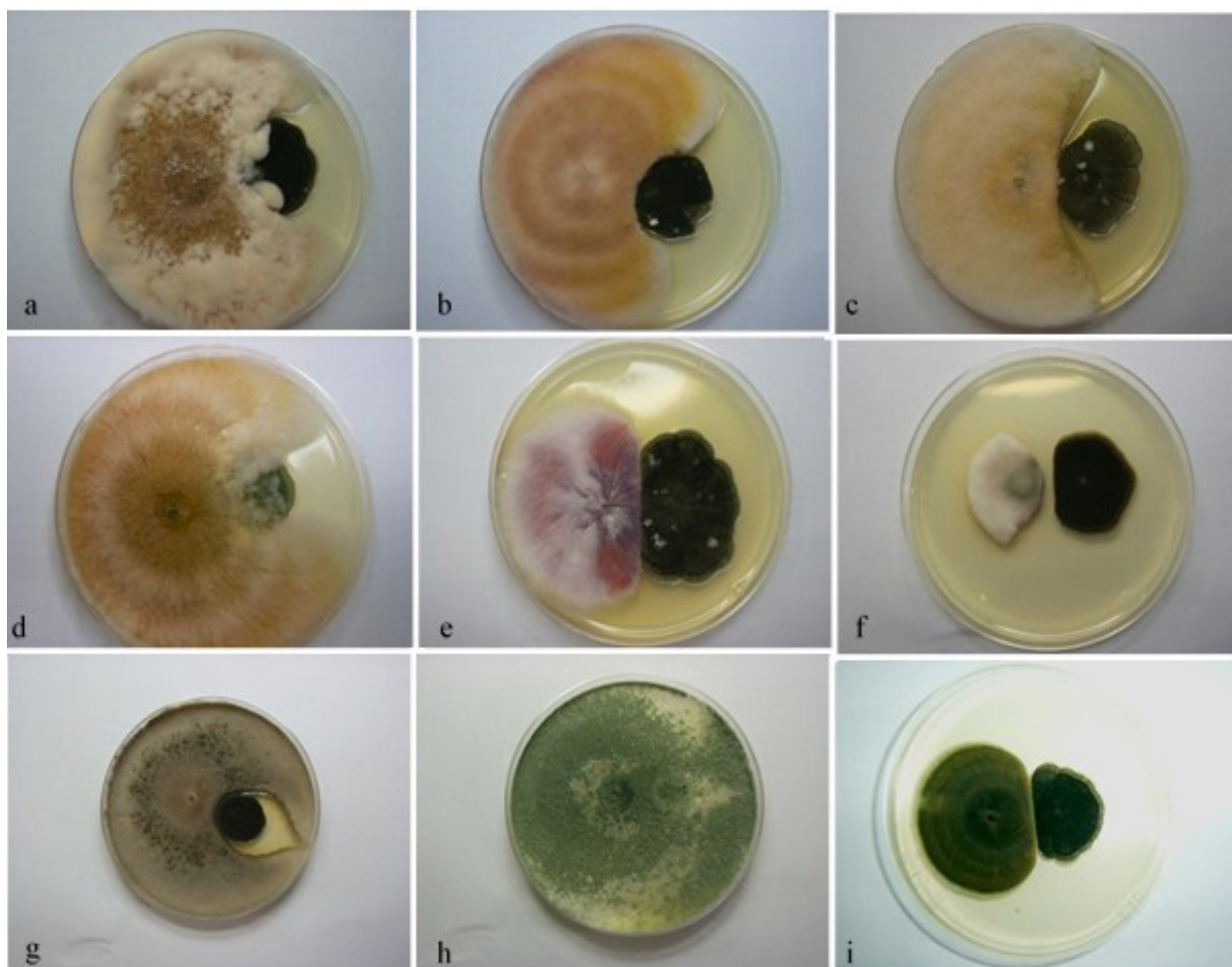

Fig. 3. Examples of fungal interaction in dual cultures of *B. sorokiniana* with selected fungi recorded six days after co-inoculation: a) *Fusarium graminearum*, b) *F. sporotrichioides*, c) *F. poae*, d) *F. culmorum*, e) *F. oxysporum*, f) *Drechslera teres*, g) *Sordaria fimicola*, h) *Trichoderma viride*, i) *Alternaria alternata*

systemic resistance (ISR), hormonal signaling, pathogen recognition receptors, effector-triggered immunity (ETI), nitric oxide (NO) signaling, programmed cell death or microbial competition. These natural defense mechanisms, often working in concert, contribute to the cereals' ability to resist and tolerate fungal pathogens like *B. sorokiniana*. Understanding these mechanisms provides insights for breeding more resistant crop varieties and developing them [Vargas et al. 2012, Singh et al. 2019, Liu et al. 2022].

Antagonism refers to the different actions of organisms that suppress or interfere with the normal growth and activity of another organism. This can manifest in different ways through competition, antibiosis, direct parasitism (biotrophic or necrotrophic) or induced resistance. Many soil microorganisms are antagonistic [Wang et al. 2020]. They secrete a potent enzyme that destroys other cells by digesting their cell walls and degrading the cellular material, as well as releasing protoplasmic material that serves as a nutrient for the inhibitor organism. For example, *Aspergillus* has an antagonistic effect on *Penicillium* spp. and *Cladosporium* sp., *Trichoderma* spp. has an effect on actinomycetes or *Pseudomonas* which show antagonism on *Cladosporium* sp. [Rao et al. 2016]. *Trichoderma* is identified as the genus with the greatest potential comprising biocontrol agents that have been used against a number of plant fungal diseases, e.g. *Verticillium dahlia*, *R. solani*, *Sclerotinia sclerotiorum*, and *Fusarium solani* [Ordentlich et al. 1990, Abbas et al. 2017, Alizadeh et al. 2020, Thambugala et al. 2020].

However, a better solution seems to be mixed cultures of microbial antagonists, which ensures better control of plant diseases in relation to individual strains [Guetsky et al. 2001, Freeman et al. 2004, Xu et al. 2011]. Calvo et al. [2003] improved the effectiveness of biocontrol of post-harvest apple diseases (*Penicillium expansum* and *Botrytis cinerea*) using yeast mixture (*Rhodotorula glutinis*, *Cryptococcus albidus* and *C. laurentii*). The other studies have found that *Trichoderma koningii*, *T. hamatum*, *Pseudomonas fluorescens*, *P. putida*, *Tilletiopsis minor* and *T. washtonensis* were more effective against *Botryodiplodia* disease when used in combination than when used individually [Haggag and Nofal 2006].

Competition is a significant and antagonistic mechanism that plays a crucial role in ecological dynamics.

It is particularly favored by certain characteristics of biocontrol agents, such as their ecological adaptability, rapid growth rate, and developmental traits, as well as by various external factors such as soil composition, pH levels, temperature, humidity, and more [Infante et al. 2009]. In an experimental study, the authors conducted an assessment of the potential impact of competition involving *Bipolaris* and 16 other fungal species during laboratory tests. Those findings shed light on the intriguing dynamics of these interactions. On average, a noteworthy reduction was observed in the initial radial growth of *Bipolaris*, with an approximate decrease of 30.1%. This reduction in growth exhibited a wide range, spanning from a substantial 58.3% reduction (in the presence of *Trichoderma viridae*) to a peculiar –4.6% change (associated with *Fusarium oxysporum*). The latter value, intriguingly, indicated an expansion in the initial diameter of the *Bipolaris* colony, which is an unusual outcome. Of particular interest, the value of PIRG (percentage initial radial growth) observed for *Trichoderma viride* stood out significantly as the highest among all the results obtained in this experiment, which confirms its documented role as a recognized antagonist of many different species of plant pathogenic fungi. In contrast, lower PIRG values were observed for other fungi, such as *Fusarium graminearum*, *Botrytis cinerea*, and *Sordaria fimicola*.

Many studies on the species *T. viride* confirm the current observations and indicate effectiveness in reducing symptoms of diseases caused not only by *B. sorokiniana* but also by microorganisms from genera *Fusarium*, *Pythium*, *Phytophthora*, *Alternaria*, *Sclerotinia*, *Botrytis* [Tucci et al. 2011, Modrzewska et al. 2022, Tyśkiewicz et al. 2022]. The results obtained by Salehpour et al. [2005] and Singh et al. [2018] of several *Trichoderma* species indicate that these fungi produce enzymes and metabolites that can inhibit the growth of *Bipolaris sorokiniana*, e.g. *Trichoderma harzianum* produces chitinase, which breaks down the cell walls of this fungus, while *Trichoderma viride* produces proteases, which degrade the cell membrane of the pathogen.

Biological control is a natural method of pest management that uses living organisms to control pests or pathogens. In the case of *B. sorokiniana*, biological control involves the use of beneficial microorganisms

to suppress the pathogen and reduce disease incidence and severity. Biological control of this fungus can be achieved by applying beneficial microorganisms to the soil or plant surfaces, either alone or in combination, to establish a protective barrier against the pathogen, including, e.g. *Chaetomium* spp. and *Bacillus amyloliquefaciens* [Yue et al. 2018, Yi et al. 2021, Luan et al. 2023]. These natural methods of control can reduce the need for chemical pesticides, promote sustainable agriculture, and protect the environment. In the current study, it was found that several species of examined fungi had antagonistic effects against *Bipolaris sorokiniana*. These effects included the inhibition or suppression of the growth and activity of this pathogen, which could potentially be used in biological control against it. The obtained results confirm that the best species for biological control against *B. sorokiniana* is *Trichoderma viride*, but perhaps better results would be achieved by a mixture of several species with the highest antagonistic potential obtained in the study, e.g. *T. viride*, *Sordaria fimicola*, *Epicoccum nigrum* and *Nigrospora oryzae*.

This study highlights the complex and diverse nature of fungal interactions, emphasizing their significant potential for enhancing biological crop protection. The diversity in the degree of inhibition among the studied fungi underscores the complexity of ecological dynamics at play. Such insights into the intricacies of fungal interactions lay the foundation for the strategic utilization of specific fungal candidates in innovative biological protection strategies aimed at managing the growth of *B. sorokiniana* and enhancing the resilience of crops against this prevalent pathogen.

CONCLUSIONS

The hypothesis was confirmed that among the examined species of fungi inhabiting crop seeds, there are those that significantly influence the growth and development of *B. sorokiniana*, the cause of seedling blight.

Confirmation of this hypothesis will allow for further research to determine what causes inhibition of the growth of this fungus, e.g. substances produced by other fungi, competition regarding the occupied living space, or perhaps some kind of hyperparasitism.

DATA AVAILABILITY

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

SOURCE OF FUNDING

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