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SOIL AND AGRICULTURAL MICROBIOLOGY - RESEARCH PAPER

Does sorghum phenolic extract have antifungal effect?

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Abstract

This study aimed to evaluate the antifungal effect of SC319 sorghum phenolic extract (SPE) on the *Aspergillus*, *Fusarium*, *Penicillium*, *Stenocarpella*, *Colletotrichum*, and *Macrophomina* genera. SPE was extracted by 20% ethanol and used in four assays: (1) against *Fusarium verticillioides* in solid (PDA) and liquid (PD) potato dextrose media; (2) Minimum Inhibitory Concentration (MIC) assay with 16 fungi isolates; (3) Conidial Germination Rate (CGR) with 14 fungi isolates and (4) Growth Curve (GC) with 11 fungi isolates. There was no reduction in the mycelial growth (colony diameter and dry weight) and in the number of *Fusarium verticillioides* spores in assay 1 (PDA and PD). The colony's dry weight was almost six times higher in the presence than in the absence of SPE. All SPE samples presented MIC (assay 1) above the maximum concentration tested (5000 $\mu g.mL^{-1}$) for the 16 isolates. Also, there was no inhibitory effect of SPE on conidia germination rate (CGR). Oppositely, in GC assay, the control had a higher CFU count than the samples with SPE in 24 h. This result suggests that SPE can delay the fungal growth in the first hours of incubation, which is an important finding that may help reduce the severity of fungal diseases in plants. However, further studies are needed to confirm these results, including sorghum genotypes with different profiles of phenolic compounds. Although the SC319 SPE was not effective as an antifungal agent, it may have potential as a growth promoter of beneficial fungi in the food and pharmaceutical industries.

Keywords Toxigenic fungi · *Sorghum bicolor* (L.) Moench · Minimum inhibitory concentration · Biofungicide

Introduction

The use of chemical pesticides to control plant pathogens has intensified in the last decades [1]. In this group, synthetic fungicides are chemically grouped into carboxamides, triazoles, strobilurins, dithiocarbamates, and others [2]. However, as they are non-biodegradable substances, these products, besides high costs, are toxic and pose risks to the environment and human and animal health [3].

An ideal antifungal agent should have a broad spectrum of action and minimal health and environmental effects. Furthermore, the search for natural antifungal agents is rising to meet agribusiness interests, governments, and consumers. As a result, new molecules have been discovered, and new

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formulations are made available, reducing toxicity and increasing the bioavailability of active ingredients. Consequently, 25% of the substances used as antifungal agents worldwide were obtained from natural products or derivatives [3].

Plants and microorganisms are the primary sources of natural substances with antimicrobial potential [4]. Among these, phenolic compounds, secondary metabolites present in plants, have a range of biological effects, including antioxidant, antiviral, antitumor, and antibacterial effects [5, 6]. Furthermore, the inhibitory effect of phenolic compounds in spore germination, mycelial growth, and the production/activity of microbial enzymes vary among the different groups of phenols [7]. Tannins, for example, have well-known antimicrobial properties for many microorganisms, including bacteria, yeasts, and molds [8, 9].

Regarding the fungicide action, Elsherbiny et al. (2016) [10] observed that the methanol extract of pomegranate peel has a significant antifungal effect on the spore germination and mycelial growth of *Fusarium sambucinum*. Kharchoufi et al. (2018) [11] studied the antifungal effect of pomegranate peel extract and verified, by optic microscopy, significant changes in the shape of *Penicillium digitatum* hyphae, which appeared wilted and coiled.

Sorghum [*Sorghum bicolor* (L.) Moech] is a cereal that stands out for its agronomic advantages such as greater tolerance to water stress and high temperatures, its lower market price, and, lately, its nutritional and functional properties [12–14]. Depending on the genotype, its grains can be a rich source of various phenolic compounds, such as phenolic acids, anthocyanins, and tannins, which are concentrated in the pericarp [15]. Some studies have reported the effectiveness of sorghum phenolic extract as an antibacterial agent. Kil et al. (2009) [16] reported sorghum phenolic extracts' antioxidant and antibacterial effect on *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhimurium*, *Klebsiella pneumoniae,* and *Bacillus subtilis*. However, studies demonstrating its antifungal potential were not found in the literature.

Thus, the objective of this study was to evaluate the antifungal effect of a sorghum phenolic extract against phytopathogenic fungi that cause severe plant diseases.

Material and methods

Phytopathogenic isolates

Assays to evaluate the antifungal effect of sorghum phenolic extract were performed at the Food Security and Phytopathology Laboratories of Embrapa Milho e Sorgo, Sete Lagoas, MG, and the Mycology Laboratory of the Institute of Biological Sciences of the Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, MG.

Phytopathogenic fungi strains of the *Fusarium*, *Aspergillus*, *Penicillium*, *Stenocarpella*, *Colletotrichum* and *Macrophomina* genera from the Mycological Collections of the Universidade Federal de Lavras (UFLA), Lavras, MG, Brazil, and Embrapa Milho e Sorgo, Sete Lagoas, MG, Brazil, were used in the experiments (Table 1).

Obtaining and chemical characterization of sorghum phenolic extract

The phenolic extract was obtained from the bran of the SC 319 sorghum genotype, which has high levels of phenolic compounds [17, 18], selected from a panel of 100 sorghum lines of the Sorghum Genetic Improvement Program of Embrapa Milho e Sorgo (unpublished data, stored in an Embrapa database, under confidentiality). The sorghum bran (pericarp) was obtained by decortication of the grains in a rice processing machine and further ground in a ball mill (Brand: Retsch, model: MM200) until obtaining a granulometry of approximately 10 µm.

The extraction of sorghum phenolics was performed according to Singleton et al. (1999) [19], with modifications. Alternative extraction solutions were tested to replace the conventional solvent (methanol), which is quite toxic and leaves polluting residues, to other less toxic. Water at room temperature (RT), 40 °C and 90 °C and ethanol (10, 20, and 30%) at RT and 40 °C, both with or without 3% citric acid, were used in the test (Table 2 of "Results and Discussion"). The ethanol/water (20/80%) at RT was selected because it uses a less toxic reagent, has lower energy expenditure (RT) and has shown equivalent results to the conventional method (methanol 1%

Table 1 Identification and Taxonomy of the microorganisms

Nº	Identification	Taxonomy
1	BRM 051202	*Fusarium graminearum*
2	CML 2743	*Fusarium verticillioides*
3	CML 2825	*Fusarium proliferatum*
4	CML 2793	*Fusarium andiyazi*
5	BRM 034978	*Aspergillus* sp.
6	BRM 035055	*Aspergillus* sp.
7	BRM 032174	*Aspergillus terreus*
8	BRM 038161	*Penicillium citrinum*
9	BRM 032157	*Penicillium pinophilum*
10	BRM 035045	*Penicillium* sp.
11	BRM 035317	*Fusarium verticillioides*
12	BRM 051204	*Fusarium verticillioides*
13	EMS01	*Stenocarpella maydis*
14	EMS02	*Stenocarpella maydis*
15	EMS03	*Colletotrichum* sp.
16	EMS04	*Macrophormina* sp.
17	SC5314	*Candida albicans* (control)
18	H99	*Cryptococcus neoformans* (control)

Table 2 Extraction of total phenolic compounds from sorghum using different solvents, at room temperature (RT) or at 40 °C, with or without 3% of citric acid (CA)

Solvent		Total Phenolic (mg GAE.g^{-1})	
1	Methanol 1% HCl RT	18,77	A
2	Ethanol 20% RT	17,49	A
3	Ethanol 20% RT 3% CA	17,30	A
4	Ethanol 20% 40 °C	18,11	A
5	Ethanol 20% 40 °C 3% CA	16,97	A
6	Ethanol 50% RT	19,02	A
7	Ethanol 50% RT 3% CA	17,90	A
8	Ethanol 50% 40 °C	18,94	A
9	Ethanol 50% 40 °C 3% CA	20,10	A
10	Water 90 °C 3% CA	12,50	C
11	Ethanol 10% RT	14,93	B
12	Ethanol 10% RT 3% CA	13,33	B
13	Ethanol 10% 40 °C	13,92	B
14	Ethanol 10% 40 °C 3% CA	14,78	B
15	Water RT	12,00	C
16	Water RT 3% CA	10,07	C
17	Water 40 °C	13,71	B
18	Water 40 °C 3% CA	10,62	C
CV (%)		19,92	

Values followed by the same capital letter in the column do not differ from each other at the 5% probability by the Scott-knott test

HCl). Then, one gram of sorghum bran was added to 100 mL of ethanol/water solution (20/80%, v/v). Next, the solution was kept under stirring at 200 rpm on a shaker table (Nova Ethics, model: 109) and subsequently centrifuged (Jouan, model: B4i) for 15 min at 2000 *g* (RCF) at room temperature. Then, the supernatant with SPE was used to analyze the phenolic compound content.

The modified Folin-Ciocalteu assay, previously described by Awika et al., (2003) [20], using gallic acid as a standard, was used to quantify the phenolic compounds of the extract. An aliquot of 100 µL of sorghum extract was taken and added with 1.1 mL of deionized water, 0.4 mL of Folin-Ciocalteu reagent solution, and 0.9 mL of ethanolamine solution. The reading was performed at 600 nm in a UV/VIS spectrophotometer (Brand: Intrutherm, model UV 2000A). The phenolic extracts were lyophilized in a freeze dryer (Brand: Terroni, model LS3000) and suspended in deionized water (1 mg.mL^{-1}), according to the technique described by Kil et al. (2009) [16].

Evaluation of the antifungal effect of sorghum phenolic extract (SPE)

Four different assays were performed to evaluate the antifungal effect of SPE: (1) In solid (PDA—potato-dextrose-agar) and liquid (PD—potato-dextrose) culture media using only *Fusarium verticillioides*; (2) Minimum inhibitory concentration (MIC), carried out with 16 different isolates of phytopathogenic fungi; (3) Conidia germination rate (CGR) carried out with 14 isolates of phytopathogenic fungi, (4) The growth curve (GC) carried out with 11 isolates of phytopathogenic fungi. These four assays are summarized in Fig. 1 and detailed below. The different concentrations were used according to each type of assay. Initially, the sorghum phenolic extract was tested only against *F. verticillioides*, in solid and liquid medium (PDA and PD). As no effect was observed in this first assay, three other new assays were used with a larger number of isolates of phytopathogenic fungi. In MIC (assay 2), 16 isolates were used and only 14 showed growth, which were used in assay 3 (CGR). Subsequently, three of these isolates were lost and only 11 were used in assay 4 (GC).

Assay 1—Antifungal effect of SPE against F. verticillioides in solid and liquid culture media

This study was carried out at the Laboratory of Phytopathology of Embrapa Milho e Sorgo, Sete Lagoas, MG, Brazil. For this assay, the fungus *Fusarium verticillioides* (strain CML 2743) was used because it is one of the most prevalent in maize and sorghum, and that causes more damage to these crops. Solid (PDA) and liquid (PD) media (Difco Laboratories, Detroit, MI, USA) were incorporated or not (control) with freeze-dried SPE at a concentration of 1 mg.mL^{-1} (266 mg gallic acid equivalent (GAE).g^{-1}), according to work carried out by Kil et al. (2009) [16]. In addition, the PD medium was evaluated without agitation (PD) and with agitation (PDag). The treatments are described below.

T1. PDA: solid medium + SPE
T2. PD: liquid medium without agitation + SPE
T3. PDag: liquid medium with agitation + SPE

In T1 (PDA), mycelium discs (± 5 mm in diameter), taken from the edges of *F. verticillioides* colonies from a seven days of culture, were transferred to the centers of the surfaces of the Petri dishes containing the culture media with or without SPE (150 mg of lyophilized SPE in 150 mL of PDA medium). For seven days, the plates were kept at room temperature (25 ± 2 °C). Mycelial growth was measured by colony diameter and evaluated every 24 h for five days, according to the methodology used at the Phytopathology Laboratory of Embrapa Milho e Sorgo, Sete Lagoas, MG.

In T2 (PD) and T3 (PDag), 40 mg of lyophilized SPE in 40 mL of PD medium (1 mg.mL^{-1}) was used. A mycelial disc (± 5 mm in diameter) of *F. verticillioides* was transferred to Erlenmeyer containing the liquid medium. The flasks were kept in the dark for 7 days at room temperature (25 ± 2 ºC), without

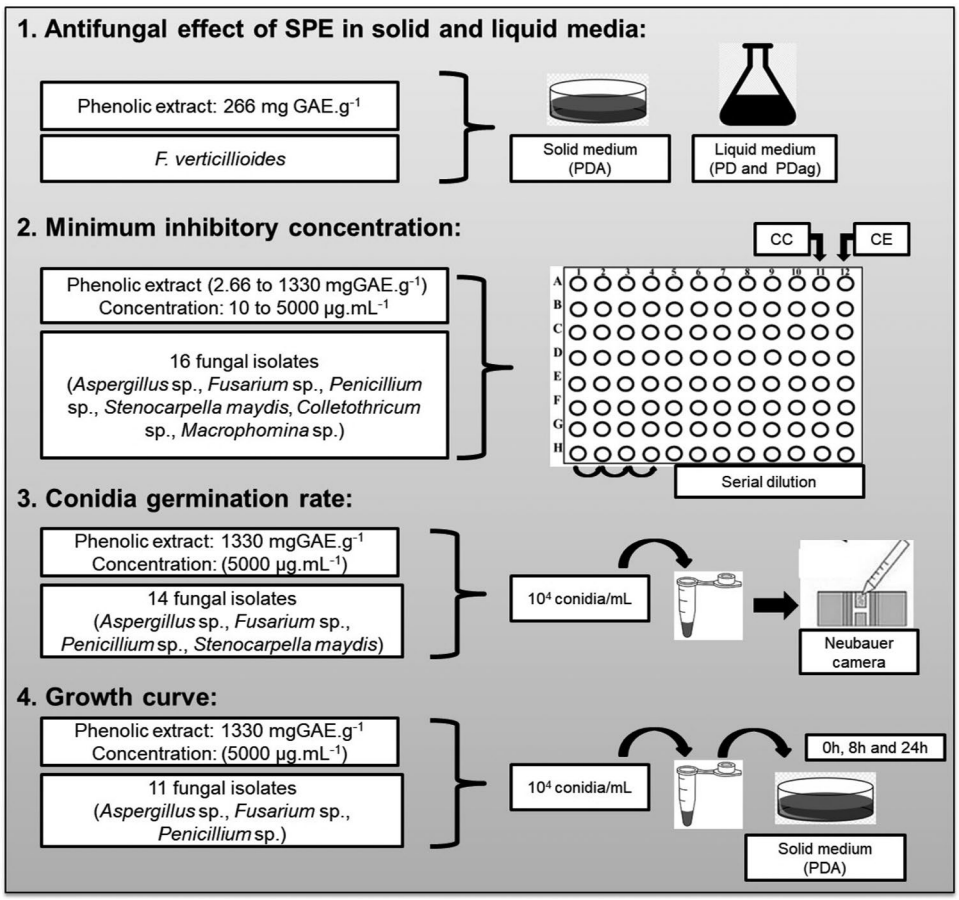

Fig. 1 Summary of assays performed to evaluate the antifungal effect of phenolic sorghum extract

(T2) or with agitation at 100 rpm (T3). After seven days of incubation, the T2 (PD) samples were filtered through qualitative filter paper (12.5 cm). Then, the mycelium was taken and dried in an oven at 51 °C until constant weight and the mycelial weight was determined. The T3 samples (PDag) were filtered through gauze, diluted in water (1:1), and the spores were counted in a Neubauer chamber with the aid of an optical microscope (Olympus BX60). The experimental design used was completely randomized, with six replications and three treatments. ANOVA was performed, with comparison of means by Tukey test at 5% probability.

Assay 2—Minimum Inhibitory Concentration (MIC)

This study was carried out at the Mycology Laboratory of the Institute of Biological Sciences of UFMG, Belo Horizonte, MG, Brazil. Sixteen isolates of phytopathogenic fungi (Table 1) from the Mycological Collection of UFLA and Embrapa Milho e Sorgo, MG, Brazil, were used for this assay. *C. albicans and C. neoformans* strains, from the Mycology Laboratory of UFMG, MG, Brazil, were used as a control. The yeast *C. albicans* was used because its growth was inhibited by a sorghum phenolic extract, at the same concentration used in the present work [16] and *C. neoformans* because it is widely used in experiments with MIC analysis at the Mycology Laboratory of UFMG.

The cultures were stored according to the procedure described by Castellani (1939) [21]. The strains were previously subcultured from the stored samples in Petri dishes containing PDA medium. The fungal inoculum was prepared from fungal cultures grown in these Petri dishes and incubated for seven days at 28 °C. The fungal colonies were covered with 3 mL of sterile saline solution (0.85% NaCl) plus 1% Tween 80 and submitted to scraping with a subculture loop to obtain a suspension and conidia count. Conidia were counted in a 10 µL aliquot in a Neubauer chamber.

For the MIC assay, the microdilution was carried out in RPMI (Roswell Park Memorial Institute) 1640 medium, which is specially formulated to use in antifungal susceptibility testing of yeasts and molds. The inoculum concentration corresponded to 2×104 and 2×103 CFU.mL^{-1} in the test and control, respectively [22].

Fungi susceptibility test to SPE: the broth microdilution method was used according to the M60 document proposed by CLSI [23]. The synthetic antifungals Itraconazole (ITRA), Fluconazole (FLU), and Amphotericin B (ANFB) were used as controls. SPE solutions were prepared from dilutions of phenolic extract at a concentration of 40 mg.mL^{-1} (10,640 mg GAE.g^{-1})

in distilled water. Stock solutions at 2000 μg.mL^{-1} of ANFB or ITRA were prepared in dimethylsulfoxide (DMSO) (Gibco-BRL, Grand Island, NY, USA) and for FLU in distilled water. Then, the ANFB, ITRA, FLU and SPE solutions were diluted in RPMI 1640, respectively, in the following proportions: 64:1936 μL; 256:1744 μL; 512:1448 μL; 500:1500 μL. Subsequently, 100 μL of these solutions were used to start the serial dilution process in sterile 96-well flat-bottomed microplates containing 100 μL of RPMI 1640, obtaining concentrations ranging from 0.2 to 128 μg.mL^{-1} for itraconazole, from 0.5 to 256 μg.mL^{-1} for fluconazole, from 0.06 to 32 μg.mL^{-1} for amphotericin B and from 10 to 5,000 μg.mL^{-1} for SPE (2.66 to 1,330 mg GAE. g^{-1}). The antifungal agents were tested in a range of final concentrations in which the growth inhibition of filamentous fungi occurred, based on the MIC values found in the literature [24, 25].

The experiment was performed in duplicate, for each of the four treatments (FLU, ITRA, AFNB, SPE), by visually observing the growth inhibition in each well compared to the growth observed in the positive control well, where there was only the inoculum, in the absence of the antifungal and SPE. MIC was considered the lowest concentration capable of inhibiting 80% of fungal growth for Itraconazole and Fluconazole. For amphotericin B and SPE, it was considered the lowest concentration capable of inhibiting 100% of growth. These 80% and 100% inhibition percentages are recommended in the CLSI manual [23].

Assay 3—Effect of sorghum phenolic extract on Conidial Germination Rate (CGR)

This study was carried out with the fungal isolates 1 to 14 (Table 1) at the Mycology Laboratory of the Institute of Biological Sciences of the Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, MG, Brazil. Fungal samples were prepared, adjusted to 10^4 conidia.mL^{-1}, and inoculated in the PD broth, without (control) or with SPE at the concentration of 5000 μg.mL^{-1} (1 mL SPE:1 mL medium), the experiment was performed in triplicate. Then, samples were homogenized by vortex for 15 s and incubated at 28 ºC for 24 h. After incubation, aliquots of each sample were taken, and the number of germinated and non-germinated conidia was determined using a Neubauer chamber. The percentage of germinated conidia (CGR) was calculated for each fungal isolate [26].

Assay 4—Effect of sorghum phenolic extract on growth curves

This study was carried out at the Mycology Laboratory of the Institute of Biological Sciences of Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil. Eleven fungal isolates were selected for this assay (from nº 2 to 12) as showed in Table 1. From the inoculum of the different fungi, previously prepared and adjusted to 10^4 conidia.mL^{-1}, were added to RPMI 1640 medium without or with SPE (5000 μg.mL^{-1}) and incubated in conical propylene tubes (50 mL) at 28 ºC. Plating aliquots evaluated the fungal growth at 0, 8, and 24 h after adding the fungal inoculum in the RPMI 1640 medium. The experiment was performed in triplicate. For further evaluation, an aliquot of 50 μL of each sample was seeded in a Petri dish containing PDA and incubated at 28 ºC for further evaluation. After 48 h of growth, the colonies obtained in colony-forming units (CFU. mL^{-1}) were counted.

Statistical analysis

Data referring to the evaluation of different solvents for the extraction of total sorghum phenols and the antifungal effect of SPE in solid and liquid media were analyzed by analysis of variance with the aid of the SISVAR software [27]. The means were submitted to the Tukey or Scott-Knott test at 5% probability.

For the analysis of germination rate and growth curve data, the Prism 5 5.01 Software (GrapPad Inc., San Diego, CA, USA) was used with the analysis of variance, followed by Tukey's test and, for the growth, the area under the curve was determined. P-value < 0.05 were considered statistically significant.

Results

Obtention and chemical characterization of sorghum phenolic extract

There were significant differences ($p > 0.05$) in the phenolic compounds levels from sorghum extracted with different solvents, which varied between 10.07 and 20.1 mg GAE.g^{-1} (Table 2). However, solvents 1 to 9 showed similar results and had better extraction efficiency of phenolic compounds than the others. Therefore, the solvent 20% ethanol at room temperature was selected and used in the present work due to generating lower chemical residues and lower costs than the conventional method (1% methanol HCl RT) and similar extraction capacity. Also, the selected method uses less energy (RT) than the others that use higher temperatures. The final concentration of freeze-dried sorghum phenolic extract was 266 mg GAE.g^{-1}), which was used in the antifungal assays of this work.

Evaluation of the antifungal effect of sorghum phenolic extract (SPE)

Assay 1—Antifungal effect of SPE for F. verticillioides in solid (PDA) and liquid (PD) culture media

In the PDA treatment, with the application of sorghum phenolic extract and control, there was no difference on *F.*

verticillioides colonie diameters which means that there was no effect of SPE on fungal growth (Table 3).

In the liquid medium without agitation (PD), the colony dry weight was almost six times higher in the treatment with SPE (2.15 g) than the control (0.37 g) and in the PDag, the spores.mL^{-1} was almost twice higher (257×10^5 spores.mL^{-1}) than the control (155×10^5 spores.mL^{-1}) (Table 3). Thus, no antifungal effect of SPE was observed on the *F. verticillioides* development in PD and PDag.

Assay 2—Evaluation of the susceptibility of phytopathogenic fungi to sorghum phenolic extract—Minimum Inhibitory Concentration (MIC) of SPE

After seven days of growth all SPE samples presented MIC above 5000 µg.mL^{-1} for the 16 fungi analyzed. For synthetic antifungals the MIC values of the samples varied between 0.50 and above 32 for amphotericin B, 0.25 and above 128 for itraconazole, 8.0 and above 256 for fluconazole (Table 4). Therefore, it was possible to notice that the phenolic extract of sorghum SC 319 did not present an inhibitory effect on the growth of the species analyzed since all samples obtained normal growth compared to those that received synthetic antifungals.

Assay 3—Effect of sorghum phenolic extract on Conidial Germination Rate (CGR)

The percentage of germinated conidia (CGR) for the 14 fungal species evaluated after 24 h in PD Broth, in the absence and presence of SPE (5000 µg.mL^{-1}) is shown in Fig. 2. In the presence of SPE, the species represented by *F. verticillioides* CML 2743 (B), *F. proliferatum* CML 2825 (C), *F. andiyazi* CML 2703 (D) and *A. terreus* BRM 32174 (G) showed higher percentages of germination when compared to samples without SPE. This result corroborates those observed in the preliminary assay (assay 1) for the species *F. verticillioides* CML 2743 (B), in which the values of the SPE treatment were higher than those in control. There was no significant difference in the presence and absence of the extract for the other species. Thus, the results demonstrate no inhibitory effect of SPE on conidia germination rate (CGR) of the tested species.

Assay 4—Effect of sorghum phenolic extract on growth curves (GC)

The effect of SPE on growth curves (CFU.mL^{-1}) during 24 h for 11 phytopathogenic fungi isolates (from nº 2 to 12 of Table 1) is shown in Fig. 3. There was a significant difference in the growth of fungi in the presence and absence of SPE. *F. verticillioides* CML 2743 in medium with SPE kept the CFU. mL^{-1} over 24 h, while the control increased from 15,000 to 450,000 CFU.mL^{-1}. *F. proliferatum* CML 2825, *F. andiyazi* CML 2793, *Aspergillus sp.* BRM 34978 and *P. pinophilum* BRM 32157 had similar behavior. In the first 8 h, there was a reduction in the CFU.mL^{-1}, both in the absence and in the presence of SPE, and a much higher increase in the control samples between 8 and 24 h, especially for *F. andiyazi* CML 2793, whose CFU value was 40 times higher in samples without SPE. In the case of *A. terreus* BRM 32174, *Penicillium sp*. BRM 35045, and *F. verticillioides* BRM 35317, after eight h of growth, the CFU in the samples with SPE was more significant than in control. However, after 24 h, the growth curves were inverted, with the control samples showing much higher growth than those with SPE. For *F. verticillioides* BRM 51204, with and without SPE, they had similar curves during the 24 h. Thus, the result of SPE application may depend on the species of fungus, although more work must be done to confirm this hypothesis.

Table 3 Effect of sorghum phenolic extract (SPE) on the growth of *Fusarium verticillioides:* colony diameter (cm) in solid medium PDA, colony dry weight and number of spores in liquid medium without agitation (PD) and with agitation (PDag), respectively

Treatment	Repetition	PDA						PD		PDag	
		Colony diameter (cm)						Colony dry weight		Number of spores	
		Day 1	Day 2	Day 3	Day 4	Day 5	Mean	g	Mean	nº ($\times 10^5$)	Mean
Control*		7.5	9.3	11.1	13.2	13.8	13.8 a	0.373	0.37 b	155	155 b
SPE	1	8.1	8.4	9.3	12.0	13.5	13.9 a	4.87	2.15 a	223	257 a
	2	7.5	8.7	9.9	12.3	12.9		0.78		245	
	3	7.2	8.4	10.2	12.0	13.5		2.32		222	
	4	8.1	8.7	11.4	13.8	14.7		2.36		251	
	5	7.5	9,0	11.1	13.2	14.7		1.04		290	
	6	8.1	8.7	9.9	12.0	14.1		1.53		311	
CV (%)							0,5		99,9		35,0

Values followed by the same lowercase letter in the column do not differ from each other at the 5% probability by Tukey's test

*Without SPE

Table 4 Minimum inhibitory concentration (MIC, μg.mL^{-1}) of sorghum phenolic extract (SPE) and synthetic antifungals (amphotericin B, itraconazole and fluconazole), for different phytopathogenic fungi species and *C. albicans* and *C. neoformans* (controls), determined by the broth microdilution method

Fungi species		SPE	Amphotericin B	Itraconazole	Fluconazole
1	*F. graminearum*	> 5000	2,0	128	> 256
2	*F. verticillioides*	> 5000	16,0	128	> 256
3	*F. proliferatum*	> 5000	16,0	> 128	> 256
4	*F. andiyazi*	> 5000	> 32	> 128	> 256
5	*Aspergillus* sp.	> 5000	2,0	> 128	> 256
6	*Aspergillus* sp.	> 5000	32,0	0,50	> 256
7	*A. terreus*	> 5000	4,0	0,25	> 256
8	*P.citrinum*	> 5000	4,0	0,25	> 256
9	*P. pinophilum*	> 5000	4,0	128	> 256
10	*Penicillium* sp.	> 5000	1,0	0,50	> 256
11	*F. verticillioides*	> 5000	> 32	128	> 256
12	*F. verticillioides*	> 5000	> 32	> 128	> 256
13	*S. maydis*	> 5000	> 32	0,25	> 256
14	*S. maydis*	> 5000	> 32	0,25	32,0
15	*Colletotrichum* sp.	> 5000	0,50	0,25	8,0
16	*Macrophomina* sp.	> 5000	16,0	0,25	256
	C. albicans	> 5000	0,06	16,0	4,0
	C. neoformans	> 5000	0,06	0,25	4,0

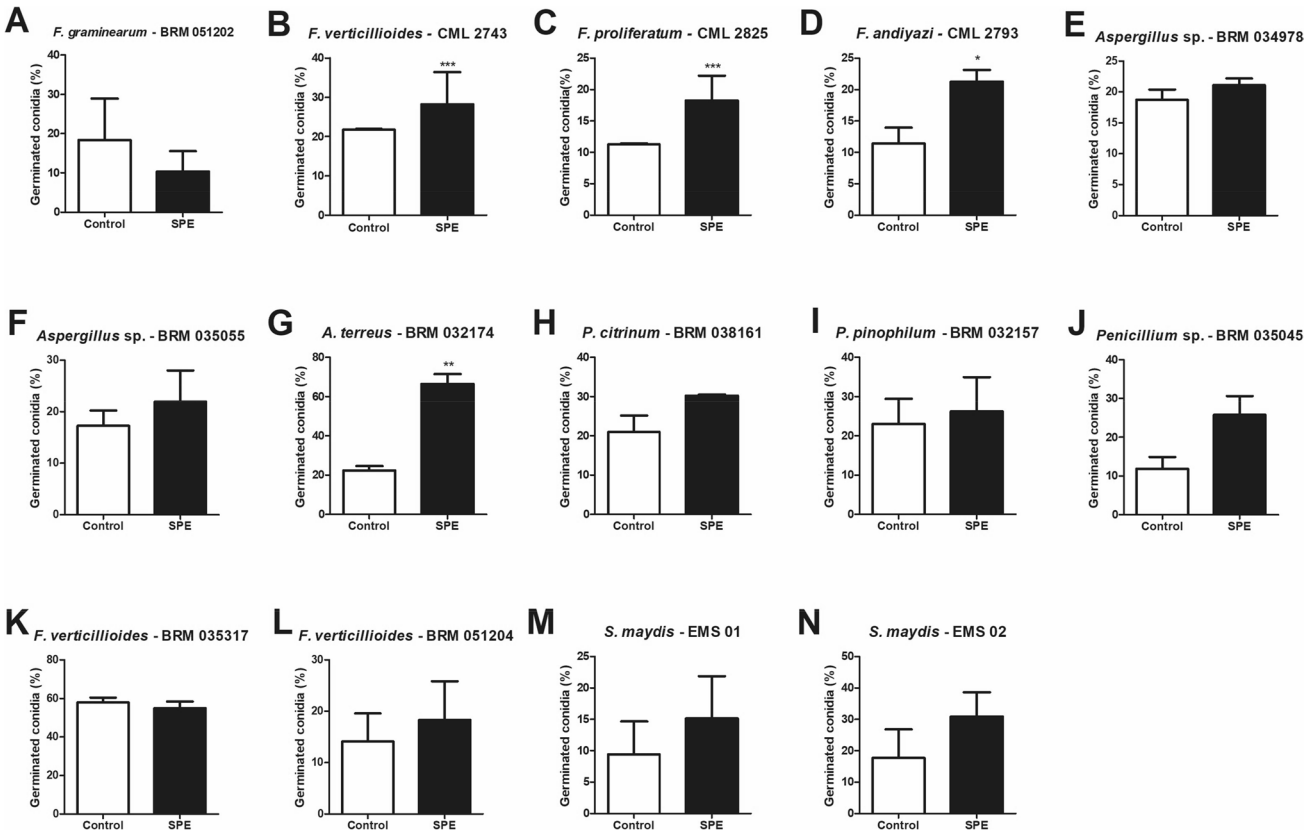

Fig. 2 Effect of sorghum phenolic extract (SPE) on conidia germination rate (CGR). Percentage of germinated conidia for fungal species (**A**—**N**) after 24 h in PD medium in the absence (control) and in the presence of SPE (5000 μg.mL.$^{-1}$). * Significant difference ($p < 0.05$) in relation to the absence of SPE **($p < 0.01$); *** ($p < 0.001$)

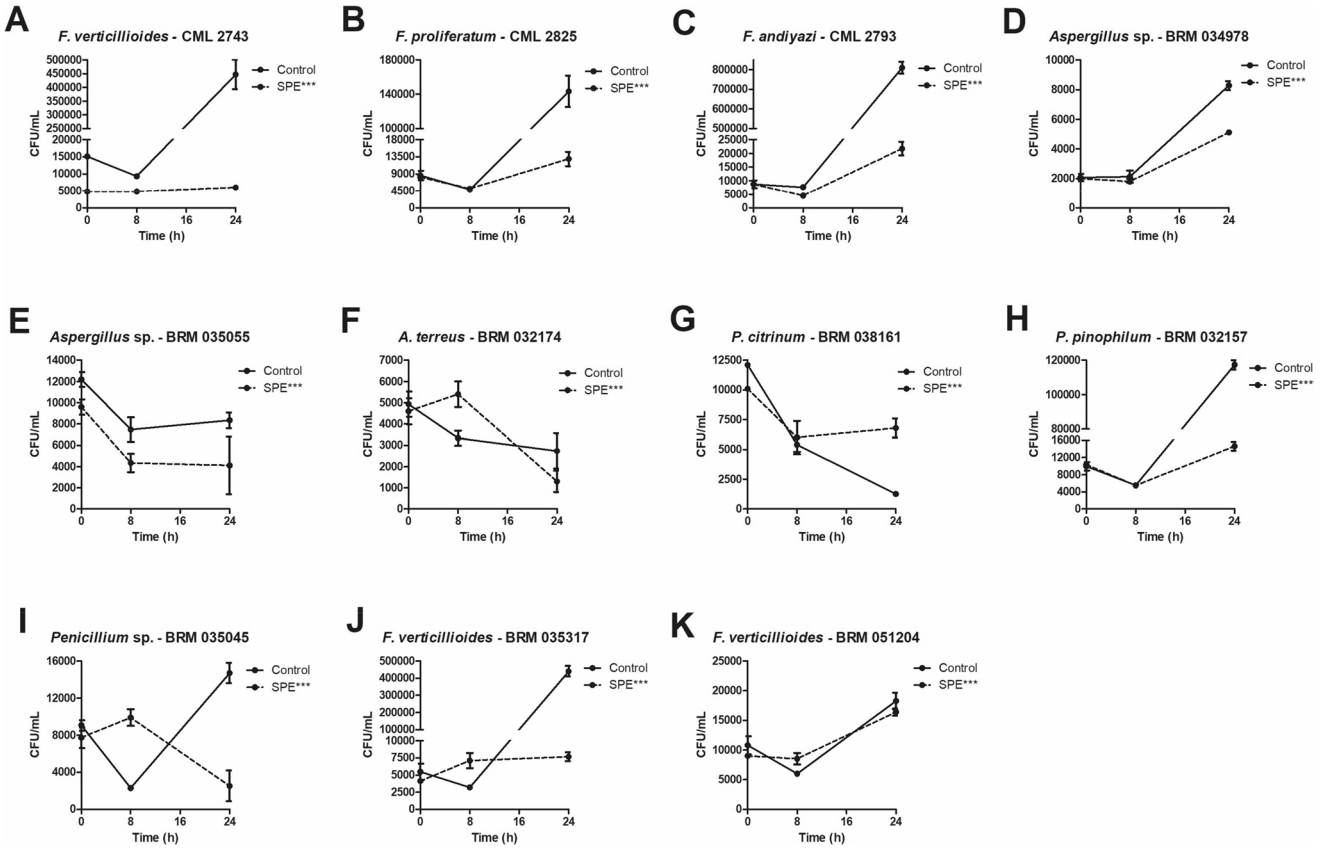

Fig. 3 Effect of sorghum phenolic extract (SPE) on the growth curve (GC) of fungal species. Total colony-forming unit (CFU.mL^{-1}) of the fungal species (**A**—**K**) at 0, 8 and 24 h in the absence (control) and presence of SPE (5000 µg.mL.$^{-1}$). Significant difference in relation to the absence of the SPE *($p < 0.05$); **($p < 0.01$); ***($p < 0.001$)

Discussion

There was no reduction in the mycelial growth rate (colony diameter and dry weight), as well as in the number of spores of *Fusarium verticillioides* (CML 2743) when applied phenolic sorghum extract (SPE) in the solid and liquid growth media (assay 1). Likewise, the SC 319 SPE did not inhibit the growth of the 16 fungal species analyzed in the Minimum Inhibitory Concentration (MIC) (assay 2) and the conidia germination rate (CGR) (assay 3) of those14 fungus species tested (from nº 1 to 14, Table 1). Contrary to expectations, there was more remarkable fungus development in the treatments with sorghum phenolic extract in these three assays.

However, these results differ from those found in the growth curve (GC) of the assay 4, whose control samples showed higher growth than those with SPE. It is speculated that this result may be due to the growth time of the fungi; that is, in the growth curve, the evaluation was performed after 48 h and in the MIC after 168 h, suggesting that the SPE can delay the fungal growth in the first hours, since there was growth but, slowly. This result is relevant because the reduction in CFUs may reduce the amount of initial inoculum, which may reflect the onset of infection and the severity of diseases caused by these pathogens.

Ataei Azimi et al. (2007) [28] showed high antifungal activity of phenolic extracts from seeds and leaves of *Sorghum bicolor* (0 – 25 mg.L^{-1}) on *Fusarium poae* and *F. solani*. In the present work, it was investigated the effect of SC 319 sorghum phenolic extract on *F. verticillioides*, which may be a more resistant species than *F. poae* and *F. solani*.

The work performed by Javaid et al. (2012) [29] investigated the antifungal potential of an allelopathic grass, *Sorghum halepense*, to manage *Macrophomina phaseolina* isolated from cowpea plants, infected with charcoal rot. The authors demonstrated that different concentrations of methanolic extracts (from 0.5 to 3.0 g.100^{-1} mL) from shoots, roots, and inflorescences of *S. halapense* had antifungal activity against *M. phaseolina* by reducing the fungal biomass significantly. Naeem et al. (2021) [30] examined the in vitro efficacy of different concentrations of methanolic leaf extract of *Sorghum halepense* (L.) Pers. against target pathogenic mycotoxin producing fungal species (*Trichoderma viride* Pers., *Trichoderma harzianum* Rifai. and *Cladosporium cladosporioides*). For this they tested different concentrations 2%, 4%, 6%, 8%, and 10%

of methanolic leaf extract for their antifungal potential. All the applied concentrations of *S. halepense* leaf extract inhibited the growth of all the tested fungal strains.

A similar work by Ratnavathi & Sashidhar (2007) [31] evaluated the effect of phenolic extracts from six sorghum cultivars (IS 620, AON 486, LPJ, IS 17779, SPV 86, and SPV 462) with total phenolic contents ranging from 0.45 to 9.39 µg of tannic acid.g^{-1}. The concentrations of 0.01, 0.1, and 1.0% reduced the growth and biomass of *Aspergillus parasiticus*.

Kil et al. (2009) [16] evaluated the antimicrobial effect of four sorghum cultivars (Gumeunchalsusu, Bulkeunchalsusu, Jangsususu, and Neulsusu), extracted with methanol and further fractioned with n-hexane, ethyl acetate, n-butanol, and water. For the MIC analysis, the methanolic extract of the Bulkeunchalsusu cultivar exhibited the highest level of antimicrobial activity against all tested bacteria (*Bacillus subtilis*, *S. aureus*, *E. coli*, *Salmonella typhimurium*, and *K. pneumoniae*). However, none of the cultivars showed antifungal or anticandidal activity. This result corroborates the findings of the present work.

Funnell-Harris et al. (2017) [32] observed that *F. thapsinum* cultivated in a medium with sorghum extract (bmr6) showed significantly faster growth than the control and the media added with sugar. This result also corroborates those obtained in the present work, in which *F. verticillioides* showed greater growth than the control, in the preliminary assay and in the conidia germination assay, in which some species showed higher germination rates in the presence than in the absence of SPE.

Funnell-Harris et al. (2014) [33] evaluated the effect of ferulic, vanillic, sinapic, syringic, and caffeic acids (phenolic compounds) extracted from sorghum, on the growth of some *Fusarium* species in vitro, and *F. thapsinum* was tolerant to these compounds. However, the authors observed inhibition of *F. verticilioides*, *F. proliferatum*, and *M. phaseolina* even at the lowest concentration of ferulic acid (0.5 mM). Thus, they concluded that ferulic acid could inhibit several fungi and be used in plant resistance to fungal pathogens. Recently, Schöneberg et al., (2018) [34] also observed that the increasing concentrations of ferulic acid substantially inhibited the growth of *Fusarium graminearum* (FG), *F. langsethiae* (FL) and *F. poae* (FP). In contrast, p-hydroxybenzoic acid, vanillic acid, quercetin, and rutin slightly stimulated mycelium growth. The effect of different phenolic acids and flavonoids on the mycelium growth assay, using cereal based media, varied largely depending on the *Fusarium* species and the concentration levels tested. There are a few studies available on the in vitro effect of these antioxidants on Fusarium species.

Awika & Rooney (2004) [12] suggest that the antimicrobial activity of sorghum may be due to the presence of tannins and other phenols that may synergistically contribute to its antimicrobial power. According to Gordana et al. (2007) [35], the antimicrobial activity in plant extracts depends on several secondary metabolites. Gauthier et al. (2016) [36] report that phenolic compounds isolated from natural sources have valuable antifungal properties, but their effectiveness as an antifungal agent is often strain and molecule dependent. Furthermore, Dzhavakhiya et al. (2012) [37], Paul et al. (2011) [38] and Da Silva Bomfim et al. (2015) [39] suggest that the combination of natural compounds with other phenolic acids, or with essential oils, or with conventional fungicides, may be a possible strategy to improve the bioactivity of these compounds, resulting in increased antifungal activity. According to Paul et al. (2011) [38] and Da Silva Bomfim et al. (2015) [39] essential oils combined with phenolic compounds can improve their bioavailability, increasing the permeability of phenolic compounds in mitochondria and the fungal plasma membrane.

Although the SC 319 genotype in the present work contains high levels of tannins [18], this genotype may have a low content of ferulic acid, and its antifungal activity was demonstrated by Funnell-Harris et al. (2014) [33]. Furthermore, the study by Kil et al. (2009) speculated that the cultivar might affect the inhibition of fungal growth. Thus, it is suggested to carry out further work with different sorghum genotypes with different phenolic compound profiles to elucidate which phenolic is more effective as a fungicide to evaluate its inhibitory effect on different species of fungi.

Conclusions

Contrary to expected, the sorghum phenolic extract (SPE) from the bran of the SC319 genotype stimulated the growth of some fungal species in the PDA and PD media, MIC, and Conidia Germination Rate assays. Instead, in the Grow Curve assay, SPE had an inhibitory effect on some fungi growth in 24 h, suggesting that the SPE can delay the fungal growth in the first hours. However, further studies are necessary to confirm these effects, and the use of other sorghum varieties with different phenolic compound profiles is suggested.

Although the results of this study were not conclusive regarding the antifungal potential of SC319 sorghum phenolic extract, it can be used to enhance the growth of beneficial fungi commonly used in the food and pharmaceutical industries.

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Data availability All data generated or analysed during this study are included in this published article.

Declarations

Conflict of interest The authors declare no competing interests.

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