Current Research in Environmental & Applied Mycology (Journal of Fungal Biology)
14(1): 122–132 (2024) ISSN 2229-2225
www.creamjournal.org **Article**
Doi 10.5943/cream/14/1/7

Antifungal activity against *Fusarium oxysporum* and *Sclerotinia sclerotiorum* of *Penicillium citrinum* extracts isolated from *Anticarsia* larvae

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Shiraishi IS, Daniel JFS, Berto TM, Oliveira WL, Abreu LM, Evaristo JAM, Evaristo GC Pontes FLD 2024 – Antifungal activity against *Fusarium oxysporum* and *Sclerotinia sclerotiorum* of *Penicillium citrinum* extracts isolated from *Anticarsia* larvae. Current Research in Environmental & Applied Mycology (Journal of Fungal Biology) 14(1), 122–132, Doi 10.5943/cream/14/1/7

Abstract

Fungi have the ability to act as a biocontrol agent against plant diseases, providing an alternative to chemical control. The objective of this study was to demonstrate the *in vitro* antifungal activity of the crude extracts of *Penicillium citrinum*, against two phytopathogens of agronomic importance, *Fusarium oxysporum* and *Sclerotinia sclerotiorum*, and identify the metabolites of the most bioactive extract. Chloroform extract of rice solid medium showed excellent growth inhibition activity (65–100%) on two phytopathogens. This extract inhibited 86.9% and 100% of *F. oxysporum* and *S. sclerotiorum*, respectively. The metabolites citrinin, citrinin H–1, fusarindin, redoxcitrinin, emodin, 2,4–dihydroxy–3,5,6–trimethylbenzoic acid, sclerotinin A, and 2,4,5–trimethylbenzene–1,3–diol were identified from chloroform extract by LC–HRMS. The metabolite fusarindin was identified for the first time in the fungal species. The use of organic extracts has the potential for successful biological control, mitigating the excessive use of fungicides that may pose a risk to human health and the environment.

Keywords – antagonistic activity – biocontrol agent – phytopathogenic fungus – polyketides

Introduction

Diseases caused by phytopathogenic fungi are responsible for the loss of vegetable and fruit crops throughout the world (Teng et al. 1984, Avery et al. 2019). Brazil is a large producer of grain and fruit and one of the largest soy agricultural producers. Research aiming at the prediction of weather conditions and the strict control of invasive pests helps to maintain this important economic activity. The control of problematic microorganisms, together with the increasing resistance of plant pathogens to these fungicides lead to their unrestrained use in several crops (Singh 2014). In addition, the indiscriminate use of these compounds does not ensure the

Submitted 30 July 2024, Accepted 02 September 2024, Published 25 September 2024
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requirements of this increasingly demanding market, which prioritizes the quality of products. Thus, due to the increased negative environmental and health effects of current chemical crop control, strategies are needed to reduce the level of exposure to agrochemicals together with providing safer food (Avery et al. 2019).

Fusarium species rank among the most economically destructive plant pathogens, causing plant diseases including *Fusarium* head blight or cereal scab, sudden death syndrome in soybean, maize ear rot, and maize and pea root rot (O'Donnell et al. 2013). *Fusarium oxysporum* species complex is extremely common in numerous soils and plants, producing vascular wilts, damping off and crown and root rot in a wide range of hosts (Aoki et al. 2014), such as cucumber, tomato, sweet basil and melon (Maher et al. 2008).

Another significant plant pathogen, *Sclerotinia sclerotiorum*, is the cause of irreparable damage and is known as "white mold disease" in a number of plants, including cabbage, potatoes, and beans (Bolton et al. 2006). The dormant state produced by this fungus, denominated sclerotia, can survive in soil over several years, with continued metabolic events, causing new infection cycles (Mitchell & Wheeler 1990). Sclerotia germinate carpogenically or myceliogenically, initiating different types of disease. Carpogenic germination produces apothecia and later ascospores to infect different host plants and cause diseases. The myceliogenic germination of sclerotia produces vegetative hyphae that extend to different plant tissues leading to infection (Bolton et al. 2006).

Moreover, the use of fungi isolated in the environment as biocontrol agents is an important goal for researchers aiming at environmentally friendly agriculture increases in the quality of crops, and the development of green agriculture approaches (Das et al. 2019). *Penicillium citrinum*, is a filamentous fungus that grows in a variety of environments, including soils and plant waste, as well as endophytic hosts in plants (Ting et al. 2012), marine habitats (Kawahara et al. 2012), and more recently, entomopathogenic fungi (Wu et al. 2022, Idrees et al. 2023), was isolated in the current study. Research revealed that *P. citrinum* can be used to manage pests since it is known that the metabolite, citrinin, and the fungal culture filtrate are both efficient against the phytopathogenic fungus *Botrytis cinerea* (Sreevidya et al. 2015). Ting et al. (2012) showed a lower rate of disease occurrence and disease severity caused by *F. oxysporum* using banana plantlets inoculated with *P. citrinum*. Biocontrol capacity comprises the antagonistic potential of the fungus, as well as its ability to produce metabolites and lytic enzymes (Singh 2014). The detection of these natural products in organic extracts and their possible use in the environment are important to contribute to the safety of agriculture, decreasing health and ecological risks. This study evaluated the potential of organic extracts of the strain *P. citrinum* (JUANT028), isolated from a caterpillar of *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) to control the phytopathogenic fungi *S. sclerotiorum* and *F. oxysporum*.

Materials & Methods

Microorganisms

The JUANT028, JUANT029, and JUANT079 fungi were isolated from *A. gemmatalis* larvae collected in a soybean crop (Brazil), coordinates: 23º18'5"S and 51º6'21" W. A total of 81 individuals were isolated and designated using the JUANT coding prefix (Yada et al. 2019). *Sclerotinia sclerotiorum* was isolated from different soil samples (0–20 cm depth) they were taken from fields of corn crops (Sumida et al. 2018).

DNA sequencing and phylogenetic analyses

The JUANT028 strain was cultivated on potato–dextrose–agar (PDA; Himedia) for five days at 25°C. The genomic DNA was extracted using the Wizard® Genomic DNA Purification Kit (Promega). The polymerase chain reaction was conducted with the primers T1 and T2 (Liu et al. 1999) under the conditions of the partial amplification of the Beta-tubulin (*tub2*) gene. The purified PCR product was sequenced by a commercial service (Moreira et al. 2016).

Blast searches conducted with the *tub2* DNA sequence of JUANT028 suggested that it belonged to the Section Citrina of *Penicillium*, and an alignment was composed with TUB2 sequences of reference strains of this section. A Maximum likelihood (ML) phylogenetic tree was estimated using the RAxML program (v. 8.12) implemented in the Cipres portal (www.phylo.org), with the "rapid bootstrap analysis/search for the best–scoring ML tree" option.

Screening of fungal isolates

The antagonistic activity of JUANT028, JUANT029 and JUANT079 fungi was tested in dual–culture confrontation assays against *S. sclerotiorum* on PDA medium. Agar discs (8–mm diam.) were taken from the edge of 7–day old colonies of fungi and *S. sclerotiorum* was taken from the edges of actively growing colonies of fresh fungal cultures, and placed on the surface of a fresh PDA plate at a spacing of 6 cm. The radial growth of the fungal mycelium was measured until the control completely covered the Petri dish (5 days). The plates were incubated in a Biochemical Oxygen Demand (BOD) incubator at 20°C with a photoperiod of 12 h. The experiments were conducted in replicates of five and calculation of antagonism was performed after 21 days of incubation. Interactions between mycelia were scored for degree of antagonism using a scale of 1–5 (Bell et al. 1982), where 1 = JUANT028, JUANT029, and JUANT079 fungi overgrowing *S. sclerotiorum*, 2 = JUANT028, JUANT029, and JUANT079 growing on 2/3 of the dish. 3 = dish evenly occupied by both strains, 4 = *S. sclerotiorum* growing on 2/3 of the dish, and 5 = *S. sclerotiorum* overgrowing JUANT028, JUANT029, and JUANT079 fungi.

Percentage inhibition (*PI*) was calculated according to the following equation: $PI = C–T/C \times 100$, where *C* is the growth of the test pathogen in the absence of antagonist (cm) and *T* is the growth of the test pathogen in the presence of antagonist (cm).

Preparation of crude extracts from JUANT028 fungus

JUANT028 was cultivated on PDA at 28°C for 7 days. Subsequently, three agar disks (8 mm) of the strains were inoculated in 20 Erlenmeyer flasks of 1 L with 250 mL of liquid medium (ML) and twenty–five flasks (0.5–litre) containing rice medium (MR). The ML contained 30.0 g dextrose, 5.0 g bactopeptone, 3.0 g of yeast extract, 0.3 g MgSO4, 0.3 g KH_2PO_4, and 0.3 g K_2HPO_4 in 1 L of water. Each MR flask contained 90 g of rice (Uncle's Ben's®) and 75 mL of distilled water was autoclaved at 121°C for 45 min. Controls consisted of flasks containing ML broth and MR media that were not inoculated.

The fungal cultures were left stationary at room temperature. Mycelium fungi of the cultures in ML broth were separated by reduced pressure filtration and the liquid phase was yielded to liquid/liquid fractionation with ethyl acetate (EtOAc). After lyophilization, the mycelial masses were combined and extracted totally with methanol (MeOH). The organic solvents were removed by vacuum distillation at 55 and 60°C using a rotary evaporator. The yield was ethyl acetate extracts **ML**–**A** (1.16 g) and methanolic extracts **ML**–**M** (19.96 g). Cultures of JUANT028 in flasks containing MR were macerated with chloroform (3 x 500 mL), followed by extractions with ethyl acetate (3 x 500 mL), and lastly, extraction with methanol (3 × 500 mL). Yields of the extracts obtained from rice medium were 31.8 g from chloroform (**MR**–**C**), 19.9 g from ethyl acetate (**MR**–**A**), and 123.4 g from methanol (**MR**–**M**). Uninoculated controls of PD broth and MR media were extracted with ethyl acetate and methanol, respectively.

Antifungal activity of JUANT028 extracts against phytopathogenic fungi

The *in vitro* antifungal activity of the different extracts of JUANT028 was evaluated on solid media by measuring the radial mycelial growth rate of *S. sclerotiorum* and *F. oxysporum* in the absence and presence of the extracts at concentrations of 50, 150, 250, and 350 μg/mL (Quiroga et al. 2001). Dried fungal and negative control extracts were dissolved in dimethyl sulfoxide (DMSO, 10%, v/v) and Tween 80 (0.5%, w/v) to give a final concentration of 50–350 μg/mL. Aliquots of 0.8 ml of each extract were added to molten PDA medium, and then dispensed into Petri dishes. Agar disks (8 mm) taken from cultures of the two pathogenic strains were placed in the center of

the plates of *F. oxysporum* and *S. sclerotiorum*, and incubated at 20 and 25°C, respectively. The positive control containing 5.0 ppm of the commercial fungicide Frowncide 500 SC® (500 g of active ingredient Fluazinam/liter; Syngenta, Sorocaba–Brazil, Brazil) [3–chloro–N– (3–chloro–5–trifluoromethyl–2–pyridyl) –α,α,α–trifluoro–2,6–dinitro–p–toluidine] was tested. Five replicates were run simultaneously. The negative control was composed of a mixture of the solvents used for dissolving the extracts. After 5 days (growth in the control plates reached the edge of the Petri dish), the radial mycelial growth was measured. Each data point represented the mean of four measurements of a growing colony.

Growth inhibition (GI) was calculated according to the following equation: $GI(\%) = MGC – (MGPE/MGC) \times 100$, where *MGC* is mycelial growth of the negative control, and *MGPE* is mycelial growth in the presence of the fungal extracts.

The antifungal data are expressed as mean ± standard error of mean (SEM), and data were evaluated using statistical analysis (ANOVA) to determine the significance level of the differences ($p<0.05$). The statistical significance for the differences between extracts was detected by ANOVA, followed by the Tukey test. Data analysis was performed using R software (R Core Team 2013), http:/www.R–project.org/).

Chromatographic fractionation and analysis by LC–HRMS

The chloroform extract (MR–C, 31.8 g) was fractionated through Sephadex LH–20 (1.75 mm × 0.3 Ø) using MeOH as the eluent. Fraction MRC–6 was then purified by reversed-phase C18 Luna 5µ (250 x 10,0 MM), eluting with 50% MeOH to give six semi-purified fractions named MRC–6P1 – MRC–6P6. The six fractions, MRC–6P1–6P6, of the JUANT028 extract were analyzed using HPLC coupled to a high–resolution mass spectrometer with quadrupolar and time–of–flight hybrid analyzers (HPLC–QTOF–MS) (micrOTOF–Q II, Bruker Daltonics, Fremont, CA, USA).

An aliquot of 2 mg of each fraction extract was solubilized in 1 mL of HPLC-grade methanol. The sample was sonicated for 15 min, centrifuged (Eppendorf MiniSpin, Hamburg, Germany) at 13,400 rpm for 5 min, and filtered through a PVDF syringe filter with a 0.22 µm pore size (Agilent HP, CA, USA) before liquid chromatography–high resolution mass spectrometry (LC–HRMS) analysis. LC–HRMS Instrumentation and Conditions: LC–HRMS analysis was performed on a high–performance liquid chromatography (HPLC) (Prominence UFLC, Shimadzu, Tokyo, JP), with an autosampler maintained at 10°C, coupled to the quadrupole time of flight mass spectrometer (QToF–MS) (MicroToF QII, Bruker Daltonics, Fremont, CA, USA). Compound separation was achieved on an Synergi Fusion–RP 80 150 mm × 2.0 mm column, with a 4 µm particle size (Phenomenex, Torrance, CA, USA) maintained at 40°C. For positive and negative ion mode, the gradient elution with a binary mobile phase consisted of water (A) and methanol(B), both containing formic acid 0.1 %. The gradient elution modes were as follows: 0–28 min (5–95 % B), 28–38 min (95 % B), 38–48 min (95 – 5 % B), and 48–55 min (5 % B). The flow rate was 200 µL /min and the sample injection volume was 5 µL. HRMS analyses were performed in an electrospray (ESI) ion source using the following instrument settings: nitrogen as the nebulizer gas at 2.0 bar; nitrogen as the drying gas (flow of 6.0 L/min) at 180°C; capillary potential of 4.5 kV for the positive and 3.5 kV for the negative mode. Acquisition range was m/z 100–1000. The instrument was calibrated with sodium formate. Data acquisition was carried out using Compass Data Analysis 4.1 software (Bruker Daltonics, Fremont, CA, USA).

Data processing and metabolite identification

Metabolite identification was based on MSDIAL 5.1 using the internal database for positive and negative ionization modes. Some compounds could be identified only by their elemental composition, and others were completely unknown. The raw data from the LC/MS were transformed into the "ibf" format and then analyzed using MS–Dial software (version 5.1.0), which is freely available. The following parameters were used in the analysis: MS1 tolerances of 0.01 Da, a minimum peak height of 15,000, and a mass slice width of 0.05. Databases from MSDIAL were

utilized for metabolite identification. These databases, which were downloaded in December 2022, include positive charged spectra (ESI(+)–MS/MS from authentic standards, containing 16,481 unique compounds, VS17) and negative charged spectra (ESI(–)–MS/MS from authentic standards, containing 9,033 unique compounds_VS17).

Results

Fungal identification

The Maximum Likelihood phylogenetic tree constructed with partial TUB2 DNA sequences grouped only the isolate JUANT028 (continuation of study) inside a well–supported monophyletic group with the ex–type and other reference strains of *P. citrinum*, confirming the identification of the fungus (Fig. 1).

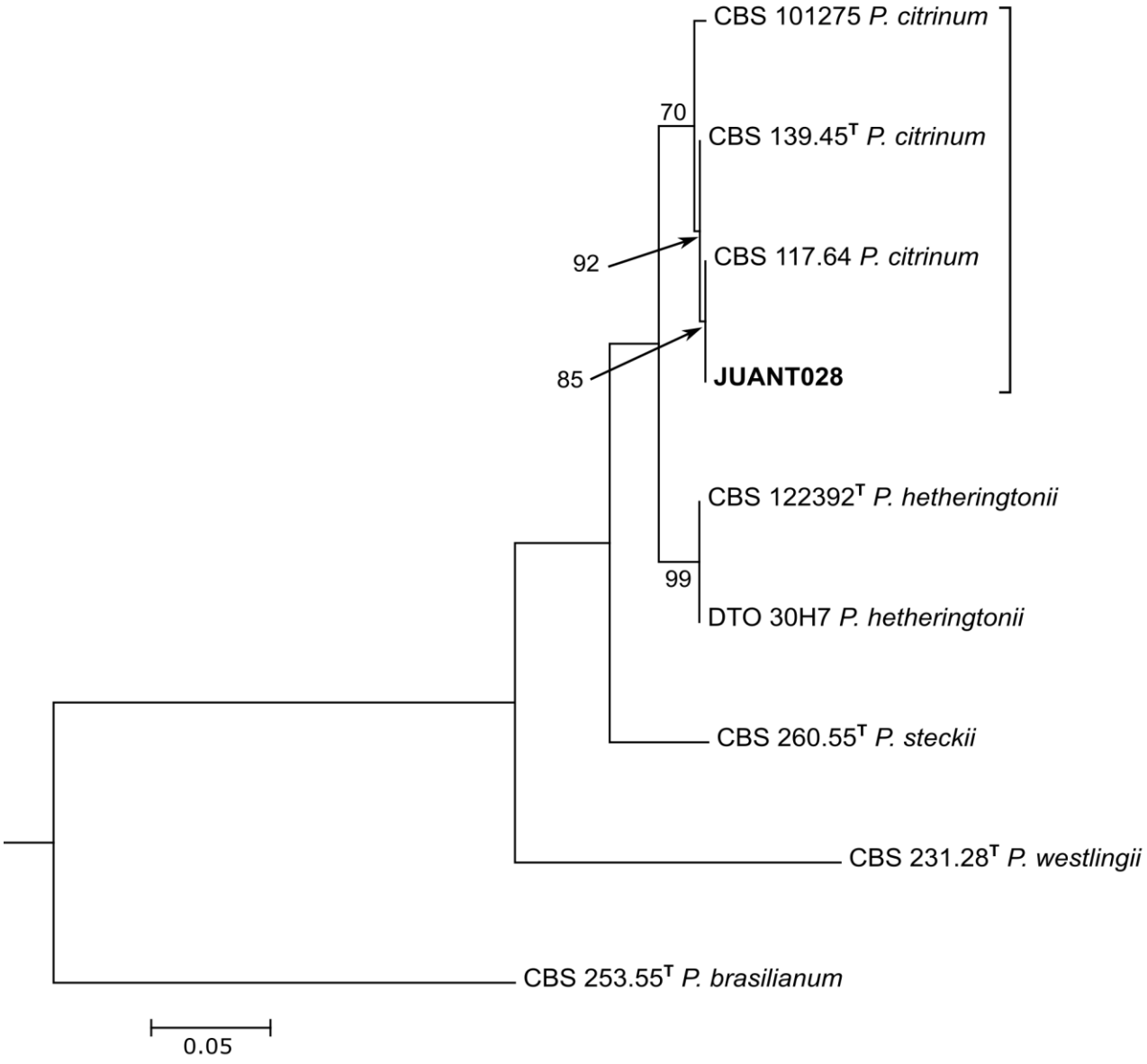

Fig. 1 – Maximum Likelihood tree of partial *tub2* gene showing the phylogenetic relationships of JUANT028 isolate and reference strains of *Penicillium* Section Citrina. Bootstrap values > 70% are shown by the nodes. *Penicillium brasilianum* strain CBS 253.55 was used as the outgroup. T identifies ex–type strains.

Growth inhibition assay by dual culture technique

In the dual culture assays, *P. citrinum* (JUANT028) showed significant antagonism to soil-borne *S. sclerotiorum* (Table 1). A reduction in mycelial growth was observed during the dual culture method. The radial growth of *S. sclerotiorum* was inhibited by *P. citrinum* (JUANT028), JUANT029, and JUANT079 with 75.3%, 57.9%, and 28.4% inhibition, respectively, after 7 days of incubation. This indicates that all three entomopathogenic isolates tested inhibited the soil-borne phytopathogen. *Penicillium citrinum* had the lowest scores according to Bell's classification and was the most effective antagonist, so it was chosen for further study. Radial growth of *S. sclerotiorum* was inhibited by *P. citrinum* (JUANT028), in a class 2 interaction of dual culture assay.

Table 1 Mycelial growth inhibition by entomopathogenic isolates after 7 days of inoculation in dual culture. Values are the mean of four replicates ± standard error.

Isolates	*S. sclerotiorum* inhibition (%)	Bell's classification
JUANT028	75.3 ± 0.6	2
JUANT029	57.9 ± 0.3	3
JUANT079	28.4 ± 0.2	4

Antifungal activity of *P. citrinum* extracts using PDA well diffusion assay against phytopathogenic fungi

As shown in Table 2, all extracts tested at doses above 150 µg/mL reduced the development of *F. oxysporum* and *S. sclerotiorum*. At 350 µg/mL, the MR–C extract showed the highest level of inhibition (86.9%) against *F. oxysporum*, whereas the MR–M extract showed the lowest levels of inhibition against this phytopathogen.

When compared to the other extracts, the MLM extract for S. *sclerotiorum* exhibited the least inhibition at all tested doses (up to 25.8%). With inhibitions of 77.9, 90.2, 100, and 100% at doses of 50, 150, 250, and 350 µg/mL, respectively, the MR–C extract showed the greatest results. Shiraishi et al. (2019) already published a description of the preliminary findings for these extracts.

Identification of *P. citrinum* metabolites by UHPLC and HRMS/MS in tandem mode

Eight active polyketide natural products from entomopathogenic *P. citrinum* chloroform extract were identified in this study (Fig. 2): citrinin H–1, redoxcitrinin, fusarindin, emodin, 2,4–dihydroxy–3,5,6–trimethylbenzoic acid, 2,4,5–trimethylbenzene–1,3–diol and sclerotinin A. Table 3 exhibits the mass accuracy and characterization of the specified ions in the metabolites.

Discussion

Penicillium citrinum is a widely distributed filamentous fungus found globally. This species has been isolated from a variety of substrates, including soil, tropical cereals, plants, spices, human tissues, indoor environments, and insects (Houbraken et al. 2010, Belmont–Montefusco et al. 2020). *Penicillium citrinum* was previously reported as a biocontrol agent of *Botrytis cinerea*, which causes Botrytis Gray Mold, as well as being antagonistic to *Macrophomina phaseolina* (Sreevidya et al. 2015). This fungus was able to inhibit the development of *Alternaria alternata, Colletotrichum gloeosporioides, Fusarium solani, Pestalotiopsis theae,* and *S. sclerotiorum,* in a class 3 interaction of dual culture assay (Sharma et al. 2021). These studies collaborate with our results and encourage the continuation of studies with this microorganism.

In the well diffusion assays, *P. citrinum* extracts were highly effective against phytopathogens. Extract MR–C achieved 65–87% inhibition of *F. oxysporum*, a range that aligns with the maximum inhibition (77.7%) reported in a similar investigation (Yogalakshmi et al. 2021), which evaluated the ethyl acetate extract from *Trichoderma atroviride* culture against the same pathogen.

Table 2 Growth inhibition (%) of *Fusarium oxysporum* and *Sclerotinia sclerotiorum* by organic extracts of *Penicillium citrinum*.

Extracts[1]	Concentration (µg/ml)	***F. oxysporum***		***S. sclerotiorum***	
		Radial mycelial growth (cm)	Growth inhibition (%)	Radial mycelial growth (cm)	Growth inhibition (%)
ML-A	50	7.7[a, ◆](a)	0	3.8[a(*),b,g](b)	40.8
	150	6.6[b,d](a)	15.5	3.4 [b,&](b)	49.8
	250	6.0[b(*),e](a)	24.9	3.4 [a*,b, ◆](b)	51.0
	350	5.9 [b*,e,#, ❖](a)	25.3	2.6[&,f,h,i](b)	66.3
ML-M	50	8.4[c](a)	0	5.9[c](b)	0
	150	5.8 [b*,e, ❖](a)	27.0	5.2[c,d,e](b)	11.9
	250	5.6 [b*e,f](a)	30.0	4.9[d,e,#](b)	19.6
	350	5.5 [b*,f](a)	32.3	4.6[e,#](b)	25.8
MR-C	50	3.3[g](a)	64.8	2.1[h,j, ❖](b)	77.9
	150	3.0[g](a)	70.5	1.5[f, h,j](a)	90.2
	250	2.0[h](a)	84.9	1.0[k](a)	100
	350	1.9[h](a)	86.9	1.0[k](b)	100
MR-A	50	7.3[a,&] (a)	4.6	5.6[c, #](b)	4.8
	150	6.7[b,&,#] (a)	14.6	4.3[b,g](b)	30.6
	250	5.6 [b*,f](a)	31.2	2.9[a*,&,◆❖](b)	59.6
	350	5.0,[f](a)	39.5	2.6[&,◆,❖](b)	67.7
MR-M	50	8.1[c, ◆](a)	0	4.3[b,e](b)	31.3
	150	7.3[d,&] (a)	5.1	3.6[a*,b, ◆](b)	46.7
	250	7.3[a,&] (a)	4.5	2.4[&,f,h](b)	71.9
	350	6.4[b, ❖](a)	18.7	2.1[f, h, ❖](b)	78.1
Frowncide[2]	5ppm	1.8[h](a)	85.0	1.0[k](b)	100
Negative control[3]		7.5[a](a)		5.8[c,d](b)	

Notes: Values followed by the same letter in the line and superscripts in the column for *P. citrinum* separately do not differ statistically according to the Scott–Knott test ($p \leq 0.05$).

[1]Extracts – ML–M: mycelial methanolic extract; ML–A: ethyl acetate filtrate extract; MR–C: chloroform rice extract. MR–A: ethyl acetate rice extract. MR–M: methanolic rice extract.

[2]Frowncide 500 SC®: fungicide (5 ppm).

[3]Control used to calculate inhibition: DMSO 5%, three drops of Tween 80.

Extract MR–C also resulted in total inhibition (100%) of *S. sclerotiorum* at concentrations of 250 and 350 µg/mL. These results indicate that the polyketides and other mycotoxins with bioinsecticide activity were biosynthesized by *P. citrinum*. Growth of *S. sclerotiorum* was also completely inhibited when exposed to the volatile metabolites produced by the fungus *Clonostachys rosea*, as demonstrated in research conducted by Rodriguez et al. (2011).

Although the results of this research were not significantly different from those achieved by the insecticide Frowncide, which contains the active compound fluazinam, it's important to note that fluazinam may have adverse environmental effects. The lack of extensive research on the consequences of indiscriminate pesticide use often leads to an underestimation of their harmful impacts and the effects of higher concentrations. This work aims to address the need for safer alternatives to agrochemical controls, which pose significant risks to both human health and the environment. By exploring and developing these alternatives, we hope to enhance food safety and reduce environmental harm.

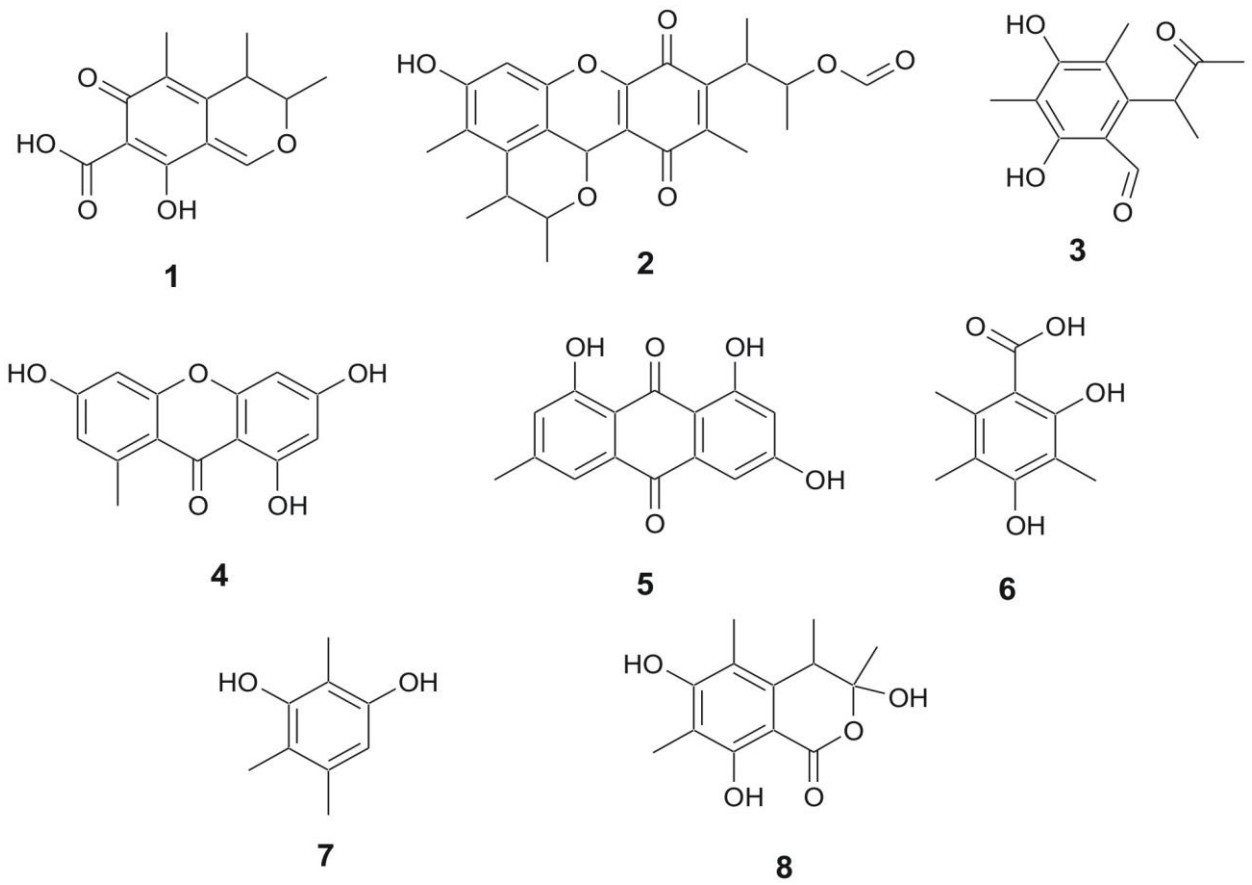

Fig 2 – *Penicillium citrinum* metabolites citrinin (1), citrinin H–1 (2), redoxcitrinin (3), fusarindin (4), emodin (5), 2,4–dihydroxy–3,5,6–trimethylbenzoic acid (6), 2,4,5–trimethylbenzene–1,3–diol (7), and sclerotinin A (8).

Table 3 Characterization (mass accuracy) of named ions of the metabolites of chloroform extract of *Penicillium citrinum.*

Metabolite	**Molecular formula**	**Calcd. $[M+H]^+$**	**Found $[M+H]^+$**	**Calcd. $[M-H]^-$**	**Found $[M-H]^-$**	**Error / ppm**
Citrinin	$C_{13}H_{14}O_5$	251.091950	251.09000			-7.8
Citrinin H–1	$C_{24}H_{26}O_7$	427.175680	427.17746			4.2
Redoxcitrinin	$C_{13}H_{16}O_4$	237.112685	237.11214			-2.3
Fusarindin	$C_{14}H_{10}O_5$	259.060650	259.06219			5.9
Emodin	$C_{15}H_{10}O$	271.060650	271.06439			13.8
2,4–dihydroxy–3, 5,6–trimethylbenzoic acid	$C_{10}H_{12}O_4$	197.081385	197.08110			-1.4
2,4,5–trimethylbenzene –1,3–diol	$C_9H_{12}O_2$			151.07590	151.08182	39.0
Sclerotinin A	$C_{13}H_{16}O_5$			251.09195	251.10054	34.2

Extracts and metabolites of natural products to control fungal diseases are necessary to reduce the use of chemical insecticides and explore new natural bioinsecticides, since plant diseases pose a serious threat to economically significant crops globally, with fungus being the primary pathogen responsible for plant disease.

Eight active polyketide were identified from the chloroforms extract in this study. It is well known that polyketides are a broad class of secondary metabolites synthesized by microorganisms with various biological activities. The polyketide citrinin, a fungal metabolite, is a well-known mycotoxin produced mainly by *P. citrinum* and several *Aspergillus* species (Vrabcheva et al. 2000).

All these compounds have been identified previously from *P. citrinum* (Lu et al. 2008; Lai et al. 2013; Luo et al. 2019; Wang et al. 2019), except fusarindin isolated from *Penicillium patulum* and other species of the genus *Penicilium* (Broadbent et al. 1975; Pastre et al. 2007). The eight identified metabolites exhibited biological activities. Citrinin and emodin showed inhibition on hypha growth of tested plant pathogenic fungi (*Alternaria citri, Alternaria oleracea, Bipolaris maydis, Colletotrichum capsici, Ceratocystis paradoxa, Cochliobolus miyabeanus, Diaporthe citri, Exserohilum turcicum, Pestalotiopsis theae, Phytophthora parasitica var. nicotianae*) with IC_{50} values ranging from 3.1 to 123.1 μg/mL and 3.0 to 141.0 μg/mL, respectively (Luo et al. 2019).

Citrinin, citrinin H–1, and 2,4,5–trimethylbenzene–1,3–diol revealed antioxidative activity against DPPH radicals (Lu et al. 2008). Citrinin and citrinin H–1 were investigated for their antibacterial activity. For *E. coli*, citrinin H–1 demonstrated bactericidal activity up to a concentration of 15.63 μg L^{-1}, which was comparable to the action of citrinin against other bacteria (Pastre et al. 2007). *P. citrinum* extract and its metabolites have antifungal efficacy against *Aspergillus fumigatus, A. niger, Candida albicans,* and *C. neoformans*. Comparable to citrinin at 100 μg/disc, the extract's results were obtained at a concentration of 2.5 mg/disc (Wakana et al. 2006). In the current study, extracts produced by *P. citrinum* showed significant antifungal activity in inhibiting two phytopathogenic fungi. Considering this approach, polyketides with verified biological activity were found in the chloroform extract, which inhibited phytopathogens.

Conclusion

Two phytopathogenic fungi, *F. oxysporum* and *S. sclerotiorum*, were inhibited by the chloroform rice extract produced by *P. citrinum*, demonstrating antifungal activity. Citrinin, citrinin H-1, fusarindin (3,6,8-trihydroxy-1-methylxanthone), emodin, redoxcitrinin, 2,4-dihydroxy-3,5,6-trimethylbenzoic acid, sclerotinin A, and 2,4,5–trimethylbenzene–1,3–diol were the eight metabolites identified in this extract. All of these metabolites, except fusarindin, have been produced previously by *P. citrinum*. The findings open up new directions for an investigation into these extracts, which may have been use in agriculture as natural antifungal agents to manage phytopathogens.

Acknowledgements

This work was supported by Diretoria de Pesquisa e Pós-Graduação (EDITAL 2019 – PROPPG – Dr J.F.S. Daniel) of Federal Technological University of Paraná, Brazil.

The authors express their gratitude to Dr. Ciro H. Sumida and Dr. Solange Monteiro de Toledo Piza Gomes Carneiro for kindly providing *S. sclerotiorum* and *F. oxysporum* strains, respectively.

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