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Natural Products Obtained from Argentinean Native Plants Are Fungicidal against Citrus Postharvest Diseases

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Abstract: Natural products obtained from plants constitute an alternative to chemically synthesized fungicides, whose improper use might have caused the development of resistant fungal strains. In the present work, 40 products obtained from 20 native Argentinean plant species were tested against three citrus postharvest pathogens: *Penicillium digitatum*, *Penicillium italicum*, and *Geotrichum citri-aurantii*. Natural products were obtained by classical solvent extraction methods and the fungicidal evaluation was carried out by agar diffusion tests using commercial fungicides as negative controls and dimethyl sulfoxide as a positive one. The inhibition percentages were determined 7 and 14 days post inoculation of each fungus. Most of the products tested showed inhibition percentages higher than 50% for *G. citri-aurantii*, but only 20% of them were active against *P. digitatum* and *P. italicum*. The most promising products which inhibited (100%) the growth of at least one of the three phytopathogens were extracted from the following plants: *Orthosia virgata*, *Petiveria alliacea*, *Funastrum clausum*, *Solanum caavurana*, and *Solanum pilcomayense*. These products were tested over inoculated oranges and there were no statistically significant differences between the treatments with a commercial fungicide and the methanolic extract in the control of fruit rot. The products extracted from native plants have fungicide potential, but further studies are required.

Keywords: natural products; post harvest; citrus diseases

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1. Introduction

Fruits of *Citrus* spp. (Rutaceae) are cultivated in more than one hundred countries and their fruits are widely consumed throughout the world. Postharvest handling tries to achieve the highest quality fruits, increasing their postharvest life and reducing production losses, thus obtaining commercially suitable fruits [1]. According to the latest estimates by Federcitrus (2022), Argentina produces 1,038,168 tons of oranges; 77,000 of them are destined for the export market [2]. Postharvest losses produced by phytopathogenic origin (approximately 30% of the production) are considered of economic importance. Green and blue molds, caused by *Penicillium digitatum* (Pers.: Fr.) Sacc. and *Penicillium italicum* Wehmer, respectively (order Eurotiales, Aspergillaceae family), and sour rot caused by *Geotrichum citri-aurantii* Ferraris (order Saccharomycetales, Dipodascaceae family), are the most economically important postharvest diseases as they affect not only the quantity but also the quality of citrus fruits which, once infected, must be discarded from the production lot [1–5]. *Penicillium digitatum* and *P. italicum* cause citrus diseases by invading wounds in the fruit rind. Wounds occur during the harvest and subsequent handling of fruit in the

packinghouse or during commercialization, but some infections can occur before harvest through injuries, cracks, or wounds made by insects in the field. In this case, the fruit infected long before harvest usually drops from the tree, but fruit infected three days before harvest cannot be detected and may be harvested, becoming a source of contamination [2]. A circular area surrounding the infection site (rind wound) appears water-soaked, soft, and decolorized. In the meantime, the fungus grows, and an aerial white mycelium develops in the center of the lesion and expands radially. In the case of green mold, after 7–8 days the central area of the lesion becomes olive green colored and surrounded by a broad band of dense and non-sporulating white mycelium. In contrast, blue mold presents a central sporulating area, blue or bluish green colored [1].

Geotrichum citri-aurantii causes sour rot; this fungus lives in dead plant tissue or orchard soils and it may cause diseases in fruits. Symptoms include maceration and disintegration of the fruit cuticle and pulp. Once the fruits rot, they generate a strong fermented and rancid smell. If high humidity conditions are present, a very thin white mycelium appears on the fruits, rapidly disappearing [3,4].

These three postharvest diseases have been controlled worldwide for many years solely by the application of conventional fungicides after harvest such as (1) Imazalil (IMZ) [(RS)-1-(*β*-allyloxy-2,4-dichlorophenethyl) imidazole], which constitutes the most common fungicide employed postharvest by the citrus industry; (2) Thiabendazole (TBZ) [2-(1,3-thiazol-4-yl) benzimidazole], which belongs to the benzimidazole group of fungicides and has been used for the control of citrus molds; (3) Sodium ortho-phenylphenate (SOPP) [sodium (1,1′-biphenyl)-2-olate], which is currently used in combinate fungicide formulations; (4) Guazatine and propiconazole, which have been used in Europe, South Africa, and Australia for the control of *G. citri-aurantii*; and finally, pyrimethanil (PYR) (4,6-dimethyl-N-phenyl-2-pyrimidinamine], fludioxonil (FLU) [4-(2,2-difluoro-1,3-benzodioxol-4-yl)-1H-pyrrole-3-carbonitrile, azoxystrobin (AZX) [methyl-(2*E*)-2-{2-[6-(2-cyanophenoxy) pyrimidin-4-yloxy] phenyl}-3-methoxyacrylate], and Trifloxystrobin (TFX) (methyl-(*E*)-methoxyimino-{(*E*)-*α*-[1-(*α*,*α*,*α*-trifluoro-*m*-tolyl) ethylideneaminoxy]-*o*-tolyl}acetate) are classified as "reduced-risk" fungicides [1,5]. IMZ and TBZ are used alone, in mixtures, or separately applied in sequence. These have constituted the most widely used treatments for more than 25 years, which has contributed to the proliferation of resistant fungal strains [6]. Consequently, the excessive or inadequate use of these fungicides represents a risk to human and animal health, due to food contamination and the accumulation of toxic residues in the environment. Due to marketing globalization and climate change, the problem is growing at an accelerated pace [7,8].

In this context, there are some new alternatives for the control of diseases that occur in citrus postharvest, such as biological control, natural products obtained from different sources, and thermotherapy [9]. Biological control is based on the utilization of antagonistic microorganisms to reduce/keep the population of some phytopathogens below the levels that cause economic losses [10]. Another alternative is the use of natural products obtained from plant extracts or essential oils, which have revealed non-toxic action and biodegradability but are potent antibiotics against phytopathogens [8,11,12]. Finally, through cycles of considerable temperature changes on fruits, fungal infections can be reduced [9].

Plants provide unlimited opportunities for evaluating and isolating new antifungal compounds because of their vast chemical diversity; in fact, numerous antifungal compounds currently used against human pathogens have been isolated from them [13,14].

The aim of this study consisted of the discovery of new botanical products obtained from native Argentinean species with potential fungicide properties. For this, we selected 20 native plants from the central zone of Argentina which were evaluated for their fungicide properties against morphologically and molecularly characterized strains of *P. italicum*, *P. digitatum*, and *G. citri-aurantii* (isolated from citrus that presented the correspondent disease symptoms), by using a high-throughput and simple bioassay. Data were collected 7 and 14 days after fungal inoculation and each sample was classified according to its fungal growth inhibition percentage. Statistical analyses were performed based on the part of each

plant used and the type of extract in which the most active compounds might be present. The methanolic extract obtained from *S. pilcomayense* was evaluated over postharvest oranges artificially inoculated with *P. digitatum*. Finally, a discussion on the most active species, their ethnopharmacological uses, and phytochemistry, was accomplished.

2. Materials and Methods

2.1. Plant Material

The plants were collected from farms and at the side of roads in areas surrounding the Litoral region of Argentina, between the years 2019 and 2020. Each plant sample was identified, and a *Voucher Specimen* was deposited in the Herbarium of the FCA-UNL "Arturo Ragonese" (Herbario SF), Kreder 2805-(3080HOF)-Esperanza, Argentina (Table 1). After harvesting, the plants were dried in a suitable environment (a dark room with low relative humidity), while their different parts (leaves, flowers, fruits, or the whole plant) were separated according to the type of extract that had to be prepared. Three species (*Orthosia virgata*, *Solanum argentinum*, and *Solanum pilcomayense*) were collected in different zones to detect differences in their bioactivities which may be associated with phytochemical changes determined by the environment.

Table 1. Plant species used for extracts preparation to be tested against the orange pathogens *Penicillium digitatum*, *P. italicum*, and *Geotrichum citri-aurantii*. Botanic family, *Voucher specimen*, collection data, and place of collection or acquisition are also reported.

Family	Plant Scientific Name	*V. specimen*	Collection Data and Place of Acquisition
Apocynaceae	*Araujia brachystephana* (Griseb.) Fontella & Goyder	Pensiero et Zabala 13800	13/08/2019, San Javier, Santa Fe, 30°03′23.2″ S 59°51′34.8″ W
	Forsteronia glabrescens Müll. Arg.	Pensiero et Zabala 13804	13/08/2019, Gral. Obligado, Santa Fe, 28°42′54.8″ S 59°23′13.7″ W
	Orthosia virgata (Poir.) E. Fourn.	Pensiero et Zabala 13802	13/08/2019, San Javier, Santa Fe, 30°03′23.2″ S 59°51′34.8″ W
	Orthosia virgata (Poir.) E. Fourn.	Pensiero et Zabala 13805	13/08/2019, Gral. Obligado, Santa Fe, 28°42′54.8″ S 59°23′13.7″ W
	Funastrum clausum (Jacq.) Schltr.	Pensiero et Zabala 13797	13/08/2019, San Javier, Santa Fe, 30°03′23.2″ S 59°51′34.8″ W
Bromeliaceae	*Tillandsia tricholepis* Baker	Pensiero et Zabala 13798	13/08/2019, San Javier, Santa Fe, 30°03′23.2″ S 59°51′34.8″ W
Fabaceae	*Albizia inundata* (Mart.) Barneby & J.W. Grimes	Pensiero et Zabala 13816	14/08/2019, Gral. Obligado, Santa Fe, 28°12′53.9″ S 59°08′58.3″ W
	Vachellia caven (Molina) Seigler & Ebinger	Derita M. 39	17/03/2019, Las Colonias, Santa Fe, 31°26′30.3″ S 60°56′24.7″ W
Phytolaccaceae	*Petiveria alliacea* L.	Pensiero et Zabala 13975	13/08/2019, San Javier, Santa Fe, 30°03′23.2″ S 59°51′34.8″ W
Polygonaceae	*Polygonum stelligerum* Cham.	Derita M. 55	16/03/2019, San Pedro, Bs As, 33°40′11.6″ S 59°39′38.4″ W
	Polygonum lapathifolium L.	Derita M. 54	16/03/2019, San Pedro, Bs As, 33°40′11.6″ S 59°39′38.4″ W
Ranunculaceae	*Clematis montevidensis* Spreng.	Pensiero et Zabala 13824	17/08/2019, Vera, Santa Fe, 29°28′17.6″ S 60°05′27.7″ W
Nictaginaceae	*Pisonia zapallo* Griseb	Pensiero et Zabala 13808	13/08/2019, Gral. Obligado, Santa Fe, 28°42′54.8″ S 59°23′13.7″ W
Solanaceae	*Solanum argentinum* Bitter & Lillo	Pensiero et Zabala 13820	16/08/2019, Tapenagá, Chaco, 27°53′17.9″ S 59°51′39.4″ W
	Solanum argentinum Bitter & Lillo	Pensiero et Zabala 13817	16/08/2019, Lib. San Martín, Chaco, 26°07′13.9″ S 59°56′25.1″ W
	Solanum granulosum-leprosum Dunal	Pensiero et Zabala 13806	13/08/2019, Gral. Obligado, Santa Fe, 28°42′54.8″ S 59°23′13.7″ W
	Solanum pilcomayense Morong	Pensiero et Zabala 13815	14/08/2019, Gral. Obligado, Santa Fe, 28°13′42.2″ S 59°06′52.6″ W
	Solanum pilcomayense Morong	Pensiero et Zabala 13801	13/08/2019, San Javier, Santa Fe, 30°03′23.2″ S 59°51′34.8″ W
	Solanum caavurana Vell.	Pensiero et Zabala 13813	14/08/2019, Gral. Obligado, Santa Fe, 28°13′42.2″ S 59°06′52.6″ W
Verbenaceae	*Lantana megapotamica* (Spreng.) Tronc.	Pensiero et Zabala 13796	13/08/2019, San Javier, Santa Fe, 30°03′23.2″ S 59°51′34.8″ W

2.2. Extraction Process

For the preparation of the extracts, air-dried aerial parts of each species (100 g) were pulverized and successively macerated with 250 mL of dichloromethane (DCM) and 250 mL of methanol (MeOH) under mechanical stirring (3 × 24 h each) to obtain the corresponding extracts after filtration and evaporation. These solvents were selected based on their differential extraction capability of less polar metabolites (DCM) and more polar metabolites (MeOH). In this way, most of the compounds present in the plant species under study were extracted.

2.3. Fungal Strains

Monosporic strains of *Geotrichum* and *Penicillium* spp. called NA1, NA2, and NA3 were obtained from orange fruits that presented typical symptoms of sour rot, green mold rot, and blue mold rot, and cultivated in Potato-Dextrose-Agar medium (PDA) [15–18]. The strains were morphologically characterized and conserved in the Mycology Reference Center (CEREMIC-UNR, Rosario, Argentina) under the codes CCC-5-2019 (NA1), CCC-102 (NA2), and CCC-101 (NA3). Isolates were also conserved at −20 °C on dried filter paper in the mycological collection of ICiAgro Litoral, UNL, CONICET, FCA, Argentina.

2.4. Molecular Characterization

The identity of the isolates was confirmed through molecular characterization. For that, fungal genomic DNA was extracted from 7-day-old cultures grown on PDA at 20–25 °C as described in Gupta et al. [15] and was used as the template for PCR amplification of a segment of the ITS (Internal Transcribed Spacer) region of ribosomal nuclear DNA (rDNA) using the primers ITS4 (TCCTCCGCTTATTGATATGC) and ITS5 (GGAAGTAAAAGTCG-TAACAAGG) [16]. PCR reactions were performed on a Techne TC-312 thermal cycler (Techne, Cambridge, UK) in 20 µL reaction mixtures containing 1× PCR buffer, 2.5 mM $MgCl_2$, 0.4 µM each primer, 0.2 mM dNTPs, 1 U of Taq DNA polymerase (PB-L, Productos Bio-Lógicos®, Rosario, Argentina), and 100 ng of genomic DNA. Amplifications were programmed to carry out an initial denaturation at 94 °C for 5 min, followed by 36 cycles, each consisting of a denaturation step at 94 °C for 30 s, an annealing step, and an extension step at 57 °C for 30 s and at 72 °C for 30 s. The final extension was carried out at 72 °C for 7 min. PCR products were visualized under ultraviolet light on 1.5% (*w*/*v*) agarose gel in 1× TAE buffer stained with GelRed (Biotium Inc., Fremont, CA, USA). A UST-30M-8E, Biostep transilluminator (Biostep, Jahnsdorf, Germany) was used. The amplified products were purified and sequenced with the same primers in Macrogen (Seoul, Republic of Korea). The identification was performed by comparing the sequences with all fungal sequences of the GenBank Nucleotide Database hosted by the National Center for Biotechnology Information (NCBI, https://www.ncbi.nlm.nih.gov/ accessed on 15 December 2022) using BLASTn (Nucleotide Basic Local Alignment Search Tool) [17]. All the sequences generated in this study were deposited in GenBank.

The inoculum for bioassays was obtained according to the Clinical & Laboratory Standards Institute reported procedures and adjusted to 1×10^4 Colony Forming Units (CFU) mL^{-1} [18].

2.5. In Vitro Susceptibility Test

Diffusion tests were carried out using 9 cm diameter sterile Petri dishes provided with four divisions so that each sample was tested in quadruplicate. Plant extract solutions were prepared at a concentration of 50 mg mL^{-1} in DMSO and once dissolved, 400 µL of this stock solution was diluted in 20 mL of molten PDA culture medium. After vigorous shaking and before the mixture was solidified, 5 mL was poured into each of the four compartments of the Petri dishes and cooled down. A conidia concentration between 10^4 and 10^5 CFU mL^{-1} was inoculated inside a well located in the center of each compartment once the medium containing 1000 ppm of each plant extract was solidified, according to our previous methodology developed and published [7,13]. A negative control was prepared

using the commercial fungicide Imazalil® and the solvent DMSO without plant extract served as a positive (growth) control. Once the mycelium of the control plates completely covered the surface of the medium (approximately 7 days), the mycelium diameter of each plant-treated plate was carried out by scanning the plates with ImageJ® software [19]. The average inhibition percentages with their standard deviations were determined at 7 and 14 days after inoculation.

2.6. Fungicidal Assays on Wounded Oranges Using S. pilcomayense Methanolic Extract

Oranges (cv. "Salustiana") were harvested from the Experimental Field of Intensive and Forestry Crops (*Facultad de Ciencias Agrarias*, *Universidad Nacional del Litoral*) at the mature stage and sorted based on size and absence of physical injuries or disease infection. They were artificially inoculated with *P. digitatum* following the methodology described by Di Liberto et al. [20]. Three groups of 10 oranges were used: (1) control with sterile water, (2) treatment with 3000 ppm aqueous solution of *S. pilcomayense* methanolic extract, and (3) treatment with 3000 ppm aqueous solution of Imazalil®. The treatments were carried out after 2 h of inoculation, by 5 s immersion of each fruit into a beaker with the corresponding solutions mentioned above. For the in vivo test, the 3000 ppm concentration of the extract was chosen because we did not have a good response when we treated the fruits with a 1000 ppm concentration. So, we decided to hardly increase the fungicidal concentration resulting from the in vitro assays.

The fruits were stored at 25 °C and 85% RH for 14 days. After storage, the degree of *P. digitatum* sporulation on the surface of decayed fruits was evaluated on a 0 to 4 scale (sporulation index). With this scale, the severity of the disease was visually quantified assuming the following values: 0 (negligible sporulation); 1 (fruit with lesions on up to 10% of its surface); 2 (fruit with injuries on between 10 and 30% of its surface); 3 (fruit with lesions on between 30 and 50% of its surface); and 4 (refers to dense fungal sporulation over the entire fruit that infected more than 50% of its surface) [20]. The sporulation index for each fruit was treated as a replicate, and each treatment mean was subjected to statistical analysis.

2.7. Statistical Analysis

For the in vitro test, the differences in the mean percentage of fungal growth in the presence of each extract were compared with positive and negative controls by statistical analysis with a 95% Confidence Interval (CI) according to our published results [13].

For the in vivo test, experimental data were analyzed statistically by one-way ANOVA followed by Tukey's multiple comparison tests ($\alpha = 0.05$) using the GraphPad Prism 7.0 software and according to Di Liberto et al. [20].

3. Results

3.1. Plant Extract Yields

The part of each plant used and the corresponding DCM and MeOH extract yields (g/100 g of dried plant material) are provided in Table 2. For the cases of duplicated species under study, *O. virgata* showed similar crude extract percentages (2.4 and 2.3 for DCM extracts; 4.1 and 5.0 for MeOH extracts), but *S. argentinum* and *S. pilcomayense* presented differences in DCM yields and both (DCM and MeOH) yields, respectively.

Table 2. Part of each plant used and the corresponding DCM and MeOH extract yields (g/100 g of dried plant material).

Plant Scientific Name	Part Used	DCM Extract Yield (%)	MeOH Extract Yield (%)
A. brachystephana	Aerial parts	2.4	10.7
F. glabrescens	Aerial parts	1.9	7.3
O. virgata	Aerial parts	2.4	4.1
O. virgata	Aerial parts	2.3	5.0
F. clausum	Aerial parts	3.7	7.2
T. tricholepis	Whole plant	3.4	4.4
A. inundata	Aerial parts	1.1	3.4
V. caven	Flowers	1.3	7.3
P. alliacea	Aerial parts	0.4	3.0
P. stelligerum	Leaves	0.7	2.8
P. lapathifolium	Leaves	0.6	3.0
C. montevidensis	Aerial parts	1.0	10.2
P. zapallo	Aerial parts	1.3	7.7
S. argentinum	Aerial parts	0.7	7.0
S. argentinum	Aerial parts	3.5	7.7
S. granulosum-leprosum	Aerial parts	3.2	5.0
S. pilcomayense	Aerial parts	1.3	7.6
S. pilcomayense	Aerial parts	0.5	3.2
S. caavurana	Aerial parts	1.4	10.3
L. megapotamica	Aerial parts	0.4	4.5

3.2. Fungicidal Evaluation of the Selected Plant Extracts against P. digitatum, P. italicum, and G. citri-aurantii

Fungal isolates NA1, NA2, and NA3 were morphologically identified by CEREMIC-UNR as *G. citri-aurantii* (CCC-5-2019), *P. digitatum* (CCC-102), and *P. italicum* (CCC-101), respectively. Figure 1 shows the typical symptoms and signs caused by each fungus on oranges, the morphological characteristics of the colonies developed after isolation, and the microscopic features of their conidia. Typical green, blue, and white sporulation were observed above the rots and in the plates.

To confirm morphological identification, DNA was extracted from the three fungal isolates, and the ITS region was amplified and sequenced. BLASTn searches revealed that the fungal isolate sequences NA1, NA2, and NA3 presented 99.4%, 100%, and 100% of identity with previously characterized strains of *G. citri-aurantii* (EU131181, [21]), *P. digitatum* (MT448740, [22]), and *P. italicum* (MK736929, [23]), respectively. The nucleotide sequences were deposited in GenBank (accession numbers OQ132875, OQ132876, and OQ132877 for NA1, NA2, and NA3 isolates, respectively).

Table 3 shows the fungicidal activities (percentages of inhibition in quadruplicate using the agar diffusion test) of 40 extracts obtained from different parts of 20 plant species evaluated against the orange phytopathogens *P. digitatum*, *P. italicum*, and *G. citri-aurantii* determined 7 days post inoculation. Table 4 shows the fungicidal activities of the plant extracts under study, but now determined 14 days post inoculation.

From Tables 3 and 4, many comments should be highlighted: Among all the DCM extracts obtained from the 20 species evaluated against *P. digitatum*, none of them presented 100% of inhibition, but the most active ones after 7 days of incubation were obtained from *T. tricholepis*, *A. inundata*, and *P. stelligerum* (80.0, 79.3, and 75.2% of fungal growth inhibition). These percentages decreased after 14 days of incubation to 76.8, 67.2%, and 50.2%, respectively. *Penicillium italicum* inhibition by DCM extracts was lower than for *P. digitatum* since no extract of this type exceeded 70% growth inhibition at 7 days post inoculation. In contrast, many DCM extracts showed high inhibition against *G. citri-aurantii* (*O. virgata*, *T. tricholepis*, *A. inundata*, *P. lapathifolium*, and some species of *Solanum* diminished the fungal growth by more than 70%) and remarkably, *F. clausum* and *P. alliacea* completely

interrupted the growth of *G. citri-aurantii* even 14 days after inoculation, representing potent fungicide products.

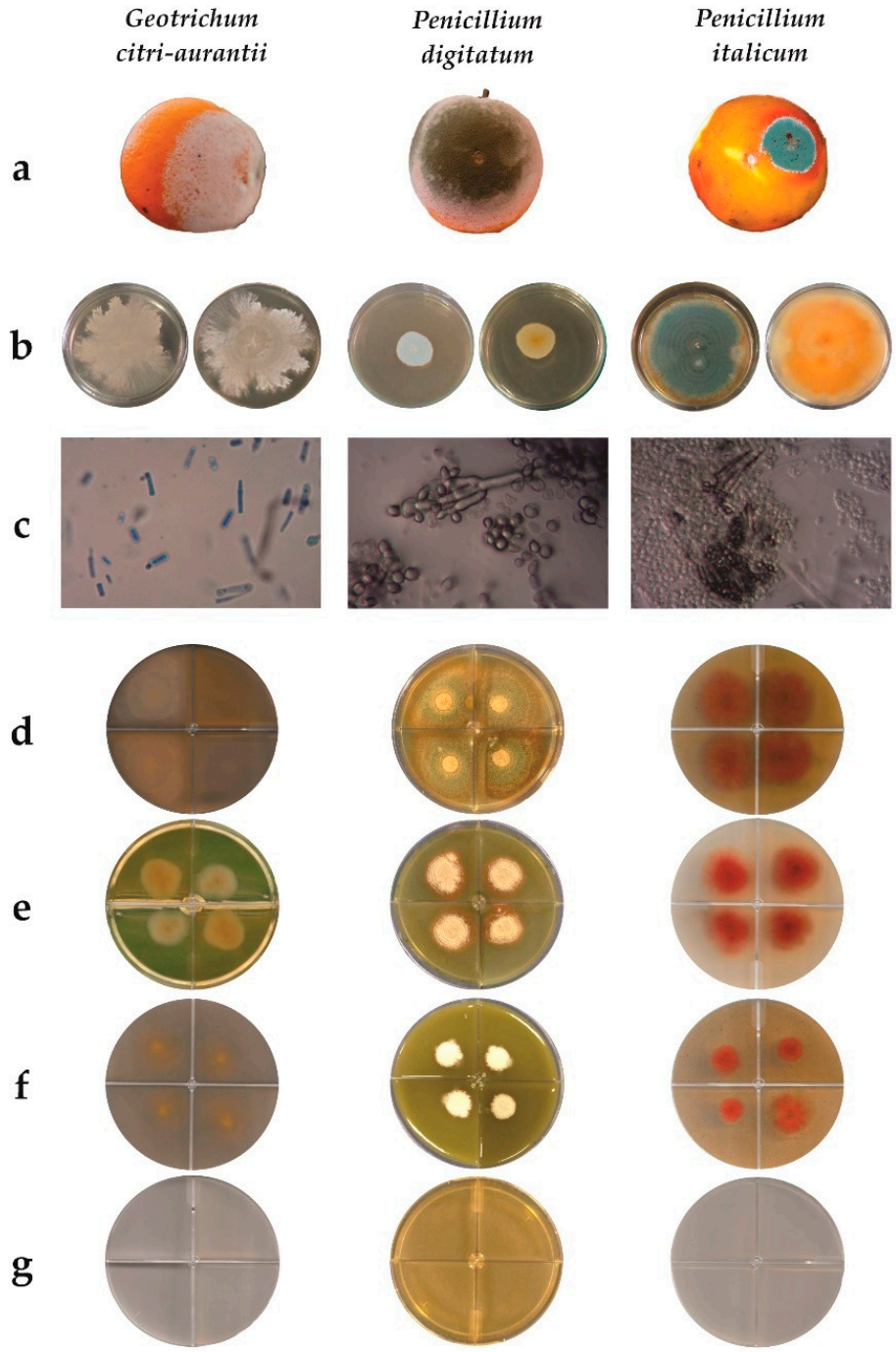


Figure 1. (**a**) Symptoms and signs caused by *Geotrichum citri aurantii*, *Penicillium digitatum*, and *Penicillium italicum* in orange; (**b**) morphological characteristics of morphologically and molecularly characterized colonies isolates on PDA media; (**c**) typical microscopic conidia features observed at magnification 40×; (**d**) tested plates with 0% inhibition; (**e**) tested plates with 50% inhibition; (**f**) tested plates with 80% inhibition; and (**g**) tested pates with 100% inhibition. Note: in some cases, the coloration of the medium may be due to the plant extract dissolved in it.

Table 3. Fungicidal activity % of inhibition (calculated as the average of the inhibition percentage of the four sectors in which the Petri dish was divided) of 40 extracts obtained from different parts of 20 plant species evaluated against the orange phytopathogens *Penicillium digitatum*, *P. italicum*, and *Geotrichum citri-aurantii* determined 7 days post inoculation. Different letters mean significant statistical differences.

Plant Scientific Name	*P. digitatum*		*P. italicum*		*G. citri-aurantii*		Controls	
	DCM	MeOH	DCM	MeOH	DCM	MeOH	+(DMSO)	−(IMZ)
A. brachystephana	61.0 ± 5.2 ^{c}	44.3 ± 6.2 ^{a}	30.9 ± 6.5 ^{a}	30.5 ± 5.3 ^{a}	65.4 ± 5.0 ^{c}	66.6 ± 1.8 ^{c}	0.0 ± 0.0 ^{a}	100.0 ± 0.0 ^{b}
F. glabrescens	52.9 ± 7.1 ^{c}	75.1 ± 1.5 ^{c}	45.8 ± 6.9 ^{a}	54.1 ± 5.4 ^{c}	71.0 ± 0.4 ^{c}	70.1 ± 2.8 ^{c}	0.0 ± 0.0 ^{a}	100.0 ± 0.0 ^{b}
O. virgata	55.3 ± 2.9 ^{c}	50.9 ± 5.9 ^{c}	33.2 ± 6.5 ^{a}	48.4 ± 6.5 ^{a}	88.0 ± 4.9 ^{b}	50.8 ± 1.9 ^{c}	0.0 ± 0.0 ^{a}	100.0 ± 0.0 ^{b}
O. virgata	70.6 ± 2.0 ^{c}	86.3 ± 3.8 ^{b}	31.6 ± 4.9 ^{a}	48.5 ± 4.7 ^{a}	99.2 ± 0.7 ^{b}	100.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{a}	100.0 ± 0.0 ^{b}
F. clausum	62.4 ± 3.8 ^{c}	81.5 ± 1.5 ^{b}	40.8 ± 4.4 ^{a}	43.1 ± 6.9 ^{a}	100.0 ± 0.0 ^{b}	33.0 ± 5.2 ^{a}	0.0 ± 0.0 ^{a}	100.0 ± 0.0 ^{b}
T. tricholepis	80.0 ± 2.2 $^{b\,c}$	85.9 ± 0.7 ^{b}	60.8 ± 7.0 ^{c}	67.1 ± 3.6 ^{c}	87.8 ± 1.5 ^{b}	54.9 ± 1.0 ^{c}	0.0 ± 0.0 ^{a}	100.0 ± 0.0 ^{b}
A. inundata	79.3 ± 2.1 $^{b\,c}$	100.0 ± 0.0 ^{b}	35.7 ± 5.2 ^{a}	100.0 ± 0.0 ^{b}	79.3 ± 7.0 $^{b\,c}$	97.3 ± 0.5 ^{b}	0.0 ± 0.0 ^{a}	100.0 ± 0.0 ^{b}
V. caven	59.5 ± 4.0 ^{c}	76.8 ± 3.5 ^{c}	36.2 ± 3.8 ^{a}	58.1 ± 6.5 ^{c}	56.5 ± 5.1 ^{c}	32.3 ± 4.2 ^{a}	0.0 ± 0.0 ^{a}	100.0 ± 0.0 ^{b}
P. alliacea	72.5 ± 1.7 ^{c}	83.3 ± 3.8 ^{b}	53.2 ± 2.8 ^{c}	81.3 ± 2.2 ^{b}	100.0 ± 0.0 ^{b}	62.9 ± 3.9 ^{c}	0.0 ± 0.0 ^{a}	100.0 ± 0.0 ^{b}
P. stelligerum	75.2 ± 2.3 ^{c}	80.1 ± 2.8 ^{b}	32.8 ± 6.5 ^{a}	57.0 ± 5.3 ^{c}	76.0 ± 2.1 ^{c}	38.4 ± 4.1 ^{a}	0.0 ± 0.0 ^{a}	100.0 ± 0.0 ^{b}
P. lapathifolium	64.2 ± 6.4 ^{c}	80.7 ± 0.9 $^{b\,c}$	33.0 ± 3.2 ^{a}	43.8 ± 5.2 ^{a}	82.1 ± 3.5 ^{b}	40.2 ± 3.9 ^{a}	0.0 ± 0.0 ^{a}	100.0 ± 0.0 ^{b}
C. montevidensis	49.4 ± 5.2 ^{a}	56.4 ± 5.1 ^{c}	24.6 ± 3.2 ^{a}	45.8 ± 2.7 ^{a}	65.5 ± 2.8 ^{c}	52.7 ± 4.6 ^{c}	0.0 ± 0.0 ^{a}	100.0 ± 0.0 ^{b}
P. zapallo	73.2 ± 4.0 ^{c}	81.7 ± 2.2 ^{b}	37.5 ± 4.7 ^{a}	51.8 ± 4.7 ^{c}	90.6 ± 7.9 ^{b}	70.0 ± 4.2 ^{c}	0.0 ± 0.0 ^{a}	100.0 ± 0.0 ^{b}
S. argentinum	45.2 ± 6.3 ^{a}	62.3 ± 5.3 ^{c}	29.0 ± 3.3 ^{a}	62.7 ± 5.1 ^{c}	63.8 ± 0.9 ^{c}	73.3 ± 1.9 ^{c}	0.0 ± 0.0 ^{a}	100.0 ± 0.0 ^{b}
S. argentinum	73.3 ± 4.0 ^{c}	70.0 ± 5.6 ^{c}	27.9 ± 4.0 ^{a}	51.6 ± 4.8 ^{c}	75.0 ± 6.8 ^{c}	48.6 ± 4.9 ^{a}	0.0 ± 0.0 ^{a}	100.0 ± 0.0 ^{b}
S. granulosum-leprosum	54.7 ± 6.0 ^{c}	49.5 ± 4.5 ^{a}	44.6 ± 4.7 ^{a}	40.0 ± 4.3 ^{a}	41.3 ± 5.4 ^{a}	61.5 ± 5.1 ^{c}	0.0 ± 0.0 ^{a}	100.0 ± 0.0 ^{b}
S. pilcomayense	44.5 ± 4.9 ^{a}	100.0 ± 0.0 ^{b}	15.3 ± 3.4 ^{a}	100.0 ± 0.0 ^{b}	74.4 ± 2.4 ^{c}	100.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{a}	100.0 ± 0.0 ^{b}
S. pilcomayense	46.4 ± 4.7 ^{a}	100.0 ± 0.0 ^{b}	41.9 ± 5.3 ^{a}	100.0 ± 0.0 ^{b}	71.0 ± 1.9 ^{c}	100.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{a}	100.0 ± 0.0 ^{b}
S. caavurana	60.0 ± 5.6 ^{c}	94.6 ± 1.5 ^{b}	35.5 ± 3.5 ^{a}	87.8 ± 1.3 ^{b}	79.8 ± 4.5 ^{c}	100.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{a}	100.0 ± 0.0 ^{b}
L. megapotamica	75.0 ± 2.2 ^{c}	56.3 ± 4.4 ^{c}	34.9 ± 4.8 ^{a}	41.6 ± 3.4 ^{a}	70.3 ± 1.2 ^{c}	62.9 ± 5.1 ^{c}	0.0 ± 0.0 ^{a}	100.0 ± 0.0 ^{b}

Table 4. Fungicidal activity % of inhibition (calculated as the average of the inhibition percentage of the four sectors in which the Petri dish was divided) of 40 extracts obtained from different parts of 20 plant species evaluated against the orange phytopathogens *Penicillium digitatum*, *P. italicum*, and *Geotrichum citri-aurantii* determined 14 days post inoculation. Different letters mean significant statistical differences.

Plant Scientific Name	*P. digitatum*		*P. italicum*		*G. citri-aurantii*		Controls	
	DCM	MeOH	DCM	MeOH	DCM	MeOH	+(DMSO)	−(IMZ)
A. brachystephana	40.8 ± 2.5 ^{a}	18.4 ± 3.4 ^{a}	0.0 ± 0.0 ^{a}	7.2 ± 2.3 ^{a}	13.7 ± 3.2 ^{a}	39.5 ± 4.3 ^{a}	0.0 ± 0.0 ^{a}	100.0 ± 0.0 ^{b}
F. glabrescens	38.0 ± 3.8 ^{a}	38.8 ± 4.3 ^{a}	30.6 ± 4.6 ^{a}	10.3 ± 2.1 ^{a}	26.1 ± 3.7 ^{a}	37.2 ± 4.9 ^{a}	0.0 ± 0.0 ^{a}	100.0 ± 0.0 ^{b}
O. virgata	37.9 ± 5.2 ^{a}	33.7 ± 4.3 ^{a}	7.7 ± 2.1 ^{a}	33.8 ± 4.6 ^{a}	80.0 ± 4.4 $^{b\,c}$	29.7 ± 3.2 ^{a}	0.0 ± 0.0 ^{a}	100.0 ± 0.0 ^{b}
O. virgata	45.9 ± 3.9 ^{a}	57.0 ± 3.6 ^{c}	0.0 ± 0.0 ^{a}	16.9 ± 3.2 ^{a}	90.4 ± 6.0 ^{b}	100.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{a}	100.0 ± 0.0 ^{b}
F. clausum	50.3 ± 4.5 $^{a\,c}$	53.6 ± 3.7 ^{c}	15.1 ± 3.6 ^{a}	31.2 ± 4.6 ^{a}	100.0 ± 0.0 ^{b}	16.0 ± 1.2 ^{a}	0.0 ± 0.0 ^{a}	100.0 ± 0.0 ^{b}
T. tricholepis	76.8 ± 2.7 ^{c}	69.1 ± 1.8 ^{c}	55.0 ± 2.7 ^{c}	39.1 ± 6.5 ^{a}	26.7 ± 1.7 ^{a}	46.9 ± 2.8 ^{a}	0.0 ± 0.0 ^{a}	100.0 ± 0.0 ^{b}
A. inundata	60.3 ± 2.7 ^{c}	100.0 ± 0.0 ^{b}	34.3 ± 6.2 ^{a}	100.0 ± 0.0 ^{b}	71.5 ± 5.3 ^{c}	92.5 ± 0.2 ^{b}	0.0 ± 0.0 ^{a}	100.0 ± 0.0 ^{b}
V. caven	42.3 ± 3.7 ^{a}	33.0 ± 6.1 ^{a}	30.0 ± 5.8 ^{a}	11.0 ± 3.0 ^{a}	0.0 ± 0.0 ^{a}	22.0 ± 5.1 ^{a}	0.0 ± 0.0 ^{a}	100.0 ± 0.0 ^{b}
P. alliacea	67.2 ± 2.9 ^{c}	58.8 ± 6.7 ^{c}	41.0 ± 2.0 ^{a}	57.8 ± 3.3 ^{c}	100.0 ± 0.0 ^{b}	34.4 ± 5.8 ^{a}	0.0 ± 0.0 ^{a}	100.0 ± 0.0 ^{b}
P. stelligerum	50.2 ± 1.5 $^{a\,c}$	42.3 ± 4.3 ^{a}	0.0 ± 0.0 ^{a}	0.0 ± 0.0 ^{a}	59.1 ± 4.1 ^{c}	21.5 ± 1.6 ^{a}	0.0 ± 0.0 ^{a}	100.0 ± 0.0 ^{b}
P. lapathifolium	37.8 ± 6.5 ^{a}	33.0 ± 5.3 ^{a}	0.0 ± 0.0 ^{a}	0.0 ± 0.0 ^{a}	22.1 ± 3.6 ^{a}	20.5 ± 1.2 ^{a}	0.0 ± 0.0 ^{a}	100.0 ± 0.0 ^{b}
C. montevidensis	41.4 ± 6.2 ^{a}	10.4 ± 2.1 ^{a}	0.0 ± 0.0 ^{a}	11.6 ± 4.5 ^{a}	0.0 ± 0.0 ^{a}	31.6 ± 4.3 ^{a}	0.0 ± 0.0 ^{a}	100.0 ± 0.0 ^{b}
P. zapallo	52.9 ± 5.9 ^{c}	39.5 ± 4.4 ^{a}	18.3 ± 4.4 ^{a}	0.0 ± 0.0 ^{a}	10.3 ± 2.7 ^{a}	44.6 ± 5.3 ^{a}	0.0 ± 0.0 ^{a}	100.0 ± 0.0 ^{b}
S. argentinum	29.5 ± 4.3 ^{a}	40.2 ± 6.7 ^{a}	0.0 ± 0.0 ^{a}	13.8 ± 2.3 ^{a}	0.0 ± 0.0 ^{a}	37.5 ± 5.8 ^{a}	0.0 ± 0.0 ^{a}	100.0 ± 0.0 ^{b}
S. argentinum	50.1 ± 6.3 $^{a\,c}$	50.1 ± 5.2 $^{a\,c}$	0.0 ± 0.0 ^{a}	27.8 ± 3.5 ^{a}	39.3 ± 4.5 ^{a}	33.1 ± 2.8 ^{a}	0.0 ± 0.0 ^{a}	100.0 ± 0.0 ^{b}
S. granulosum-leprosum	38.4 ± 4.3 ^{a}	10.4 ± 3.7 ^{a}	29.9 ± 4.7 ^{a}	0.0 ± 0.0 ^{a}	0.0 ± 0.0 ^{a}	37.4 ± 3.6 ^{a}	0.0 ± 0.0 ^{a}	100.0 ± 0.0 ^{b}
S. pilcomayense	32.4 ± 4.7 ^{a}	100.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{a}	100.0 ± 0.0 ^{b}	17.8 ± 4.7 ^{a}	100.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{a}	100.0 ± 0.0 ^{b}
S. pilcomayense	32.8 ± 4.8 ^{a}	100.0 ± 0.0 ^{b}	27.4 ± 3.7 ^{a}	100.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{a}	100.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{a}	100.0 ± 0.0 ^{b}
S. caavurana	50.4 ± 5.5 $^{a\,c}$	88.3 ± 2.2 ^{b}	18.2 ± 3.2 ^{a}	55.7 ± 4.3 ^{c}	71.0 ± 3.9 ^{c}	100.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{a}	100.0 ± 0.0 ^{b}
L. megapotamica	62.9 ± 5.4 ^{c}	23.6 ± 5.3 ^{a}	34.6 ± 4.2 ^{a}	22.5 ± 4.4 ^{a}	0.0 ± 0.0 ^{a}	34.6 ± 3.4 ^{a}	0.0 ± 0.0 ^{a}	100.0 ± 0.0 ^{b}

Regarding MeOH extracts, it can be observed that they were more active than DCM extracts. *Orthosia virgata*, *F. clausum*, *T. tricholepis*, *P. alliacea*, and *S. caavurana* methanolic extracts inhibited the growth of *P. digitatum* by more than 80%. Moreover, *A. inundata* and the two collections of *S. pilcomayense* presented 100% *P. digitatum* inhibition even more than 14 days after inoculation. *P. italicum* inhibition by methanolic extracts was not as high as *P. digitatum* inhibition: *P. alliacea* and *S. caavurana* extracts inhibited 81.3 and 87.8%, respectively, but this activity diminished after 14 days. Remarkably, *A. inundata* and both collections of *S. pilcomayense* maintained their *P. italicum* 100% inhibition activity for more than 14 days. Concerning *G. citri-aurantii* inhibition by methanolic extracts, it was 100% inhibited for more than 14 days by *O. virgata* and both collections of *S. pilcomayense* and *S. caavurana*, with the activity of *A. inundata* also being remarkable, which showed more than 90% inhibition even at day 14 post inoculation.

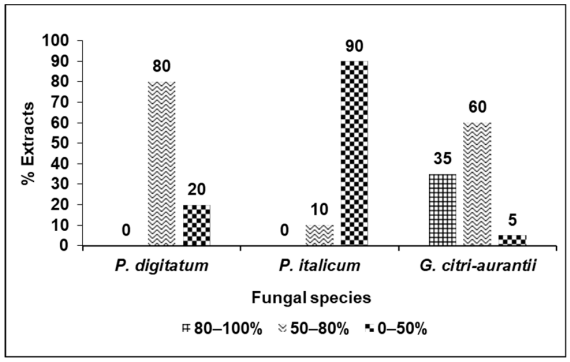
An interesting topic to remark on is the case of *O. virgata* and *S. pilcomayense* which were collected from two different locations on the same date. The high fungicidal activity of *O. virgata* was only observed for one of the collections (Gral. Obligado, Santa Fe, 28°42′54.8″ S 59°23′13.7″ W); meanwhile, the potent activity of *S. pilcomayense* was shown for both collections (Gral. Obligado, Santa Fe, 28°13′42.2″ S 59°06′52.6″ W and San Javier, Santa Fe, 30°03′23.2″ S 59°51′34.8″ W).

3.3. Analysis of the Results by Categories of Fungicidal Action

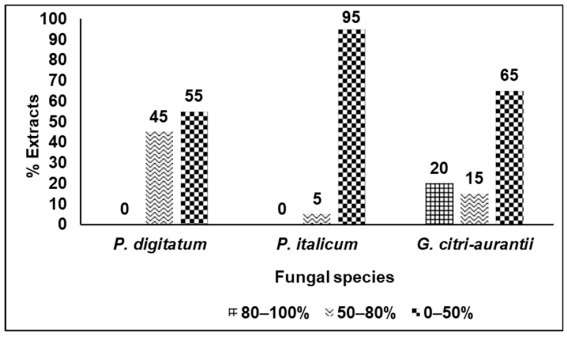
The results were divided into the following categories for percentage analysis: inactive extract (fungal growth inhibition less than 50%), moderately active extract (fungal growth inhibition between 50 and 80%), and active extract (fungal growth inhibition more than 80%).

None of the DCM extracts tested against *P. digitatum* were rated as active according to the above classification, but 80% of them were moderately active and 20% appeared to be inactive after 7 days of inoculation. The percentage of moderately active DCM extracts decreased from 80% to 45% and the percentage of inactive ones increased from 20% to 55%. These results may suggest that some DCM extracts could be fungistatic for a short period but not fungicidal. For *P. italicum*, most of the DCM extracts were inactive (90%), with only 10% of them moderately active after 7 days. This fungus represents the most resistant to DCM extracts among the three phytopathogenic strains under study. In contrast, *G. citri-aurantii* was the most susceptible phytopathogen to DCM extracts, since 35% of them were active at 7 days but decreased to 20% at 14 days after inoculation, 60% were moderately active after 7 days and decreased to 15% after 14 days, and the percentage of inactive DCM extracts against *G. citri-aurantii* was 5% after 7 days, increasing to 65% after 14 days (Figure 2).

Regarding MeOH extracts (Figure 3), 55% were active, 35% were moderately active, and 10% were inactive against *P. digitatum* 7 days post inoculation, and after 14 days 20% were active and 25% of them were moderately active. Additionally, 25, 35, and 40% of MeOH extracts were active, moderately active, and inactive, respectively, against *P. italicum* 7 days after inoculation; meanwhile, the percentages of active and moderately active extracts decreased after 14 days to 15 and 10%, respectively. The percentages of active MeOH extracts against *G. citri-aurantii* were the same 7 and 14 days post inoculation (25%), but the moderately active extracts after 7 days (50%) decreased to 0% after 14 days, increasing the percentages of inactive extracts. These results may suggest that the MeOH extracts that displayed strong activities against *G. citri-aurantii* have potential as fungicides due to their fungicidal activity being maintained for more than 14 days.

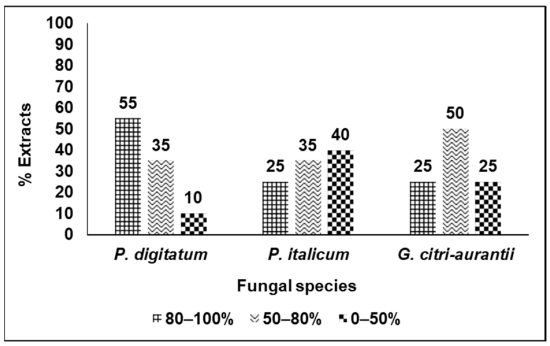


(**a**)

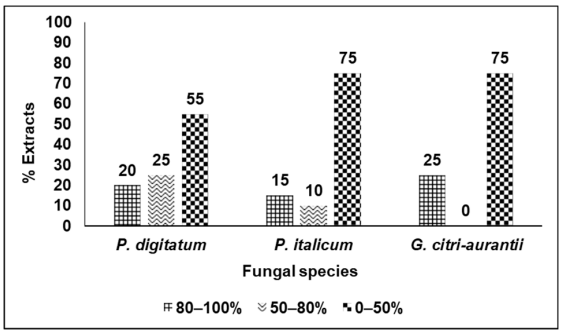


(**b**)

Figure 2. Percentage of active, moderately active, and inactive DCM extracts evaluated (**a**) 7 days and (**b**) 14 days after inoculation.



(**a**)



(**b**)

Figure 3. Percentage of active, moderately active, and inactive MeOH extracts evaluated (**a**) 7 days and (**b**) 14 days after inoculation.

3.4. Curative Effect of S. pilcomayense Methanolic Extract over Oranges Infected with P. digitatum

The effect of the most active extract from *S. pilcomayense* was examined on postharvest fresh oranges cv. "Salustiana" infected with an inoculum of *P. digitatum* (Figure 4), where the wound-inoculated fruits were treated with the bioactive extract by dipping as described in the Section 2. After 14 days of the treatment applications, no significant differences in the disease severity were observed for both treatments (3000 ppm aqueous solution of *S. pilcomayense* methanolic extract, and 3000 ppm aqueous solution of Imazalil®). The control fruits (those which had been only inoculated with the pathogen, but not treated with any fungicide) showed marked symptoms of the disease caused by the pathogen (see the sporulation index in each fruit of the control set). Contrarily, both treatments (*S. pilcomayense* methanolic extract and commercial imazalil) reduced the green mold sporulation index significantly ($p = 0.03$) with respect to the control oranges, showing no significant differences between them.

(**a**) (**b**) (**c**)

Figure 4. Sporulation index (0 to 4 scale, in which the value 0 was assigned to negligible sporulation and 4 referred to dense fungal sporulation over the entire fruit) after 14 days of inoculation of *P. digitatum* on wound-inoculated oranges: (**a**) control fruits, (**b**) fruits treated with 3000 ppm aqueous solution of *S. pilcomayense* methanolic extract, and (**c**) fruits treated with 3000 ppm aqueous solution of commercial imazalil.

4. Discussion

Although a considerable number of native Argentinean plant species were evaluated in this work for their fungicidal properties against three economically important citrus pathogens, only six of them have demonstrated high fungal inhibition. Additionally, the place of collection played an important role in the bioactivity of *O. virgata*, indicating the importance of environmental conditions on the production of bioactive secondary metabolites [24]. MeOH extracts obtained from all the species produced higher yields than DCM extracts, and this fact may be due to the number and type of molecules that can be extracted by the solvent methanol [24]. Moreover, MeOH extracts were more active than DCM extracts, especially against both species of *Penicillium*, the two phytopathogens which were more difficult to control in this work. Regarding *Geotrichum*, both types of extracts (DCM and MeOH) displayed more than 50% inhibition.

A literature review of the six most active species lets us state that they have been hardly evaluated against other microbiological activities, and in some cases a few references were found. Remarking on the importance of our native sources' knowledge, a discussion about their biological activities and phytochemical studies found in the literature is presented below:

Orthosia virgata (Figure 5a) belongs to the Apocynaceae family and it is commonly known as "*Liana de leche*" [25]. To the best of our knowledge, there are no reports about the

bioactivities and phytochemistry of this species and even its genus, which turns *O. virgata* into an interesting source to go on studying. The Apocynaceae family has been reported widely for indole-containing compounds which are under clinical use including vinblastine and vincristine (anticancer), atevirdine (anti-HIV), yohimbine (erectile dysfunction), reserpine (antihypertension), ajmalicine (vascular disorders), ajmaline (anti-arrhythmic), and vincamine (vasodilator). The main genera of the Apocynaceae family are *Alstonia*, *Rauvolfia*, *Kopsia*, *Ervatamia*, and *Tabernaemontana*. These genera consist of 400 members which represent 20% of Apocynaceae species, but only 30 (7.5%) species were investigated, whereas the rest are promising to be investigated. *Orthosia* does not belong to the most studied genus of this family, a fact that makes it even more attractive. Monoterpene Indole Alkaloids (MIAs) present in this family, deserve the curiosity and attention of researchers due to their chemical diversity and biological activities. These compounds were considered an impending source of drug lead [26].

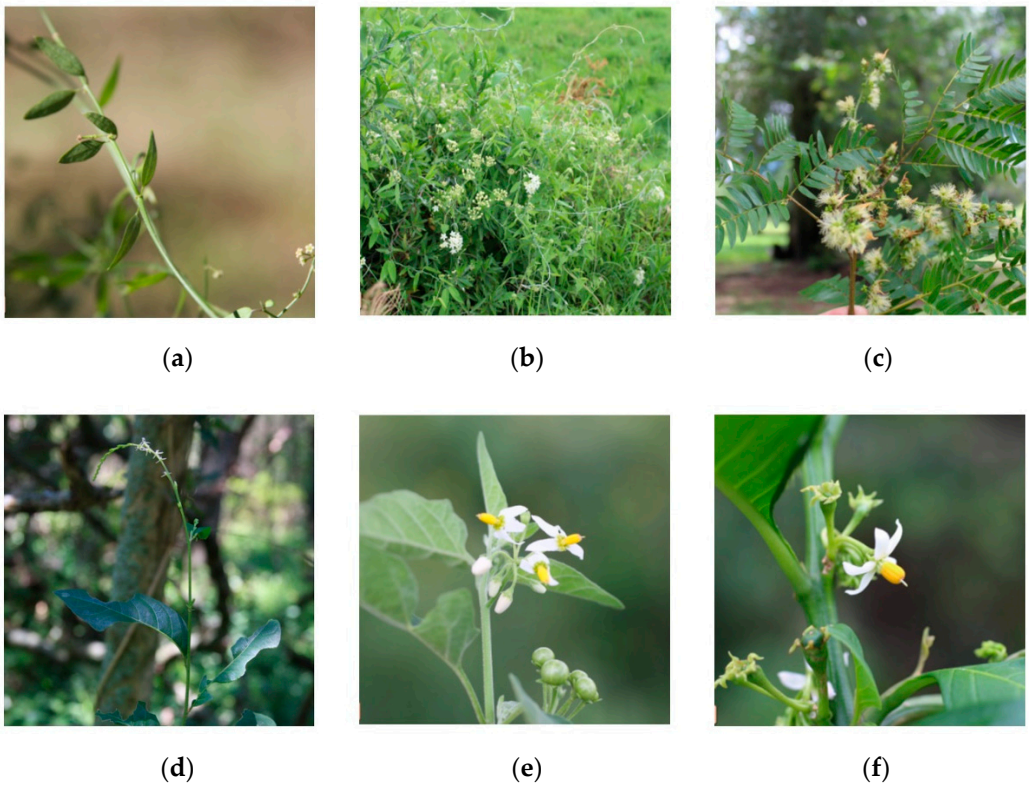


Figure 5. Pictures of the most active plant species acting as fungicides in the present study: (**a**) *Orthosia virgata*, (**b**) *Funastrum clausum*, (**c**) *Albizia inundata*, (**d**) *Petiveria alliacea*, (**e**) *Solanum pilcomayense*, and (**f**) *Solanum caavurana*. Source: database Irupé [20].

Funastrum clausum (Figure 5b) also belongs to the Apocynaceae family and it is commonly known as "*Bejuco sapo*" [27]. These plants exude sticky white latex containing the endopeptidases funastrain and funastrain c II which exhibit cysteine proteolytic activity [28]. This fact may be the reason why the products obtained from this species provided potent fungicidal action during the assays developed in this work. This was the only report found in the literature for this species and its genus, so it constitutes one of the promising species belonging to the Apocynaceae family mentioned above [26].

Albizia inundata (Figure 5c) belongs to the Fabaceae family and it is commonly known as "*Pacará*", "*Timbó blanco*" or "*Palo flojo*" [29]. The *Albizia* genus is phytochemically characterized by the presence of lignanoids, flavonoids, and phenolic glycosides [30]. Moreover, olean-type triterpene saponins isolated from *A. inundata* exhibited cytotoxicity against melanoma cells and human head and neck squamous cell carcinoma [31,32]. Also, these compounds showed high anti-plasmodial and anticandidal activities probably due

to triterpene saponins' capacity for disrupting cellular membranes [33]. So, it is possible that saponins from *A. inundata* may be responsible for the anti-phytopathogenic activities found in this work.

Petiveria alliacea (Figure 5d) belongs to the Phytolaccaceae family and it is commonly known as "*Guiné*", "*Pipi*" or "*Mucuracaá*" [34,35]. Pharmacological studies reported that extracts obtained from different parts of *P. alliacea* showed diverse effects on animal behavior affecting the central nervous system [35]. Rosado-Aguilar et al. [36] demonstrated that methanolic extracts obtained from the stem and leaves presented 100% larvae and adult mortality of *Rhipicephalus microplus*. This result proposed that *P. alliacea* may be a promising acaricide against resistant strains of *R. microplus* [36]. The antimicrobial activities of *P. alliacea* have been widely reported: (1) the hexane extracts inhibited *Staphylococcus aureus* with a Minimum Inhibitory Concentration (MIC) of 240 μg/mL; (2) the methanolic extract showed activity against *Enterococcus faecalis* (MIC = 240 μg/mL); (3) the hydroalcoholic extract was active against *Candida parapsilosis* (MIC = 250 μg/mL), *C. kefyr* and *C. albicans* (MIC = 760 μg/mL); (4) The tearful principle (Z)-thiobenzaldehyde-S-oxide obtained from *P. alliacea* demonstrated antimicrobial activity against *C. albicans*, *Klebsiella pneumoniae*, *Escherichia coli*, *S. aureus* and *S. agalactiae* [37]. Moreover, thiosulfonates isolated from this plant, and their degradation products inhibited at low concentrations (MIC values $\leq$ 64 μg/mL) the following microorganisms: *Bacillus cereus*, *Mycobacterium smegmatis*, *Micrococcus luteus*, *S. agalactiae*, *S. aureus*, *E. coli*, *Stenotrophomonas maltophila*, *K. pneumoniae*, and the fungus *Aspergillus flavus*, *Mucor racemosus*, *Pseudallescheria boydii*, *C. albicans*, *C. tropicalis* and *Issatchenkia orientalis* [37,38]. A compound named DTS, which is a polysulfide often identified in *P. alliacea*, led to a reversible disassembly of microtubules through the decrease of the total expression of tubulin (0.1 μM) and caused a decrease in phosphorylation of erk1/erk2 protein kinases (0.5 μM) in SH-SY5Y cell line, what may be the mode of action for its biocidal activities [37]. Some studies affirm that the bioactivity of *P. alliacea* leaf extracts could be attributed to the main triterpene metabolite named isoarborinol [39].

Solanum pilcomayense (Figure 5e) belongs to the Solanaceae family and it is commonly known as "*Tomatillo del monte*" [40]. Few reports have been found in the literature about its components or bioactivities; the most highlighting study was performed by Muelas-Serrano et al. [41] who demonstrated some antiprotozoal activity of aerial part extracts against *Trypanosoma cruzi* and *Trichomonas vaginalis* [41]. More recently, a study examined the antioxidant compositions and their capacities in three New Zeeland tamarillo (*Solanum* spp.) cultivars. It showed that tamarillo peels possessed higher amounts of phenolic compounds, total phenolic content, and antioxidant activity than the pulps. Pulps had higher anthocyanins concentration than peels. The antioxidant capacity of tamarillos exhibited relatively high values and was strongly correlated with high total phenolic content. The presence of these bioactive compounds highlights the potential of tamarillo for further utilization in the food and pharmaceutical industries. Tamarillo remains underutilized despite its bioactive components [42].

Finally, *S. caavurana* (Figure 5f) also belongs to the Solanaceae family and it is commonly known as "*Palo agui*", "*Laranjinha do mato*", or "*Jurubeba-branca*" [40]. The ripe fruits of *S. caavurana* contain a wide variety of steroidal alkaloids such as 4-tomatiden-3-one, 5*α*-tomatidan-3-one, and caavuranamide as well as glycoalkaloids. Regarding the nutritional components of the fruit, it contains proteins (4.2%), lipids (1.5%), and carbohydrates (56.7%). The bioactive caavuranamide (steroid alkaloid) showed significant antibacterial activity (MIC = 135 μg/mL) against *Rhodococcus equi*, a similar value to the commercial chloramphenicol (MIC = 124 μg/mL) [43]. Solanaceae fruits constitute a source of food in many countries. In a recent study, the proximate composition of two Solanaceae fruits from Brazilian *Cerrado* was evaluated. The results showed that the pulp had a high moisture content (74.6–85.4 g/100 g) and soluble fiber (1.3–2.0 g/100 g) content, and low fat, protein, and ash content. Potassium is the main mineral found in both fruits. The major components revealed 24 phenolic compounds, most being hydroxycinnamic acids derivatives

and chlorogenic acid. The antioxidant capacity of the fruits ranged from 1.3 to 11.5 µmol TE/100 mL of extract [44]. These results indicated that the *Solanum* genus can be interesting for the Brazilian fruit market and that it has the potential to be exploited for agroindustry diversification of fruit products. In addition, our work demonstrated the fungicidal capacity of a related species of *Solanum*.

5. Conclusions

Many efforts have been made to promote reducing the use of chemical fungicides on postharvest fruits worldwide. In this sense, the use of microorganisms as biological controllers has been highly developed in recent decades, but their safety has sometimes been questioned. The high mutation capacity may turn a benefic bacterium into a pathogenic one which could cause plant or human diseases. On the other hand, plant species contain a complex mixture of molecules that should be explored, not only to treat human diseases but also to prevent and control fruit or plant diseases. So, this type of study should be encouraged to reduce the use of agrochemicals, especially in small or familiar farms. In this work, we isolated and characterized (by morphological and molecular means) three strains of the most important citric phytopathogens in our region. Through a high-throughput screening method, we demonstrated that six Argentinean native plants (*O. virgata*, *P. alliacea*, *F. clausum*, *A. inundata*, *S. caavurana*, and *S. pilcomayense*) inhibited 100% of the growth of at least one of the three orange phytopathogens tested (*P. digitatum*, *P. italicum*, and *G. citri-auranti*). Additionally, an aqueous solution of 3000 ppm of *S. pilcomayense* crude methanolic extract concentration was highly fungicidal on postharvest oranges inoculated with *P. digitatum*. However, more studies are needed, such as on the chemical standardization of the extracts, their evaluation against other phytopathogens to detect spectra and mechanisms of action, human cytotoxicity, and technological formulations to determine the correct dose for the control of each disease on fruits.

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