horticulturae

MDPI

Article

Antifungal efficacy and mode of action of strawberry leaf extract against fungicide sensitive and resistant fungal pathogens on postharvest citrus

Pia Di Peto[1§], Gabriela Michavila[1§], Mario A. Debes[2,3], Bjorn V. Welin[1], Nadia R. Chafoun[1], Sabrina I. Volentini[2*] and Luciana Cerioni[2*]

[1]Instituto de Tecnología Agroindustrial del Noroeste Argentino (ITANOA), Estación Experimental Agroindustrial Obispo Colombres (EEAOC)–Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) (T4101XAC), Las Talitas, Tucumán, Argentina.
[2]Instituto Superior de Investigaciones Biológicas (INSIBIO), CONICET-UNT, and Instituto de Química Biológica "Dr Bernabé Bloj", Facultad de Bioquímica, Química y Farmacia, UNT. Chacabuco 461, T4000ILI – San Miguel de Tucumán, Tucumán, Argentina.
[3]Facultad de Ciencias Naturales e Instituto Miguel Lillo, UNT. Miguel Lillo 205, T4000JFE − San Miguel de Tucumán, Tucumán, Argentina.
*Correspondence: luciana.cerioni@fbqf.unt.edu.ar (L.C.) and sabrina.volentini@fbqf.unt.edu.ar (S.I.V.); Tel: (+54381) 4248921 Int. 210.
§ These authors contributed equally to this work.

Abstract

Postharvest diseases caused by fungal pathogens lead to significant economic losses in citrus production. The intensive use of synthetic fungicides has triggered the emergence of resistant strains and environmental contamination, emphasizing the need to search sustainable alternatives. This study evaluated the antifungal efficacy of a strawberry leaf aqueous extract (PSP2) against local isolates of citrus phytopathogens such as *Penicillium digitatum* and *P. italicum* (both fungicide-sensitive and fungicide-resistant) and *Geotrichum citri-aurantii*. *In vitro* assays showed complete inhibition of mycelial growth for all pathogens on PDA plates supplemented with 0.05 g·mL^{-1} PSP2 after 5 days at 24 °C. Furthermore, total inhibition of conidial germination and loss of viability were achieved at 0.10 g·mL^{-1} following a 24 or 8-hour exposure period respectively. To elucidate the underlying mode of action, membrane integrity and cellular ultrastructure were examined. SYTOX™ Green staining revealed increased membrane permeability, and transmission electron microscopy showed marked intracellular disorganization in PSP2-treated conidia from all phytopathogens. Finally, *in vivo* assays using artificially inoculated lemons demonstrated a significant reduction in green mold incidence. Overall, PSP2 exhibited broad-spectrum efficacy against major citrus postharvest pathogens, effectively overcoming established fungicide resistance. Our results position PSP2 as a promising biocontrol agent for sustainable fruit preservation.

Keywords: lemons, *Penicillium digitatum*; *P. italicum*; *Geotrichum citri-aurantii*, biocontrol, imazalil

Academic Editor: Firstname Lastname

Received: date
Revised: date
Accepted: date
Published: date

1. Introduction

Significant economic losses worldwide result from citrus postharvest fungal diseases such as green and blue molds and sour rot, caused by *Penicillium digitatum*, *P. italicum*, and

Geotrichum citri-aurantii, respectively [1, 2, 3]. This problem is especially severe in regions with high humidity and warm temperatures that provide optimal conditions for fungal proliferation, such as Tucumán province in Northwestern Argentina. This region accounts for approximately 80% of the country's lemon production, positioning it as a leading global producer of this citrus fruit [4]. Currently, to control citrus fungal diseases, a reduced spectrum of fungicides can be applied as thiabendazole (TBZ), imazalil (IMZ), and pyrimethanil (PYR). However, their continued use results in the emergence of resistant pathogenic strains [5, 6] and serious risks to human health and the environment [7, 8]. Additionally, consumer demand for organic and safe compounds has increased. These concerns highlight the interest to search new, effective, and more environmentally friendly alternatives for managing postharvest fungal diseases [9, 10, 11] in all fresh fruit producing regions. In this context, the exploitation of plant-derived bioactive compounds emerges as a promising strategy to ensure high-quality, safe produce while meeting sustainability standards [12].

Plant-derived extracts are particularly relevant by having strong antioxidant and antimicrobial properties related to the presence of secondary metabolites [13]. These components bring them a dual role in exerting direct antifungal activity or stimulating plant defenses, which positioned as strategic candidates in the search for sustainable alternatives to synthetic fungicides to control postharvest fungal disease [14, 15, 16, 17]. Moreover, recent studies suggest that plant extracts, particularly those rich in phenolic compounds and vitamins, can help maintain fruit quality during storage [18]. To obtain plant-derived bioactive compounds, the revalorization of agricultural residues and industrial by-products represents an attractive primary source [15, 19]. In our region, strawberry cultivation is an important economic activity being the northwest of Argentina concentrates near 50% of national production (24,800 tn) [20]. Strawberry plants display organ-specific phenolic profiles dominated by ellagitannins, flavonoids, and phenolic acids, whose distribution is specific to each cultivar [21, 22]. Therefore, investigation and development of new bioinputs based on residues derived from strawberry cultivation emerges as a highly relevant and promising strategy. Our laboratory has demonstrated the capacity of different strawberry extracts and metabolites to protect plants against pathogen infections through enhanced plant immunity and direct antimicrobial activity [23, 24, 25, 26]. Among them is the ellagitannin HeT, patented in Argentina, Brazil, and Mexico, with the ability to activate defense mechanisms in plants [25]. Subsequent studies showed that aqueous extracts obtained from leaves of different strawberry genotypes exhibited *in vitro* antimicrobial activity against bacteria and fungi [27]. More recently, a new family of strawberry acylglycosides (SAGs) was described and patented [28] these compounds display antimicrobial activity against various phytopathogenic bacteria and fungi, as well as plant growth-promoting properties [29]. These findings led to the development of the new bioinput of the PSP technology ("Plant Stimulation and Protection") [30], called PSP2, based on aqueous extract of young leaves of strawberry plants. The aim of this study was to evaluate *in vitro* and *in vivo* antifungal activity and mode of action of PSP2 against local isolates of *G. citri-aurantii*, and both fungicide-sensitive and resistant *Penicillium* spp.

https://doi.org/10.3390/xxxxx

2. Materials and Methods

2.1 Chemicals

Tween 80, (Sigma-Aldrich, Chemical Co, St. Louis, MO, USA); SYTOX™ Green nucleic acid stain (Thermo Fisher Scientific Inc. Waltham, MA, USA). Other chemicals used were of analytical grade.

2.2 PSP2 extract preparation

PSP2 extract was prepared following the methodology described by Cerviño et al. [27], with minor modifications. Briefly, fully expanded young leaves of strawberry plants (*Fragaria* × *ananassa* Duch., cv. San Andreas) were collected at the end of the crop cycle from a commercial organically managed farm located in Famaillá, Tucumán, Argentina (27°01′03.34″ S, 65°22′46.54″ W). The leaves were stored at −20 °C until further processing. Extraction was carried out using distilled water as the solvent, with a solid-to-solvent ratio of 1:10 (w/v) for 24 h at 25 °C. The resulting homogenate was filtered through Whatman filter paper to obtain an aqueous extract with a final concentration of 0.1 g dry weight mL^{-1}. When required, this extract was further concentrated by lyophilization.

2.3 Fungal isolates and conidial suspensions preparation

Fungal isolates characterized from the INSIBIO-CONICET-UNT laboratory collection were used. These included fungicide-sensitive variants of *P. digitatum* F-Pd07-S (PDS) and *P. italicum* F-Pi09-S (PIS), as well as triples fungicide-resistant variants to imazalil, thiabendazole, and pyrimethanil from *P. digitatum* F-Pd17-R31 (PDR) and *P. italicum* Pi15-R29 (PIR) [31]. Additionally, one *G. citri-aurantii* (GC) isolate was included. For conidial suspension preparation, the fungi were allowed to grow on potato dextrose agar (PDA) for 5 d at 24 ± 1°C. Sterile distilled water containing 0.05% Tween 80 (Sigma-Aldrich) was used to scrape the colony surface, and the conidia were collected and filtered through two layers of cheesecloth to remove hyphal fragments. The cellular concentration was adjusted to $1x10^6$ conidia mL^{-1} following counting in a Neubauer chamber.

2.4 Fruit

Lemons (*Citrus limon* (L.) Burm. F., cv. Eureka) were harvested of commercial orchard of Tucuman province and were free from any postharvest treatment or coating. Before treatments, each lemon was superficially disinfected (70% ethanol), rinsed with tap water, and allowed to air-dry at room temperature.

2.5 Evaluation of PSP2 effect on mycelial growth, conidia germination and minimum killing conidia time.

To evaluate the antifungal effect of PSP2, mycelial growth was assessed by placing 5 µl drops of pathogens conidial suspensions of PDS, PDR, PIS, PIR and GC on PDA containing 0.05 or 0.1 gr/ml of PSP2. After 5 d at 24 ± 1°C, colony diameter was measured for each treatment. Controls consisted on PDA growth media at pH 5 without supplements were included. Conidia germination of all fungal isolates was assessed as reported by Olmedo et al. [32]. Briefly, conidial suspensions in potato dextrose broth (PDB) at pH 5, were exposed to 0.1 gr mL^{-1} of PSP2 and incubated at 24 ± 1°C for 24 h. After that, germinated and non-germinated conidia were observed under an inverted microscope (×40). Moreover, conidial suspensions were exposed for 4-8-24 and 48 h to 0.1 gr mL^{-1} of PSP2. At each time,

a 5 µL aliquot of treated suspensions were spotted onto PDA medium without supplements, to determine the minimum time necessary to kill all treated conidia of each pathogen assayed. Three replicates were performed for each condition, and the assay was done twice.

2.6 Evaluation of conidial membrane integrity

To determine the impact of PSP2 on conidial membrane integrity, the fluorescent dye SYTOXTM Green was used, following the protocol described by Olmedo et al. [32]. Conidial suspensions were treated at 0.1 gr mL^{-1} of PSP2 for 24 h and then were washed and incubated with SYTOXTM Green (5 µM) for 30 min in darkness. Conidia were visualized under a fluorescence microscope Olympus IX51 equipped with an Olympus digital camera, QColor5 (Q-imaging). The fluorescence emission was examined and photographed using a filter set of 450-490 nm for excitation and of 515-565 nm for emission. Three replicates were performed for each condition, and the assay was done twice.

2.7 Evaluation of conidia ultrastructure

For ultrastructural characterization through transmission electron microscopy, conidia were treated with 0.1 gr mL^{-1} of PSP2 for 24 h and were processed as described by Cerioni et al. [33]. Observations were made using a Hitachi HT7800 transmission electron microscope, available at the Comprehensive Center for Electron Microscopy (CME, CONICET-UNC, Córdoba, Argentina). Controls of conidia water-treated were performed in parallel. The assay was done twice.

2.8 Application of PSP2 on lemons in situ

To evaluate the potential application of PSP2 to control posharvest diseases on lemons, a trial was performed following an *in situ* protocol. Briefly, lemons were washed and sanitized with ethanol 70 %, then wounded using a stainless-steel rod (approximately 2 mm deep and 3 mm wide). Subsequently, 10 µL of PSP2 at concentrations of 0.1 g mL^{-1} was applied directly into each wound, while distilled water was used as a control. After drying for 2h at room temperature, the treated wounds were inoculated with 10 µL of conidial suspension of 10^5 conidia/ml of PDS. The fruit were incubated for 5 d in a chamber under controlled conditions (24 °C and 95% relative humidity). Disease incidence (DI) was evaluated at the end of incubation period and calculated as follows: DI (%) = (number of decayed fruit/numbers of total fruit) × 100 % [34].

2.9 Statistic analysis

For the in vitro assays, three complete sets of experiments were conducted, encompassing three repetitions for each condition. The *in situ* assays comprised two repetitions involving 10 lemons (each with two wounds) for each condition. In all cases, the data underwent variance analysis, followed by Tukey's test using Infostat software v 2020I. Differences of $p \leq 0.05$ were considered significant.

3. Results

3.1 Antifungal action of PSP2 on mycelial growth, conidia germination and time of death conidia

The antifungal activity of PSP2 against the fungicide-sensitive or fungicide-resistant phytopathogens (PDS, PDR, PIS and PIR) and GC were evaluated. **Figure 1** showed that 0.05 gr ml^{-1} of PSP2 incorporated in the PDA plate was enough that inhibited the mycelial growth of all fungal pathogens evaluated. Controls were able to grow and complete PDA plate without PSP2 at 5d. As was expected, the mycelial growth on PDA plate amended with 0.1 gr ml^{-1} PSP2 was totally inhibited.

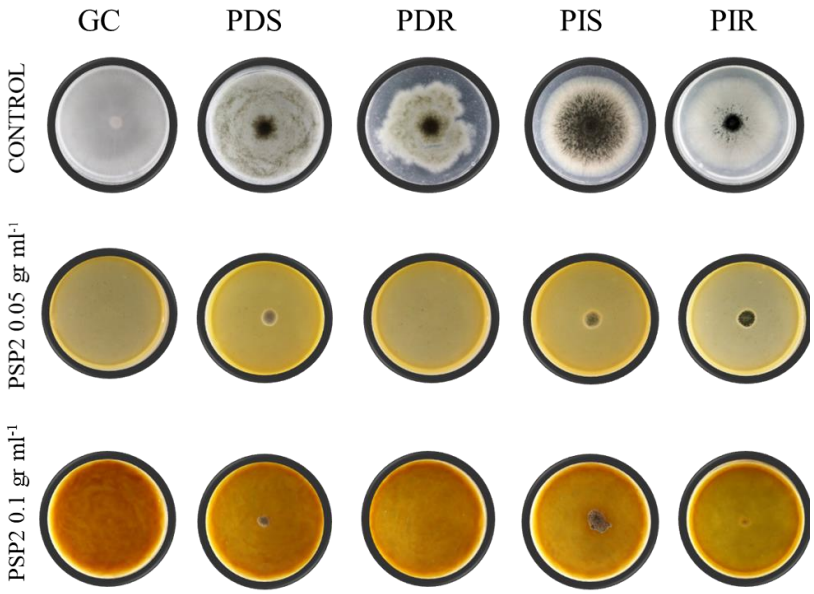

Figure 1. ***Antifungal activity of PSP2***. Mycelial growth was assessed by placing 5 µl drops of conidial suspensions (10^6 CFU mL^{-1}) of each fungal pathogen on PDA containing indicated concentration of PSP2. After 5 d incubation at 24 ± 1°C, colony diameter was measured for each condition. Controls consisting on PDA plates without supplements. The photograph is a representative image from three independent experiments.

In the minimum conidia killing time assays, the incubation of all pathogen conidia with 0.1 gr ml-1 PSP2 during 4h partially inhibits the fungal growth. With 8 h of incubation, the total inhibition was observed to GC and PIR (**Figure 2**). To PDS and PIS the same effect was observed after 24 h of incubation and in the case of PDR an incubation for 48 h was necessary to achieve the growth inhibition, this being the most resistant pathogen assayed.

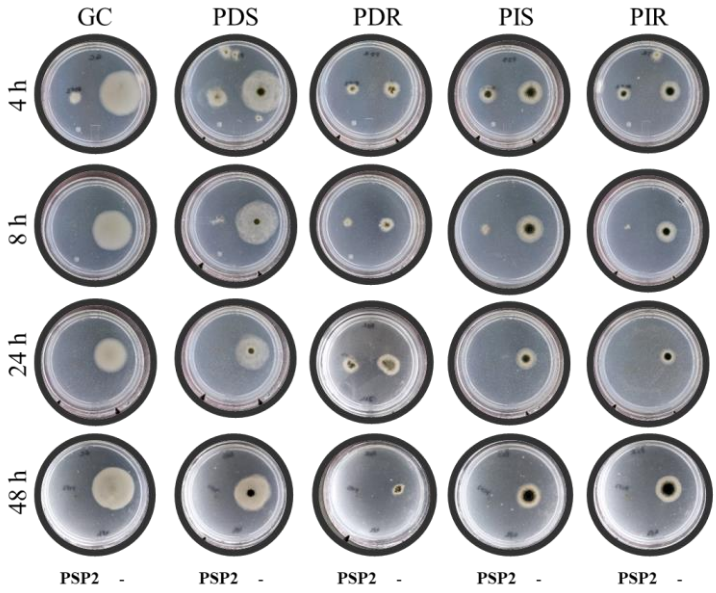

Figure 2. Minimum killing conidia time. Conidial suspensions (10^6 CFU mL^{-1}) of each pathogen were exposed to PSP2 (0.1 gr mL^{-1}). At each indicated time, a 5 µL aliquot of treated suspensions were spotted onto PDA medium without supplements. The plates were incubated 5 d at 24 ± 1°C. Controls consisting on conidial suspensions incubated with water. The photograph is a representative image from three independent experiments.

In respect to conidia germination, the results showed that 0.1 gr ml^{-1} PSP2 was able to inhibit conidia germination of all phytopathogens evaluated (**Figure 3**), while the control condition was completely germinated after 24 h.

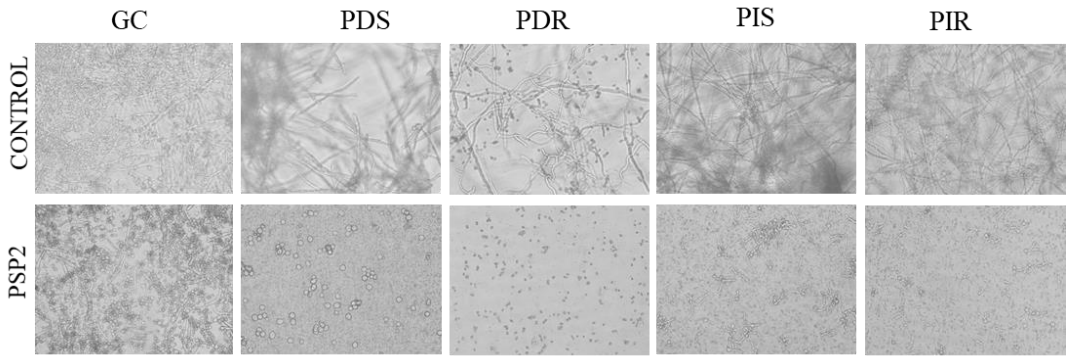

Figure 3. ***Conidia germination***. Conidial suspensions (10^6 CFU mL^{-1}) in potato dextrose broth (PDB) at pH 5 of each fungal pathogen, were exposed to 0.1 gr mL^{-1} PSP2 and incubated at 24 ± 1°C for 24 h. After that, germinated and non-germinated conidia were observed under an inverted microscope (×40). The photograph is a representative image from three independent experiments of germination and general aspect to control and treated conidia (magnification = 400x).

3.2 *Effect of PSP2 on conidial membrane integrity*

In order to analyze the effect of PSP2 over fungal conidia, a study of the integrity cell membrane was conducted using the SYTOXTM Green probe (**Figure 4 MEJORAR**). In the dark field, fluorescent conidia were observed for all fungal isolates treated with 0.1 gr ml^{-1} of PSP2 indicating membrane permeability. As positive control of membrane damages, an incubation with 600 mM H_2O_2 was included, which shows fluorescence for all the phytopathogens, similar to PSP2 treatments. No fluorescence emission was registered in samples incubated with water, as negative control.

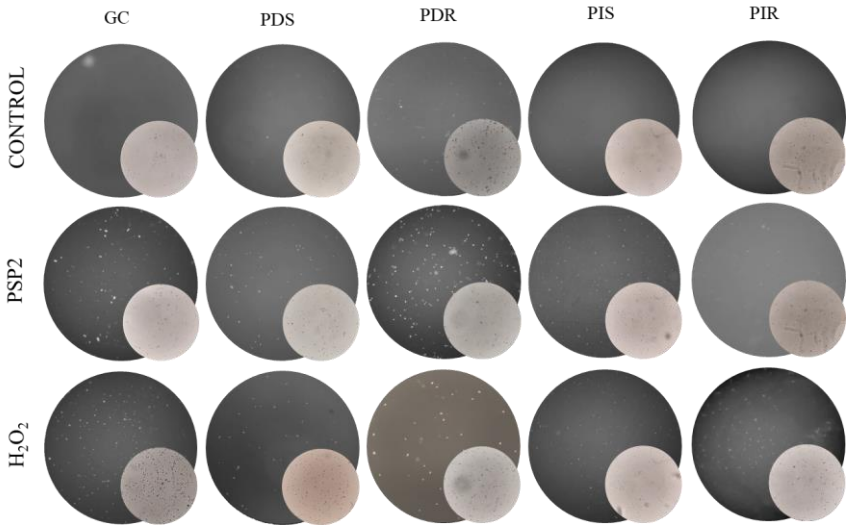

Figure 4. Effect of PSP2 on membrane integrity. Conidial suspensions (10^6 CFU mL^{-1}) of each fungal pathogen were treated with 0.1 gr mL^{-1} PSP2 for 24 h, before their incubation with SYTOXTM Green (5 µM) for 30 min in darkness. Fluorescent field images at a 40× magnification accompanied by a bright field (insets) are shown. The upper panels display negative controls and de lower panel display positive controls corresponding to conidia treated either with water or 600 mM H_2O_2, respectively. The images are representative of three independent experiments.

3.3 Internal structure conidial after PSP2 treatment

The conidia ultrastructure was examined using Transmission Electron Microscopy (TEM) (Figure 5). All evaluated fungi in control condition revealed an appropriate organization of the cell walls and cytoplasm, with conserved internal structures such as lipid bodies, mitochondria, indicating a regular conidia structure. However, after exposing the conidia to 0.1 gr ml^{-1} of PSP2 for 24 h, cellular damage was evident, including cell deformation and clear cytoplasmic disorganization, with the presence of multiple intracellular vesicles. Moreover, significant modifications in the membrane and cell wall structure occurred. These results indicate irreversible cellular damage on conidia after PSP2 treatment.

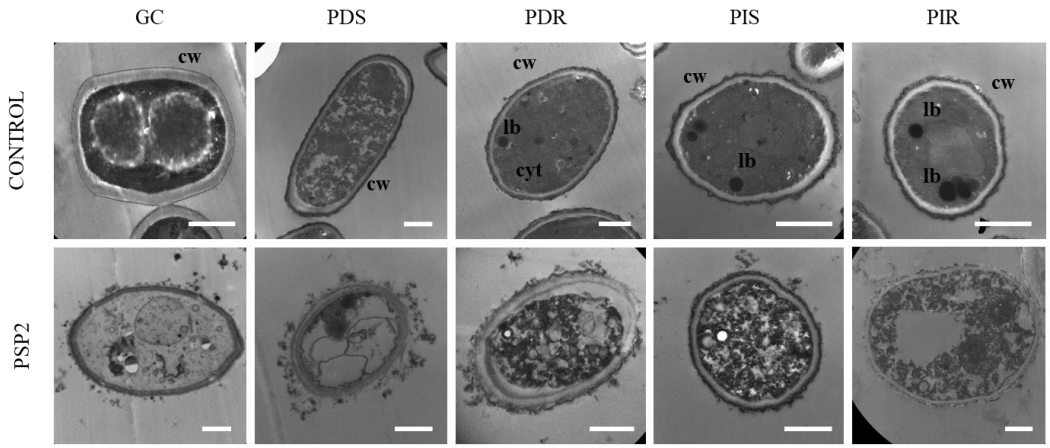

Figure 5. ***Intracellular damage induced by PSP2***. Conidial suspensions of each fungal pathogen were treated at 0.1 gr mL^{-1} of PSP2 for 24 h and then were washed and visualized by transmission electron microscopy (TEM). The tests were repeated three times for each treatment, and at least two replicates were examined. In micrographs, a scale graphic = 1 µm is represented. cw, cell wall; cyt, cytoplasm; lb, lipid body.

3.4 Effect of PSP2 in situ application over green mold incidence on lemons

The antifungal efficacy of PSP2 was evaluated by *in situ* application on artificial wounded and inoculated lemons. In control conditions, a high disease incidence to 100% was observed (**Figure 6**). The application of PSP2 demonstrated significant green mold control, achieving nearly 50% disease incidence in respect to the control without treatment. As was expected, the IMZ application was able to control green mold caused by PDS.

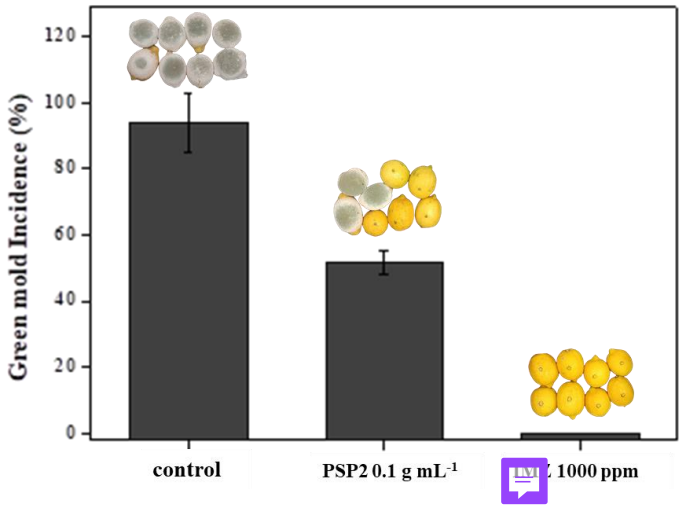

Figure 6. In situ action of PSP2 against P. digitatum on lemons. PSP2 or water (control) were added on lemon wounds before *P. digitatum* inoculation. Fruit were stored at 23 ◦C and 95 % relative humidity, and disease incidence was evaluated at 5 d post infection. Photographs inserts shown fruit aspect after incubation and are representative of three different experiments.

4. Discussion

This study, it was demonstrated that the aqueous extract from strawberry leaves PSP2 exhibits *in vitro* antifungal activity against the citrus fungal pathogens *P. digitatum*, *P. italicum*, and *G. citri-aurantii*. The extract inhibited mycelial growth at 0.05 g·mL^{-1} and completely suppresses conidia germination at 0.10 g·mL^{-1} for all isolates evaluated, regardless of their sensitivity to conventional fungicides. These inhibitory concentrations are comparable to those reported for other plant-derived extracts active against postharvest citrus pathogens. For instance, aqueous extracts of *Cistus* spp. inhibited 80–100% of *G. citri-aurantii* growth and germination at concentrations from 0.1 and 0.05 g·mL^{-1}, respectively [17]. Similarly, powders, solvent extracts, and essential oils from species such as *Thymus leptobotrys* and *Peganum harmala* have shown broad-spectrum antifungal activity against *P. digitatum*, *P. italicum*, and *Geotrichum* spp., achieving growth reductions close to complete inhibition [35, 36]. Comparable antifungal effects have also been reported for extracts from extremophile plant species, which inhibited *P. digitatum* and affected *G. citri-aurantii* growth at phenolic-adjusted concentrations [37].

To elucidate the antifungal mode(s) of action of the PSP2 aqueous extract on phytopathogenic fungi, membrane integrity assays with fluorescent probes were performed, together with ultrastructural analysis by TEM. These analyses confirmed plasma membrane permeabilization, cytoplasmic disorganization, and contraction of the cell wall, evidence of irreversible cellular damage. It is known that fungal viability relies primarily on the integrity of the plasma membrane [38, 39] which would explain the loss of viability observed with PSP2 treatment. Moreover, the pattern of structural injury is consistent with mechanisms reported for other natural metabolites, such as cinnamaldehyde, the principal component of cinnamon essential oil, which disrupts cell wall integrity [14], and natamycin, obtained from *Streptomyces natalensis* fermentation, whose action centers on plasma membrane damage [40, 41]. Plant-derived antifungal compounds acting on multiple cellular

targets are associated with a reduced risk of resistance development, as they limit adaptation to single molecular targets [14, 42, 43]. The antifungal activity of PSP2 suggests a multi-target mode of action arising from the synergistic interactions among its metabolites, conferring an advantage over single-site synthetic fungicides for resistance management. According to the criteria of the Fungicide Resistance Action Committee, multi-site fungicides are classified as low-risk tools for resistance development [44].

Exposure of fungal conidia to PSP2 (0.1 g mL-1) for 24 h induce membrane permeabilization and internal structural collapse. OuYang et al. [14] observed a similar effect after 2 hours of exposure of *G. citri-aurantii* conidia to cinnamaldehyde. The effect of PSP2 is particularly critical for controlling *G. citri-aurantii*, causal agent of sour rot, a pathogen for which commercial fungicidal options remain limited. Since total inhibition of viability in *G. citri-aurantii* (GC) and fungicide-resistant *P. italicum* (PIR) was achieved after only 8 hours of incubation with PSP2—and for other pathogens between 24 and 48 hours—future studies could focus on optimizing the minimum contact time required to induce irreversible fungitoxicity, in order to determine the operational and economic feasibility of the extract in postharvest applications.

In vivo disease control trials are a critical parameter for validating the commercial potential of biofungicide. In this work, inoculation assays on fruit under controlled conditions were conducted. *In situ* application of PSP2 on artificially wounded lemons reduced green mold (*P. digitatum*) incidence nearly 50% in respect to the control without treatment, whereas imazalil achieved total disease suppression. Compared with other plant extracts, the efficacy obtained with the PSP2 aqueous extract surpassed that reported for aqueous, methanolic, and ethanolic extracts of Cynara cardunculus in citrus fruit against *P. digitatum* [15]; only the ethanolic extract produced a reduction of approximately 10% in green mold incidence relative to the control. It is worth to note the PSP2 aqueous extract did not cause phytotoxicity in treated fruit (data no shown), representing an advantage in terms of safety and technological feasibility. Therefore, its efficacy could be further improved through formulation strategies aimed at enhancing extract concentration and retention at the wound site without compromising fruit integrity. It is also important to highlight the efficacy of the PSP2 aqueous extract against strains with multiple resistances to fungicides such as imazalil, thiabendazole and pyrimethanil. This behavior suggests that incorporating PSP2 into resistance management programs could provide strategic benefits. Its ability to control both sensitive and resistant strains positions PSP2 as a potentially valuable candidate for diversifying and strengthening the tools available for postharvest citrus disease management.

5. Conclusions

In conclusion, the strawberry leaf extract PSP2 represents a novel and effective approach for the postharvest management of citrus fungal diseases. This study demonstrated that the antifungal activity of PSP2 is mediated by plasma membrane permeabilization and irreversible intracellular damage. Furthermore, *in vivo* trials confirmed its efficacy in controlling green mold on lemons. Valorizing strawberry crop residues as a source for PSP2 production offers a sustainable and potentially profitable bioinput. Its implementation could significantly reduce the reliance on synthetic fungicides, facilitating the transition toward safer, more efficient postharvest systems that align with global food security and sustainability regulations.

Author Contributions: "Conceptualization, L.C. and S.I.V.; methodology, G.M., P.DP., M.A.D., L.C. and S.I.V.; validation, G.M. and P.DP.; formal analysis, N.R.C, L.C and S.I.V.; investigation, G.M., P.DP.; data curation, G.M. and P.DP.; writing—original draft preparation, P.DP., L.C. and S.I.V.; writing—review and editing, G.M., P.DP., M.A.D., N.R.C., B.V.W., L.C. and S.I.V; supervision, L.C. and S.I.V.; funding acquisition, N.R.C., L.C. and S.I.V. All authors have read and agreed to the published version of the manuscript."

Funding: This work was supported by grants from Agencia Nacional de Promoción Científica y Tecnológica (PICT 2019-1380 and PICT 2021-I-A-00850), CONICET (PIP 2547) and Universidad Nacional de Tucumán (PIUNT D768).

Data Availability Statement: The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding authors.

Acknowledgments: We extend our special thanks to Las Tipas farm for generously providing the lemons and to Ing. Gabriel Rodriguez for supplying lemons fruit. Additionally, we thank Agustin Padilla, Andrea Peña and Fernanda Trejo for their technical contributions.

Conflicts of Interest: Declare conflicts of interest or state "The authors declare no conflicts of interest."

References

1. Eckert, J. W.; Eaks, I. L. 1989. Postharvest disorders and diseases of citrus fruits. in: The Citrus Industry. W. Reuther, E. C. Calavan, and G. E. Carman, Eds.; Publisher: University of California Division of Agricultural Natural Resources, Oakland, CA, USA, 1989; Volume 5, pp. 179-260.

2. Pitt, J.I.; Hocking, A.D. Primary Keys and Miscellaneous Fungi. In Fungi and Food Spoilage; Pitt, J.I., Hocking, A.D., Eds.; Springer: Boston, MA, USA, 2009.

3. Wang, W.; Liu, S.; Deng, L.; Ming, J.; Yao, S.; Zeng, K. Control of Citrus Post-harvest Green Molds, Blue Molds, and Sour Rot by the Cecropin A-Melittin Hybrid Peptide BP21. Front. Microbiol. 2018, 9, 2455.

4. Federcitrus. Actividad Citrícola 2025. Available online: https://www.federcitrus.org/wp-content/uploads/2025/10/Federcitrus-Actividad-citricola-2025-02102025.pdf (accessed on 27 February 2026).

5. Kinay, P.; Mansour, M. F.; Mlikota Gabler F.; Margosan, D. A; Smilanick, J. L. Characterization of fungicide-resistant isolates of *Penicillium digitatum* collected in California. Crop Prot. 2007, 26, 647–656.

6. Sánchez-Torres, P.; Tuset, J.J. Molecular insights into fungicide resistance in sensitive and resistant *Penicillium digitatum* strains infecting citrus. Postharvest Biol. Technol. 2011, 59, 159–165.

7. Schneider, K.; Barreiro-Hurle, J.; Rodriguez-Cerezo, E. Pesticide reduction amidst food and feed security concerns in Europe. Nat. Food 2023, 6, 746–750.

8. Yin, Y.; Miao, J.; Shao, W.; Liu, X.; Zhao, Y.; Ma, Z. Fungicide resistance: Progress in understanding mechanisms, monitoring, and management. Phytopathology 2023, 113, 707–718.

9. Tilman, D.; Cassman, K.G.; Matson, P.A.; Naylor, R.; Polasky, S. Agricultural sustainability and intensive production practices. Nature 2002, 418, 671–677.

10. Palou, L.; Ali, A.; Fallik, E.; Romanazzi, G. GRAS, plant- and animal-derived compounds as alternatives to conventional fungicides for the control of postharvest diseases of fresh horticultural produce. Postharvest Biol. Technol. 2016, 122, 41–52

11. Usall, J.; Ippolito, A.; Sisquella, M.; Neri, F. Physical treatments to control postharvest diseases of fresh fruits and vegetables. Postharvest Biol. Technol. 2016, 122, 30–40.

12. Devi, M.J.; Gidado, S. Sustainable valorization of postharvest waste for disease control and quality preservation in fruits and vegetables. Waste Manag. 2026, 192, 25–38.

13. Bajaj, K.; Adhikary, T.; Gill, P.P. S.; Kumar, A. Edible coatings enriched with plant-based extracts preserve postharvest quality of fruits: A review. Prog. Org. Coat. 2023, 182, 107669.

14. OuYang, Q.; Duan, X.; Li, L.; Tao, N. Cinnamaldehyde exerts its antifungal activity by disrupting the cell wall integrity of *Geotrichum citri-aurantii*. Front. Microbiol. 2019, 10, 55.

15. Restuccia, C.; Lombardo, M.; Scavo, A.; Mauromicale, G.; Cirvilleri, G. Combined application of antagonistic *Wickerhamomyces anomalus* BS91 strain and Cynara cardunculus L. leaf extracts for the control of postharvest decay of citrus fruit. Food Microbiol. 2020, 92, 103583.

16. Hao, W.; Li, H.; Hu, M.; Yang, L.; Rizwan-ul-Haq, M. Integrated control of citrus green and blue mold and sour rot by *Bacillus amyloliquefaciens* in combination with tea saponin. Postharvest Biol. Technol. 2011, 59, 316–323.

17. Karim, H.; Boubaker, H.; Askarne, L.; Cherifi, K.; Lakhtar, H.; Msanda, F.; Boudyach, E. H.; Ait Ben Aoumar, A. Use of Cistus aqueous extracts as botanical fungicides in the control of Citrus sour rot. Microb. Pathog. 2017, 104, 139–143.

18. Bai, T.; Wang, X.; Du, W.; Cheng, J.; Zhang, J.; Zhang, Y.; Klinjapo, R.; Asavasanti, S.; Yasurin, P. Recent Advances, Challenges, and Functional Applications of Natural Phenolic Compounds in the Meat Products Industry. Antioxidants 2025, 14, 138.

19. Villamil-Galindo, E.; Van de Velde, F.; Piagentini, A.M. Strawberry agro-industrial by-products as a source of bioactive compounds: effect of cultivar on the phenolic profile and the antioxidant capacity. Bioresour. Bioprocess. 2021, 8, 61.

20. Secretaría de Agricultura, Ganadería y Pesca de la Nación. Producción de frutilla en Argentina (Informe de enero 2023). Ministerio de Economía, Presidencia de la Nación, 2023. Available online: https://www.argentina.gob.ar/sites/default/files/sagyp-informe-produccion-frutilla-enero-2023.pdf (accessed on 27 February 2026).

21. Buendía, B.; Gil, M.I.; Tudela, J.A.; Gady, A.L.; Medina, J.J.; Soria, C., Tomás-Barberán, F.A. HPLC-MS analysis of proanthocyanidin oligomers and other phenolics in 15 strawberry cultivars. J. Agric. Food Chem. 2010, 58, 3916–3926.

22. Hernanz, D.; Recamales, A.F.; Meléndez-Martínez, A.J.; González-Miret, M.L.; Heredia, F.J. Assessment of the differences in the phenolic composition of five strawberry cultivars (*Fragaria x ananassa* Duch.) grown in two different soilless systems. J. Agric. Food Chem. 2007, 55, 1846–1852.

23. Filippone, M.P.; Diaz Ricci, J.M.; Mamaní de Marchese, A.; Castagnaro, A.; Farías, R.N. Effect of fragarin on the cytoplasmic membrane of the phytopathogen *Clavibacter michiganensis*. Mol. Plant-Microbe Interact. 2001, 14, 925–928.

24. Filippone, M. P.; Diaz Ricci, J. C.; Mamani de Marchese. A., Farias, R. N.; Castagnaro, A. P. Isolation and purification of a 316 Da pre-formed compound from strawberry (*Fragaria ananassa*) leaves active against plant pathogens. FEBS Lett. 1999, 459, 115-118.

25. Mamaní, A.; Filippone, M. P.; Grellet, C.; Björn, W.; Castagnaro, A. P.; Díaz Ricci, J. C. Pathogen-induced accumulation of an ellagitannin elicits plant defense response. Mol. Plant-Microbe Interact. 2012, 25, 1430–1439.

26. Martos, G.G.; Mamaní, A.; Filippone, M. P.; Abate, P. O.; Katz, N. E.; Castagnaro, A. P.; Díaz Ricci, J. C. Ellagitannin HeT obtained from strawberry leaves is oxidized by bacterial membranes and inhibits the respiratory chain. FEBS Open Bio 2018, 8, 211–218.

27. Cerviño, A.; Grellet, C.F.; Di Peto, P.A.; Rodríguez, L.C.; Castagnaro, A.P.; Filippone, M.P.; Mamani de Marchese, A.I. Evaluación de la actividad antimicrobiana in vitro de extractos de hojas de diferentes cultivares de *Fragaria ananassa* Duch. para la formulación de bioinsumos fitosanitarios. Rev. Agron. Noroeste Argent. 2019, 39, 83–87.

28. Welin, B.; Castagnaro, A.P.; Filippone, M.P.; Grellet-Bournonville, C.F.; Mamani, A.I.; Di Peto, P. Glycoside compound of fatty acids, composition comprising it, process for its obtention and methods to apply it on plants or fruits or both at the same time. World Intellectual Property Organization, Patent No. WO2019/049044, 2019.

29. Grellet Bournonville, C.; Filippone, M. P.; Di Peto, P.A.; Trejo, M.F.; Couto, A.S.; Mamani de Marchese, A.I.; Díaz Ricci, J. C.; Björn, W.; Castagnaro, A. P. Strawberry fatty acyl glycosides enhance disease protection, have antibiotic activity and stimulate plant growth. Sci. Rep. 2020, 10, 8196.

30. Chalfoun, N.R.; Durman, S.B.; Budeguer, F.; Caro, M.D.P.; Bertani, R.P.; Di Peto, P.; Stenglein, S.A.; Filippone, M.P.; Moretti, E.R.; Díaz Ricci, J.C.; et al. Development of PSP1, a Biostimulant Based on the Elicitor AsES for Disease Management in Monocot and Dicot Crops. Front. Plant Sci. 2018, 9, 844.

31. Olmedo, G.M.; Debes, M. A.; Sepúlveda, M.; Ramallo, J.; Rapisarda, V. A.; Cerioni, L.; Volentini, S. I. Overcoming lemon postharvest molds caused by *Penicillium* spp. multiresistant isolates by the application of potassium sorbate in aqueous and wax treatments. J. Food Sci. 2023, 88, 1234–1245.

32. Olmedo, G.M.; Cerioni, L.; González, M. M.; Cabrerizo, F. M.; Rapisarda, V. A.; Volentini, S. I. Antifungal activity of β-carbolines on *Penicillium digitatum* and *Botrytis cinerea*. Food Microbiol. 2017, 62, 9–15.

33. Cerioni, L.; Volentini, S. I.; Prado, F. E.; Rapisarda, V. A.; Rodríguez-Montelongo, L. Cellular damage induced by a sequential oxidative treatment on *Penicillium digitatum*. J. Appl. Microbiol. 2010, 109, 123–130.

34. Liu, Y.; Wang, W.H.; Zhou, Y.H.; Yao, S.X.; Deng, L.L.; Zeng, K.F. Isolation, identification and in vitro screening of Chongqing orangery yeasts for the biocontrol of *Penicillium digitatum* on citrus fruit. Biol. Control 2017, 110, 18–24.

35. Ameziane, N.; Boubaker, H.; Boudyach, H.; Msanda, F.; Jilal, A.; Ait Benaoumar, A. Antifungal activity of Moroccan plants against citrus fruit pathogens. Agron. Sustain. Dev. 2007, 27, 273–277.

36. Sayago, J.E.; Ordoñez, R.M.; Kovacevich, L.N.; Torres, S.; Isla, M.I. Antifungal activity of extracts of extremophile plants from the Argentine Puna to control citrus postharvest pathogens and green mold. Postharvest Biol. Technol. 2012, 67, 19–24.

37. Boubaker, H.; Karim, H.; El Hamdaoui, A.; Msanda, F.; Leach, D.; Bombarda, I.; Vanloot, P.; Abbad, A.; Boudyach, E.H.; Ait Ben Aoumar, A. Chemical characterization and antifungal activities of four Thymus species essential oils against postharvest fungal pathogens of citrus. Ind. Crops Prod. 2024, 86, 95–101.

38. Shao, X.; Cheng, S.; Wang, H.; Yu, D.; Mungai, C. The possible mechanism of antifungal action of tea tree oil on *Botrytis cinerea*. J. Appl. Microbiol. 2013, 114, 1642–1649.

39. Tao, N.G.; OuYang, Q.L.; Jia, L. Citral inhibits mycelial growth of *Penicillium italicum* by a membrane damage mechanism. Food Control 2014, 41, 116–121.

40. He, C.; Zhang, Z.; Li, B.; Xu, Y.; Tian, S. Effect of natamycin on *Botrytis cinerea* and *Penicillium expansum* postharvest pathogens of grape berries and jujube fruit. Postharvest Biol. Technol. 2019, 151, 134–141.

41. te Welscher, Y.M.; ten Napel, H.H.; Balagué, M.M.; Souza, C.M.; Riezman, H.; de Kruijff, B.; Breukink, E. Natamycin blocks fungal growth by binding specifically to ergosterol without permeabilizing the membrane. J. Biol. Chem. 2008, 283, 6393–6401.

42. Regnier, T.; Combrinck, S.; Veldman, W.; Du Plooy, W. Application of essential oils as multitarget fungicides for the control of *Geotrichum citri-aurantii* and other postharvest pathogens of citrus. Ind. Crops Prod. 2014, 61, 151–159

43. Leiva-Mora, M.; Bustillos, D.; Arteaga, C.; Hidalgo, K.; Guevara-Freire, D.; López-Hernández, O.; et al. Antifungal mechanisms of plant essential oils: A comprehensive literature review for biofungicide development. Agriculture 2025, 15, 2303.

44. FRAC. Importance of multisite fungicides in managing pathogen resistance. Fungicide Resistance Action Committee, 2018. Available online: efaidnbmnnnibpcajpcglclefindmkaj/https://www.frac.info/media/ztqosoch/frac-statement-on-multisite-fungicides-2018.pdf (accessed on 20 January 2026).