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Article

Protective effect of *Pycnoporus sanguineus* against phytopathogenic fungi in tomato and strawberry plants

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Abstract

The search for sustainable alternatives to synthetic fungicides has increased interest in bioactive compounds from basidiomycetes, particularly *Pycnoporus sanguineus*. The present study evaluated the protective effect of aqueous extracts of *P. sanguineus* against two phytopathogens of agricultural importance: *Botrytis cinerea* in strawberries (*Fragaria ananassa*) and *Fusarium oxysporum* in tomatoes (*Solanum lycopersicum*). The tests were conducted under controlled conditions, applying different concentrations of extract (25-100 g L^{-1}). In strawberries, using an infection scale that was established *in vitro*, the severity caused by *B. cinerea* declined significantly in the treatments with the highest concentrations (50 and 75 g L^{-1}), which decreased leaf damage by 58 and 74%, respectively. In tomatoes, the treatments with the highest concentrations (50 and 100 g L^{-1}) promoted an aboveground biomass similar to that of non-infected plants (p = 0.05), which shows a positive effect against *F. oxysporum*. Nonetheless, no significant differences were observed in root biomass, indicating limited systemic activity or low mobility of the compounds. Although these findings are promising, it is necessary to optimize the formulations and validate their efficacy in field conditions.

Keywords:

Pycnoporus sanguineus, biological control, phytopathogens, secondary metabolites, synthetic fungicides.

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Introduction

The search for sustainable alternatives to synthetic fungicides has driven interest in natural products with antifungal activity; among them, basidiomycete fungi extracts have gained significant relevance (Sivanandhan *et al*., 2017; Waszczuk and Zapora, 2021). This is especially true for degrading species, given their high and complex metabolic production (Pinar and Rodríguez-Couto, 2024). In this sense, *P. sanguineus*, a saprobic fungus widely distributed in tropical and subtropical regions, including Mexico (Téllez-Téllez *et al*., 2016), has stood out for its richness in secondary metabolites, where up to 19 biosynthetic clusters have been identified in its genome, all of them related to the production of bioactive metabolites, such as clavaric acid, cinnabarin, cinnabarinic acid, as well as phenolic compounds and terpenoids (Lin *et al*., 2020). Additionally, its high enzymatic capacity, conferred by laccases, enables particularly efficient lignocellulolytic degradation, thus supporting its great metabolic versatility (Saat *et al*., 2014).

In the agricultural context, its primary derived pigments, such as cinnabarin, cinnabarin acid and tramesanguine, have been evaluated, reflecting an important antimicrobial activity against both Gram-positive and Gram-negative bacteria, including *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella* spp. and *Staphylococcus aureus* (Smânia *et al*., 1995; Smânia *et al*., 2003). Soluble formulations based on *P. sanguineus* extracts have been directly evaluated for the control of early rot and bacterial spot in tomato crops, such as *Alternaria solani*, *Xanthomonas vesicatoria* (Assi *et al*., 2017) and *Xanthomonas campestris* (Romero-Arenas *et al*., 2021). In postharvest, *P. sanguineus* is effective against relevant phytopathogens, such as *Erwinia amylovora* and *Pectobacterium carotovorum* (Cruz-Muñoz, 2021).

In addition to its effectiveness on phytopathogenic bacteria, recent studies show that the extract of this fungus can significantly inhibit the *in vitro* growth of phytopathogenic fungi, such as *B. cinerea* and *F. oxysporum* (Pérez-López *et al*., 2024); therefore, the evaluation of these effects at the *in vivo* level is very valuable, due to the commercial impact that these fungi have on strawberry and tomato production worldwide (Hassan *et al*., 2021; Heikal *et al*., 2025). Additionally, it has been determined that the extract of *P. sanguineus* has nematicidal activity and the ability to induce defense responses in plants (Brito *et al*., 2025), expanding its spectrum of usefulness in comprehensive disease management and thus reducing the ecological and health risks associated with the use of synthetic products.

The study evaluated the protective potential of aqueous extracts of *P. sanguineus* at different concentrations under controlled conditions against infections caused by *B. cinerea*, as measured by foliar severity in strawberries and by *F. oxysporum*, as measured by its impact on tomato aboveground and root biomass. This research will expand knowledge of the use of mycological resources present in Mexico within integrated agricultural disease management strategies, providing new, consistent findings and promoting their practical application.

Materials and methods

Description of the strains

Three strains were characterized and preserved in the Phytopathology Laboratory 204 of the Center for Agroecology, Institute of Sciences of the Meritorious Autonomous University of Puebla (BUAP), by its Spanish acronym. The strain of *P. sanguineus* (Basidiomycota) was isolated from a wild specimen in the vicinity of the state of Puebla, identified by morphoanatomical and molecular characterization, designated as the MA-Ps1 strain, and later registered with the National Center for Biotechnology Information (NCBI) under the accession number OR622486.1. The phytopathogen strains corresponded to *F. oxysporum* (MA-FC220) and *B. cinerea* (MA-BC20), isolated from strawberry roots and fruits and registered in NCBI under accession numbers OM473290.1 and OM473288.1, respectively.

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Protective treatment trials with *P. sanguineus*

Two protective trials were conducted to evaluate the effect of *P. sanguineus* extracts against *B. cinerea* infections in strawberries (*Fragaria* × *ananassa* Duch.) variety 'Camino Real' and *F. oxysporum* in tomatoes (*Solanum lycopersicum* L.) variety 'Río Grande'. In both cases, aqueous extracts of fruiting bodies obtained from the fruiting of the MA-Ps1 strain were applied, pulverized, and diluted to different concentrations, additionally the estimated concentration of cinnabarin derived from (Pérez-López *et al*., 2024) is reported, followed by inoculation with the corresponding pathogen (Table 1).

Table 1. Trial treatments for the application of aqueous extracts of *P. sanguineus* as a protective treatment against *B. cinerea* and *F. oxysporum* on strawberry and tomato plants, respectively.

Treatment	Protective trial against *B. cinerea* in strawberry leaves (g L^{-1})	Protective trial against *F. oxysporum* in tomato plants (g L^{-1})	Estimated amount of cinnabarin (g L^{-1})
Extract 25%	25	25	1
Extract 50%	50	50	2
Extract 75%	75	-	3
Extract 100%	-	100	4
Positive control	Untreated infected plant	Untreated infected plant	-
Negative control	Plant without infection or treatment	Plant without infection or treatment	-

In both trials, the cultivation characteristics were the same: greenhouse conditions with 8 h of light, humidity of 60%, temperature of 25-30 °C and sterilized substrate made up of 70% forest soil, 20% black soil, 5% sand and 5% perlite. Irrigation was carried out every third day. For the protective test against *B. cinerea* in strawberry leaves, a total of 225 80-day-old strawberry plants were distributed in five treatments with three replications (25 plants per replication). An *in vitro* severity index was established using sterile leaves cut and disinfected with 1% sodium hypochlorite for 5 min, then placed in Petri dishes.

These leaves were treated with a single spray with a solution of 5 ml of *P. sanguineus* at different concentrations (25% extract, 50% extract and 75% extract); then, 5 ml of *B. cinerea* suspensions was applied at a concentration of 8×10^3 conidia ml^{-1}; a positive control (untreated *B. cinerea*) and a negative control (sterile distilled water) were also considered. The progression of foliar damage was recorded daily until 100% severity was reached, with maximum severity defined as the presence of chlorotic and wilted leaves. The damage was analyzed with the Can-Eye V6.49 software, and the results were transformed into percentages; to determine the severity index, the Barremo index was used, given by the formula: $SI_{Barremo} = \sum(n_i \times v_i) / N$. Where: n_i= number of leaves in class i; v_i= severity value of class i according to Barremo (0, 1, 3 and 5); and N= total number of leaves evaluated. This index enabled the quantification of damage levels based on discrete severity categories (Table 2).

Table 2. Severity table according to the Barremo index.

Severity index	Damage description	Value (v_i)
0	Undamaged leaves (healthy green)	0
1	Minor damage (<5%, localized spots)	1
2	Moderate damage (5-25%, visible chlorotic areas)	3
3	Severe damage (>25%, wilted and necrotic leaf)	5

The trial concluded when the leaves reached 100% severity, which made it possible to compare the efficacy of the extracts against the control group with sterilized water. In the case of the tomato study, 300 120-day-old plants were used, distributed across five treatments with six replications each. Each plant was given single doses of 20 ml of *P. sanguineus* extract at different concentrations directly into the substrate, while the controls received the same volume of distilled water without *P. sanguineus*. One week after applying the extracts, the plants were inoculated with 20 ml of a suspension of *F. oxysporum* (8 × 10^3 conidia ml^{-1}).

These suspensions were obtained from cultures in PDA medium grown for one week and were adjusted by serial dilution until the desired concentration was reached, using spore counts in a Neubauer hematocytometer (Neubauer-imp®, Marienfeld, Germany). Negative controls received only distilled water without pathogens. Three days after inoculation, the plants were extracted and separated into aboveground and root parts. Both fractions were dehydrated in an oven at 40 °C until they reached a constant weight. Subsequently, dry aboveground biomass (g) and dry root biomass (g) were recorded. The expression of characteristic symptoms in plants and microscopic observation of mycelium and conidia confirmed the presence of the pathogen in both trials.

Statistical analysis

In both trials, a single-factor analysis of variance (Anova) ($\alpha = 0.05$) was performed to evaluate differences in leaf damage in strawberries and in biomass in tomatoes. Tukey's tests were applied to compare the effectiveness of the treatments. The analyses were carried out using R v4.5.0.

Results and discussion

In the *B. cinerea* infection trial on strawberry leaves, there were statistically significant differences ($p \leq 0.05$) in the severity of infection between treatments with different concentrations of the extract and the controls (Table 3).

Table 3. Analysis of variance (Anova) for the percentage of severity caused by *B. cinerea* in strawberry plants under protective treatment with *P. sanguineus* at different concentration levels.

Treatment	Average severity (%)	Severity class	*p* # 0.05
Positive control	87.1 ±5.2	3	a
Extract 25%	62.5 ±4.6	3	a
Extract 50%	36.2 ±3.8	3	b
Extract 75%	22.4 ±2.9	2	b
Negative control	0 ±0	0	c

HSD (Tukey, $p \leq 0.05$)= 14.72; CV (%)= 9.8. Average values with the same letter do not represent statistically significant differences ($p \leq 0.05$) according to Tukey's tests.

Treatments with extracts of 50 and 75 g L^{-1} significantly reduced the severity of damage (36.2 and 22.4%, respectively) compared to the positive control (87.1%), highlighting a clear dose-dependent effect (Figure 1), where higher concentrations reduce leaf damage.

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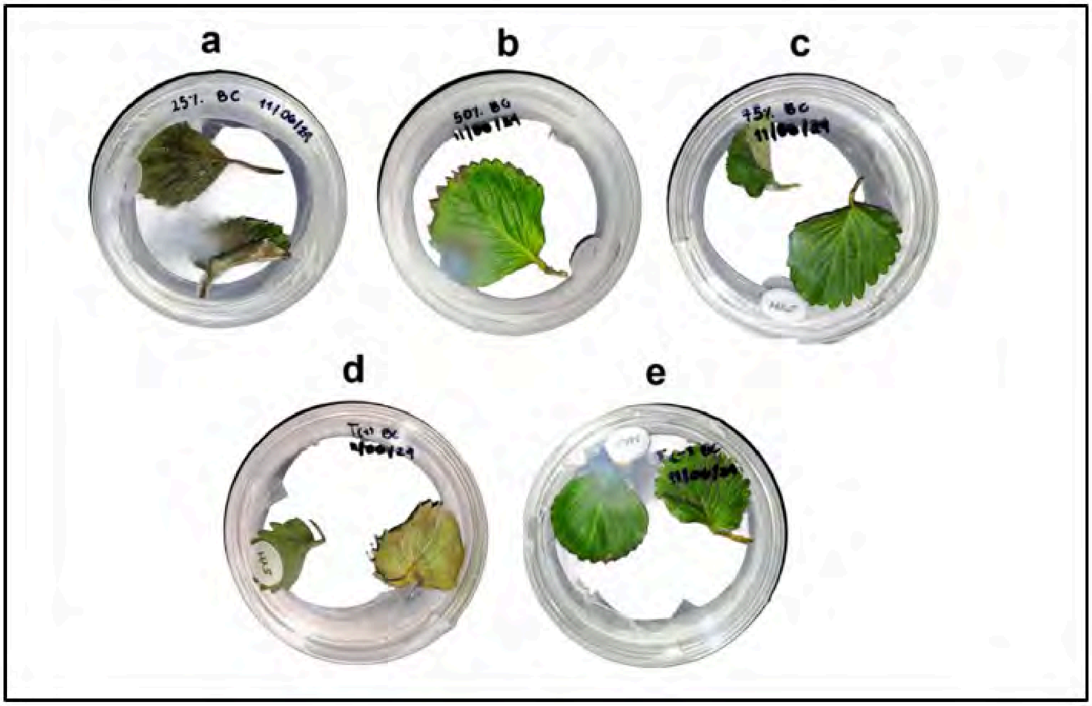
Figure 1. Trial of application of *P. sanguineus* extracts at different concentrations as a preventive treatment against foliar damage in strawberries caused by *B. cinerea*. Treatments: a) 25% extract; b) 50% extract; c) 75% extract; d) positive control; and e) negative control.



Similarly, in the trial of tomato infected with *F. oxysporum*, the Anova showed significant differences in aboveground biomass between treatments (p # 0.05), but not in root biomass (Table 4).

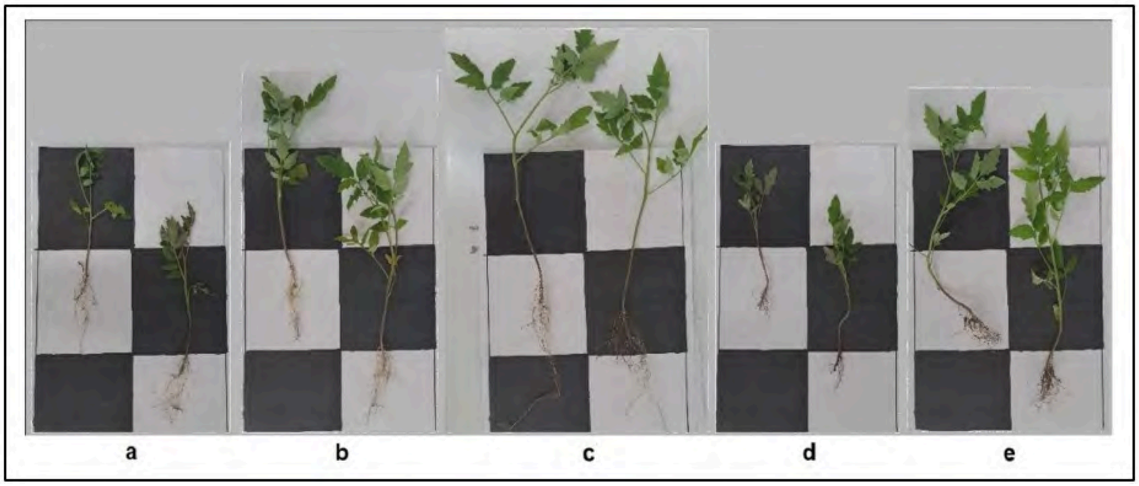
Table 4. Analysis of variance (Anova) for aboveground and root biomass in relation to damage caused by *F. oxysporum* under protective treatment with *Pycnoporus sanguineus* at different concentration levels.

Treatment	Average aboveground biomass (g)	*p* # 0.05	Average root biomass (g)	*p* # 0.05
Positive control	0.052 ±0.011	c	0.022 ±0.005	a
Extract 25%	0.117 ±0.022	a	0.049 ±0.01	a
Extract 50%	0.1 ±0.018	b	0.039 ±0.008	a
Extract 100%	0.154 ±0.025	b	0.069 ±0.012	a
Negative control	0.08 ±0.017	b	0.037 ±0.007	a

HSD (Tukey, $p \leq 0.05$): aboveground= 0.028; root= 0.019; CV (%): aboveground= 11.2; root= 14.6. Average values with the same letter do not represent statistically significant differences ($p \leq 0.05$) according to Tukey's tests.

The 50% and 100% extracts expressed values of aboveground biomass statistically comparable to those of the negative control, far outperforming the positive control, suggesting a subtly observable protective effect of the extract (Figure 2). However, the lack of differences in root biomass indicates a limited influence of the treatment on the belowground system.

Figure 2. Trial of application of *P. sanguineus* extracts at different concentrations as a preventive treatment against tomato wilt caused by *F. oxysporum*. Treatments: a) 25% extract; b) 50% extract; c) 75% extract; d) positive control; and e) negative control.



These findings confirm the preventive antifungal action of *P. sanguineus* and agree with previous studies that attribute this activity to secondary metabolites, such as cinnabarin and phenolic compounds (Elisashvili *et al*., 2009; Patel *et al*., 2009). In strawberries, treatment with the 75 g L^{-1} extract reduced leaf damage by 74.3%, which coincides with reports of the potent inhibitory activity of cinnabarin and cinnabarinic acid against bacteria and fungi (Smânia *et al*., 1995; Pineda-Insuasti *et al*., 2017).

In tomatoes, the recovery of aboveground biomass in the 50 and 100% treatments supports the hypothesis of a dose-dependent, albeit limited, protective effect in roots. The observed antifungal capacity is close to values reported in PDA media supplemented with complete extracts of *P. sanguineus*, where *B. cinerea* was more sensitive than *F. oxysporum* (Pérez-López *et al*., 2024). This suggests that crude extracts, containing diverse metabolites such as ergosterol (Téllez-Téllez *et al*., 2016), polyporins (Rosa *et al*., 2003), phenolic compounds and terpenoids (Teoh *et al*., 2011), as well as clavaric acid and laccases (Lin *et al*., 2020), act synergistically, surpassing the efficacy of isolated compounds.

Nonetheless, the absence of effect on root biomass may be due to the limited mobility of the compounds in belowground tissues, a phenomenon previously described for contact fungicides (Agrios, 2005; Brent and Hollomon, 1995). In addition, the soil microbiota can accelerate the degradation of compounds, reducing their effectiveness in the rhizosphere (Cyco# *et al*., 2017). In addition, a better selection of methodological procedures, such as pathogen infection by direct inoculation, or more efficient recording of severity through the calculation of disease progression (Gayosso-Barragán *et al*., 2021), could also influence the evaluation of the observed results.

It should be noted that, in addition to the mechanisms of direct inhibition of membranes and hyphal structures, recent studies suggest that phenolic compounds of *P. sanguineus* could induce systemic resistance in plants (Lim *et al*., 2024). This mechanism would explain the similarity observed in some treatments with negative control. Equivalent results have been described for phenol-rich plant extracts, such as *Punica granatum* and *Salvia* spp., which have been able to inhibit *F. oxysporum* in correlation with their phenolic content (Rongai *et al*., 2015; Kursa *et al*., 2022). Therefore, the efficacy of the extract could derive not only from a direct antifungal action, but also from the activation of immune responses in plants.

Consequently, *P. sanguineus* extract shows potential as a biocontrol agent within integrated management strategies, although with certain limitations, including biophysical limitations faced by both management and application of this type of formulation outside the laboratory. For example, low mobility to roots suggests the need to optimize formulations (encapsulation or emulsifiers) to improve compound distribution and persistence. Likewise, variability in environmental factors, such as humidity and wind, or exposure to light (Lim *et al*., 2024), could affect the performance of these metabolites in the field.

Conclusions

The aqueous extract of fruiting bodies of *P. sanguineus* showed potential as a protective agent against the effects of *B. cinerea* in strawberries and *F. oxysporum* in tomatoes. Nevertheless, the recovery of aboveground biomass in infected tomato plants was only observable under the highest concentrations; likewise, the significant reduction of leaf damage in strawberries was observable only in the extracts of higher concentration.

These effects are associated with the presence of metabolites such as cinnabarin and phenolic compounds, which may be responsible for the antifungal action and a possible induction of systemic defenses in plants. However, the limited response in root biomass suggests that techniques and formulations should be improved for greater effectiveness. It is recommended to evaluate their application in field conditions and to delve deeper into the characterization and mode of action of their active compounds.

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