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Original article

In vitro antagonistic effect of *Trichoderma asperellum* against *Moniliophthora perniciosa*

Efecto antagónico *in vitro* de *Trichoderma asperellum* frente a *Moniliophthora perniciosa*

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ABSTRACT: The study of phytopathogenic fungi in cacao plantations is essential for crop protection and the development of effective control strategies. The objective of this study was to evaluate the *in vitro* antagonistic effect of *Trichoderma asperellum* against *Moniliophthora perniciosa*, which causes the disease "witches' broom." Both species (*M. perniciosa* from diseased cacao (*Theobroma cacao* L.) branches and *T. asperellum* from the rhizosphere) were isolated and molecularly identified using ITS barcoding and beta tubulin techniques. Exponential growth mathematical modeling was applied to model the growth of *M. perniciosa* in the presence of the antagonistic fungus *T. asperellum*. Logistic differential calculation based on the Lotka-Volterra model for competition was applied. The trial included 5 replicates with their respective controls. Molecular analysis confirmed the presence of *M. perniciosa* at 98 % and *T. asperellum* at 100 %. *In vitro* assays demonstrated that *T. asperellum* effectively inhibits *M. perniciosa* through competition. The Lotka-Volterra model predicted *T. asperellum* dominance. In conclusion, the molecular, *in vitro,* and modeling results provide a solid basis for considering *T. asperellum* as a promising biocontrol agent for cacao witches' broom disease under controlled conditions.

Key words: Cocoa, witch's broom, phytopathogen, fungus, molecular.

RESUMEN: El estudio de hongos fitopatógenos en plantaciones de cacao es fundamental para proteger los cultivos y desarrollar estrategias de control efectivas. El estudio tuvo como objetivo evaluar el efecto antagónico *in vitro* de *Trichoderma asperellum* frente a *Moniliophthora perniciosa* causante de la enfermedad “escoba de bruja”. Se aislaron e identificaron molecularmente ambas especies (*M. perniciosa* de ramas de cacao (*Theobroma cacao* L.) enfermas y *T. asperellum* de la rizosfera) utilizando las técnicas de Barcoding ITS y Beta tubulina. Se aplicó el modelamiento matemático de crecimiento exponencial, para modelar el crecimiento de *M. perniciosa* en presencia del hongo antagonista *T. asperellum*, se aplicó el cálculo diferencial logístico basado en el modelo de Lotka-Volterra para competencia. El ensayo contó con 5 réplicas con sus respectivos controles. El análisis molecular confirmó la presencia de *M. perniciosa* 98 % y *T. asperellum* 100 % de identidad. Los ensayos *in vitro* demostraron que *T. asperellum* inhibe eficazmente a *M. perniciosa* mediante competencia. El modelo Lotka-Volterra predijo el dominio de *T. asperellum*. En conclusión, los resultados moleculares, *in vitro* y de modelado, proporcionan una base sólida para considerar a *T. asperellum* como un prometedor agente de biocontrol para la enfermedad "escoba de bruja" del cacao en condiciones controladas.

Palabras clave: Cacao, escoba de bruja, fitopatógeno, hongo, molecular.

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INTRODUCTION

Modern agriculture faces constant challenges due to fungal diseases affecting crops, causing significant economic losses and threatening global food security (1). Among these diseases, witches' broom caused by the fungus *Moniliophthora perniciosa* (formerly *Crinipellis perniciosa*) represents one of the main threats to cacao (*Theobroma cacao* L.) cultivation in various producing regions worldwide, including Ecuador (2). This pathogen is responsible for the deformation of shoots, floral cushions, and fruits, severely impacting cacao yield and quality (3).

The management of fungal diseases in traditional agriculture has largely depended on the use of synthetic fungicides (4). Although these substances provide effective short-term control, their indiscriminate application raises concerns about negative effects on the environment, human health, and the emergence of resistant pathogen strains (5). This issue has driven the search for more sustainable and ecological alternatives for disease control, with biological control emerging as one of the most promising strategies (6).

In this context, the use of beneficial microorganisms as biological control agents has gained great relevance (7). The genus Trichoderma stands out as one of the most studied and applied groups of fungi in agriculture due to its versatility and multiple antagonistic mechanisms of action against phytopathogens (8). Several Trichoderma species are recognized for their ability to promote plant growth, enhance nutrient uptake, and, most importantly, suppress disease development (9).

Within the genus Trichoderma, the species *Trichoderma asperellum* has proven particularly effective in controlling a wide range of fungal pathogens (10). Its antagonistic mechanisms include mycoparasitism, where the beneficial fungus directly attacks the pathogen (11); competition for nutrients and space, where it outcompetes the pathogen in substrate colonization (12); production of antifungal compounds such as secondary metabolites and lytic enzymes (13); and induction of systemic resistance in the host plant (14).

Cacao witches' broom, caused by *Moniliophthora perniciosa*, is a highly complex disease due to the genetic variability of the pathogen and its particular life cycle, which involves different stages in the cacao plant (15). The fungus infects meristematic tissues, causing abnormal proliferation of shoots and branches that eventually dry and die, forming the characteristic "brooms" (16). When fruits are affected, they become mummified and lose their commercial value (17).

Despite research efforts, effective and sustainable control of *M. perniciosa* remains a challenge (18). Current strategies often combine cultural practices, such as sanitary pruning, with fungicide application (19). However, these measures are not always sufficient to mitigate the impact of the disease, particularly in areas with high inoculum pressure (20). This underscores the urgent need to develop new management tools.

The aim of this study was to investigate the in vitro antagonistic effect of *Trichoderma asperellum* against *Moniliophthora perniciosa*. The results of this research will contribute to knowledge on biological interactions, providing a scientific basis for the development of more efficient and environmentally friendly biological control strategies, benefiting both producers and the cacao agroecosystem.

MATERIALS AND METHODS

Sample Collection and Fungal Isolation Sampling for the isolation of fungal agents was conducted in September 2024 at the "Hermanos Quito" farm, located in the Lorenzo Garaicoa parish of Simón Bolívar canton, Guayas province, Ecuador. Sample processing was carried out at the Microbiology Laboratory of the Agrarian University of Ecuador.

For the isolation of *T. asperellum*, the methodology proposed by Vera (7) was applied, using rice traps and subsequent inoculation on PDA medium, based on the colorimetric characteristics typical of the genus Trichoderma. For *M. perniciosa*, a cacao branch showing disease symptoms was collected, washed with neutral soap and distilled water, cut transversely into five fragments of ≈3 cm, and immersed in 1 % sodium hypochlorite for 2 minutes followed by sterile water rinses. Finally, under a biosafety cabinet, fragments were immersed in 70 % ethanol for 20 seconds, longitudinally sectioned to remove the bark, and ≈5 mm fractions of vascular cambium were placed centrally on PDA plates and incubated at 28 °C for 14 days before purification.

Molecular identification was performed through standardized procedures. DNA was extracted from 100 mg of each fungal sample. The integrity and purity of the extracted DNA were verified using microvolume spectrophotometry and 1 % agarose gel electrophoresis. DNA concentration was adjusted to 20 ng/µL for amplification by PCR (Polymerase Chain Reaction) using primers ITS1/ITS4 (21) and BtuB: Bt2a/Bt2b (22). PCR products were purified prior to sequencing by the Sanger method. Sequences obtained were bioinformatically processed and compared against the GenBank (NCBI) nucleotide database for taxonomic identification.

To determine the growth dynamics of *M. perniciosa*, a comparative experiment was designed. The control group (*M. perniciosa*) represented the absence of competition, while the treatment group incorporated the presence of microorganisms with recognized antagonistic activity (*M. perniciosa* & *T. asperellum*). The study was conducted in 90 mm Petri dishes using PDA medium as substrate for simultaneous sterile inoculation of the pathogen and its antagonist. The progress of *M. perniciosa* mycelium was recorded daily in millimeters (mm) over 7 days. Growth data were used to calculate specific growth rates for each organism and to assess the influence of antagonistic agents on pathogen growth reduction.

The methodology applied for data processing (Table 1) consisted of predictive mathematical modeling using the Lotka-Volterra system of differential equations for interspecific competition. Key parameters of the model were defined for both species (*M. perniciosa* and *T. asperellum*), including initial population size, intrinsic growth rate, environmental carrying capacity, and coefficients quantifying mutual competitive interactions between the two fungi (Table 2).

Table 1. Experimental population data

Days	*M. perniciosa* & *T. asperellum* (mm)	*M. perniciosa* Control (mm)
1	10	15
2	14	20
3	17	26
4	20	32
5	24	34
6	26	36
7	28	40

Based on the experimental data, the parameters for the intrinsic growth rate (r), carrying capacity (K), and competition coefficient (α) were estimated using statistical and numerical methods, seeking the set of parameters that best explains the observed population dynamics.

These values were applied in the Lotka-Volterra model equations to perform the growth simulations. The general Lotka-Volterra competition model used to generate the graph data consisted of two differential equations:

For Species 1 (*M. perniciosa*, denoted as N1):

$$\frac{dN_1}{dt} = r_1 N_1\left(1 - \frac{N_1 + \alpha_{12} N_2}{K_1}\right)$$

For Species 2 (*T. asperellum*, denoted as N2):

$$\frac{dN_2}{dt} = r_2 N_2\left(1 - \frac{N_2 + \alpha_{21} N_1}{K_2}\right)$$

Where:

N_1: Population of *M. perniciosa* at time t.

N_2: Population of *T. asperellum* at time t.

$\frac{dN_1}{dt}$: Rate of change of the *M. perniciosa* population with respect to time.

$\frac{dN_2}{dt}$: Rate of change of the *T. asperellum* population with respect to time.

r_1: Intrinsic growth rate of *M. perniciosa* (0.5).

r_2: Intrinsic growth rate of *T. asperellum* (0.8).

K_1: Carrying capacity of *M. perniciosa* (10000).

K_2: Carrying capacity of *T. asperellum* (15000).

α_{12}: Competition coefficient representing the effect of *T. asperellum* (N2) on *M. perniciosa* (N_1) (0.7).

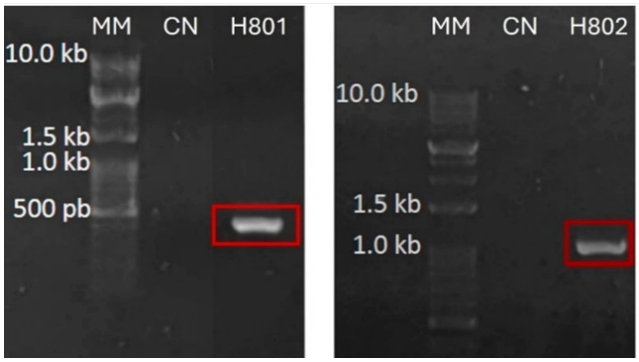
α_{21}: Competition coefficient representing the effect of *M. perniciosa* (N1) on *T. asperellum* (N2) (0.2).

With these parameters, numerical integration of the differential equations describing the population dynamics of antagonism was performed during the evaluation period, allowing the evolution of the populations of *M. perniciosa* and *T. asperellum* over time to be obtained. Finally, the simulation results were visually represented through a graph illustrating the growth curves of both species under the competitive conditions defined by the model

RESULTS

Molecular identification of fungal species

Figure 1 shows the electrophoresis readout results, revealing amplicons of approximately 400 bp and 920 bp, respectively.



MM = Molecular weight marker, CN = Negative control, kb = Kilobase, pb = Base pairs

Figure 1. 1 % agarose gel with PCR products for fragments H801 (BtuB) and H802 (ITS)

From the reads obtained through SANGER sequencing, assembled sequences were generated that allowed the identification of the species, as detailed in Table 3.

Antagonistic competition between fungal species

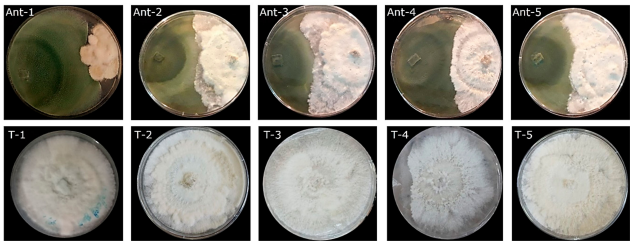
Figure 2 clearly demonstrates the antagonistic activity of *T. asperellum* against *M. perniciosa in vitro*. *T. asperellum* effectively inhibits the growth of *M. perniciosa*,

Table 2. Input parameters of the Lotka-Volterra Model for *M. perniciosa* and *T. asperellum*

Parameter	*M. perniciosa*	*T. asperellum*	Unit	Description
(N°)	100	50	CFU/mL	Initial population of each fungus.
(r)	0.5	0.8	1/day	Maximum growth rate of each fungus in the absence of competition.
(K)	10000	15000	CFU/mL	Maximum population supported by the environment for each fungus separately.
(αij)	0.7 (αMP,TA)	0.2 (αTA,MP)	Non-dimensional	Competitive effect of one species on the other (αMP,TA refers to the effect of *T. asperellum* on *M. perniciosa*; αTA,MP refers to the effect of *M. perniciosa* on *T. asperellum*).

Table 3. Molecular Identification of Fungal Isolates

Code	Organism	Fragment	Identity	Accesion
H801	*Trichoderma asperellum*	BtuB	100 %	PP596864.1
H802	*Moniliophthora perniciosa*	ITS	98 %	KX913250



Ant-1: antagonism 1 (growth of *T. asperellum* and *M. perniciosa*); Ant-2: antagonism 2 (growth of *T. asperellum* and *M. perniciosa*); Ant-3: antagonism 3 (growth of *T. asperellum* and *M. perniciosa*); Ant-4: antagonism 4 (growth of *T. asperellum* and *M. perniciosa*); Ant-5: antagonism 5 (growth of *T. asperellum* and *M. perniciosa*). T-1: Control 1 (growth of *M. perniciosa*); T-2: Control 2 (growth of *M. perniciosa*); T-3: Control 3 (growth of *M. perniciosa*); T-4: Control 4 (growth of *M. perniciosa*); T-5: Control 5 (growth of *M. perniciosa*)

Figure 2. *In vitro* antagonism assay between *T. asperellum* and *M. perniciosa* on Petri dishes with PDA medium after 7 days

probably through mechanisms such as competition for space and nutrients, mycoparasitism, or the production of antifungal compounds by the *Trichoderma* strain parasitizing the pathogenic fungus.

This suggests that *T. asperellum* has potential as a biocontrol agent against *M. perniciosa*, the causal agent of the disease known as “escoba de Bruja" (witches’ broom) in cacao.

Population dynamics under the Lotka-Volterra Model

Figure 3 presents a simulation of the competitive interaction between the pathogenic fungus *M. perniciosa* (represented by the blue line) and the antagonistic fungus *T. asperellum* (orange line) over a seven-day period under *in vitro* conditions.

The Figure 3 illustrates how the populations of both species, starting from initial densities of 100 CFU/mL for *M. perniciosa* and 50 CFU/mL for *T. asperellum*, evolve under the parameters defined by the Lotka-Volterra competition model. The most notable observation is the initial exponential growth of both populations, followed by a progressive divergence beginning around the third day. *T. asperellum* exhibits a markedly steeper growth curve and reaches a much higher final population (≈ 6400 CFU/mL) compared to *M. perniciosa* (≈ 1700 CFU/mL) at the end of the 7-day period.

DISCUSSION

This result suggests a high specificity and quality of the sequence obtained. *T. asperellum* is a fungus widely recognized for its potential as a biocontrol agent against various plant diseases (23), whereas *M. perniciosa* is the causal agent of the disease known as “Witches’ Broom” in cacao, one of the most devastating diseases affecting this crop (15).

These *in vitro* findings are promising for the development of sustainable biological control strategies against *M. perniciosa*, a devastating disease that causes significant losses in cacao production worldwide (24). Nevertheless, it is crucial that these results be validated under field conditions to confirm the efficacy of *T. asperellum* in a more complex and variable environment, where environmental factors such as temperature, humidity, and interactions with other microorganisms may influence the dynamics of antagonism (11).

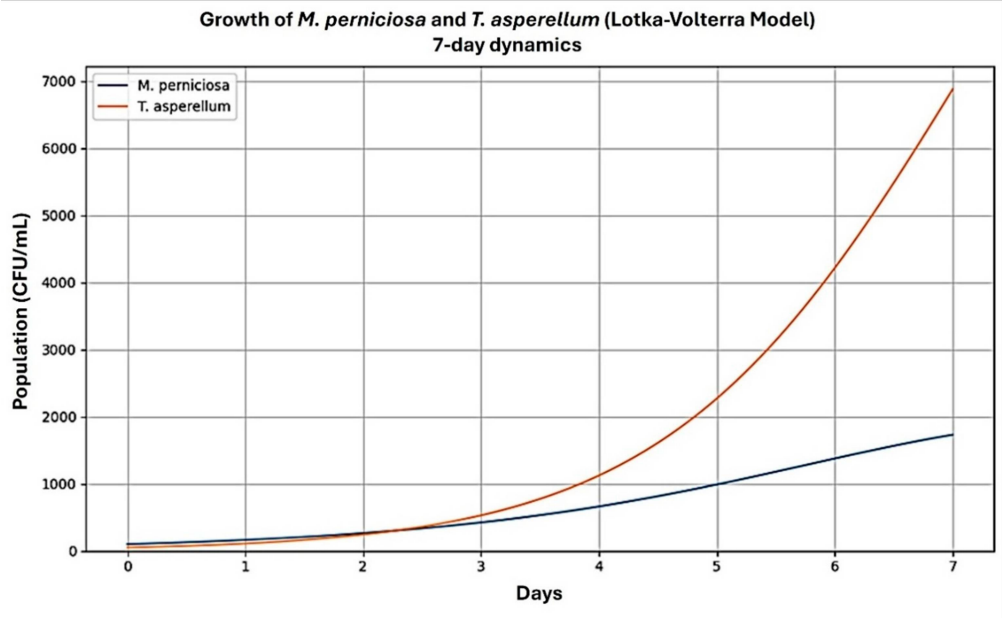


Figure 3. Population dynamics of *M. perniciosa* and *T. asperellum* during the first 7 days of competition, according to the Lotka-Volterra model

This dynamic is a direct reflection of the intrinsic growth (r) and competition (α) parameters assigned in the model. The simulation demonstrates the potential of the Lotka-Volterra model to predict and understand ecological interactions between species, a well-established concept in population ecology (25).

The *in vitro* antagonism assays unequivocally demonstrated that *T. asperellum* exerts a strong inhibitory activity on the growth of *M. perniciosa*. The simulation of population dynamics using the Lotka-Volterra model corroborates the strong antagonistic potential of *T. asperellum*. The model predicts that, even starting from a lower initial density, *T. asperellum* significantly surpasses the population of *M. perniciosa* within a short period (7 days), supporting its capacity to displace and control the pathogen in a competitive system.

CONCLUSION

The study confirmed the molecular identity of the fungal isolates used: *Trichoderma asperellum* (H801) and *Moniliophthora perniciosa* (H802), through amplification and sequencing of the BtuB and ITS genes, respectively, obtaining a high percentage of identity with reference sequences in databases. Taken together, these molecular, *in vitro*, and modeling results provide a solid basis for considering *T. asperellum* as a promising biocontrol agent for cacao Witches' Broom disease, although validation under field conditions is essential to confirm its effectiveness in real environments.

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