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Original article

Beneficial microorganisms antagonistic to *Curvularia petersonii,* which causes foliar lesions in sugar cane

Microorganismos benéficos antagónicos de *Curvularia petersonii* causante de lesiones foliares en caña de azúcar

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ABSTRACT: Foliar necrosis in sugarcane caused by phytopathogenic fungi is a serious problem; as a biocontrol alternative, beneficial microorganisms could offer a sustainable solution. The objective of this study was to evaluate the antagonistic effect of beneficial microorganisms against *Curvularia petersonii*, a phytopathogenic fungus responsible for foliar necrosis in sugar cane cultivation. A sequenced strain with 99.60% identity for *C. petersonii* (Accession No. NR_158448.1) to evaluate the percentage of inhibition (*Trichoderma harzianum*, *Trichoderma asperellum*, *Bacillus subtilis*, and *Pseudomonas fluorescens*) with 5 replicates and their respective controls over 16 days of evaluation. The mathematical modeling technique of exponential growth was used to model the development of *C. petersonii* under *in vitro* conditions and in the presence of antagonistic microorganisms using the Lotka-Volterra for dual crop confrontation. The results support that the beneficial microorganisms used had an antagonistic inhibitory effect on the phytopathogenic fungus *C. petersonii*, concluding that these microorganisms have advantages as biocontrol agents against *C. petersonii*.

Key words: Antagonism, phytopathogenic fungi, foliar necrosis.

RESUMEN: La necrosis foliar en caña de azúcar provocada por hongos fitopatógenos es un problema grave, como alternativa de biocontrol existen los microorganismos benéficos que podrían ofrecer una solución sostenible. Este estudio evalúa el potencial de microorganismos benéficos como biocontroladores efectivos. El objetivo del estudio consistió en evaluar el efecto antagónico de microorganismos benéficos frente a *Curvularia petersonii* hongo fitopatógeno responsable de necrosis foliares en cultivo de caña azucarera. Se replicó una cepa secuenciada con el 99,60 % de identidad para *C. petersonii* (Nº Accesión NR_158448.1) para evaluar el porcentaje de inhibicion (*Trichoderma harzianum*, *Trichoderma asperellum*, *Bacillus subtilis* y *Pseudomona fluorescens*) con 5 réplicas y sus respectivos controles durante 16 días de evaluación utilizado la técnica del modelamiento matemático del crecimiento exponencial, con la finalidad de modelar el desarrollo de *C. petersonii* en condiciones *in vitro* y en presencia de microorganismos antagonistas bajo la ecuación diferencial logística del modelo Lotka-Volterra para enfrentamiento por cultivo dual. Los resultados sostienen que los microorganismos benéficos utilizados tuvieron un efecto antagónico inhibitorio sobre el hongo fitopatógeno *C. petersonii,* concluyendo que estos microorganismos poseen bondades como biocontroladores frente a *C. petersonii*.

Palabras clave: Antagonismo, hongos fitopatógenos, necrosis foliar.

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INTRODUCTION

Sugarcane cultivation is considered of vital importance worldwide, as it contributes to the economy of different countries, including the development of agricultural production in Ecuador through the generation of employment and income for the agricultural sector (1). Sugarcane is exposed to multiple factors that affect its production, among them the presence of numerous pathogens that cause diseases and also impact the quality of the final product (2).

In sugarcane cultivation, different disorders have been detected during its phenological cycle, particularly in the vegetative stage. Among the main diseases, these can be caused by diverse agents such as fungi, bacteria, viruses, nematodes, among others, which negatively affect crop yield (3). Fungal diseases have been identified with causal agents belonging to the genera *Aspergillus*, *Fusarium*, and *Curvularia* (4). The genus *Curvularia* was identified by *C. lunata*, which belongs to the family Pleosporaceae of the order Pleosporales (5).

This fungal species can affect different types of grasses and crops of interest such as maize, rice, wheat, or sorghum, causing significant production losses (6). *Curvularia petersonii* is a fungus capable of generating foliar necrosis in different types of grasses, highlighting the importance of developing effective control strategies (7). The emergence of these phytopathogenic species represents a threat to sugarcane producers, leading to the need for research aimed at effective and environmentally friendly control and prevention of these diseases (8).

For the control of fungal diseases, chemical fungicides such as thiophanate-methyl, carbendazim, mancozeb, copper, and sulfur have been used for years. However, their properties have toxic effects with considerable adverse impacts on the ecosystem (9). Their persistence and accumulation in the environment can affect the food chain, posing a high risk to human health and wildlife (10). Therefore, it is essential to seek new alternatives for the control of phytopathogens (11). As a biological control strategy, the use of antagonistic microorganisms against phytopathogenic fungi is a common technique, where various species such as *Trichoderma*, *Pseudomonas*, and *Bacillus* may represent viable alternatives (12, 13).

These species of microorganisms have been widely studied in different crops (14), for disease control (15), as well as for mitigating environmental impact (16). These strains have the ability to promote plant growth and improve soil structural conditions (10). Within this context, the present study aimed to evaluate the antagonistic capacity of different beneficial microorganisms against the fungus *Curvularia petersonii* under controlled conditions.

MATERIALS AND METHODS

Location and Samples of the Assay

The study was conducted at the Biotechnology Laboratory of the State University of Milagro, Ecuador. Regarding the pathogenic fungus, a sample was taken for replication from a sequenced strain with 99.60 % identity to *Curvularia petersonii* (Accession No. NR_158448.1), isolated from necrotic foliar tissue of sugarcane variety ECU-08. To evaluate dual culture confrontation, pure strains of *Trichoderma harzianum, Trichoderma asperellum, Bacillus subtilis*, and *Pseudomonas fluorescens* were obtained from the Agricultural Microbiology Laboratory Microbiolab RUC 1713152047001 in Quito, Ecuador.

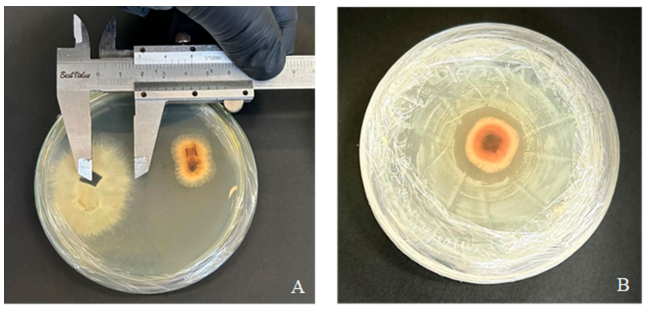
Trial management

The experiment was designed to evaluate antagonism through dual culture confrontation between four beneficial microorganisms (*T. asperellum, T. harzianum, P. fluorescens*, and *B. subtilis*) and one pathogenic strain (*C. petersonii*), with five replicates and their respective controls in PDA medium (Potato Dextrose Agar) at 39 g L, totaling 45 experimental units.

For the dual antagonistic interaction assay of *T. asperellum* and *T. harzianum* against *C. petersonii*, 5 mm portions of reserve strains were transplanted and placed at opposite ends of the plate, maintaining a distance of 20 mm from the edge. Subsequently, the plates were sealed with parafilm. Inoculation of *P. fluorescens* and *B. subtilis* was carried out by dissolving a fragment of each strain in peptone water inside a glass test tube. Then, using a micropipette adjusted to 10 µL, the suspension was dispensed onto the plate and spread over the agar surface with a Drigalski spatula. Finally, controls were inoculated at the central point of the plate. The strains were incubated at 32 °C.

Data Recording

Evaluations were performed daily over a period of 16 days, using a mechanical Vernier caliper for fungi (Figure 1A), while bacteria were evaluated based on their colony-forming units (CFU). In this section, suppression efficacy and surface propagation of microorganisms on the agar were determined (Figure 1B).



A (Evaluation of inhibition among fungal strains); B (Evaluation of inhibition among fungal and bacterial strains)

Figure 1. Evaluation of the inhibition of *C. petersonii* against beneficial microorganisms

Experimental design

To represent *C. petersonii* development under controlled environments and in the presence of its antagonists, the logistic differential equation was applied. This equation describes the dynamics of population change as a function of time under given conditions. The calculations for simulating the interactions were based on the Lotka-Volterra model for competitive interactions.

RESULTS

Effect of *C. petersonii* on *T. asperellum*

Figure 2 shows the growth curve of two fungi, *C. petersonii* (blue line) and *T. asperellum* (red line), growing together in a dual confrontation trial.

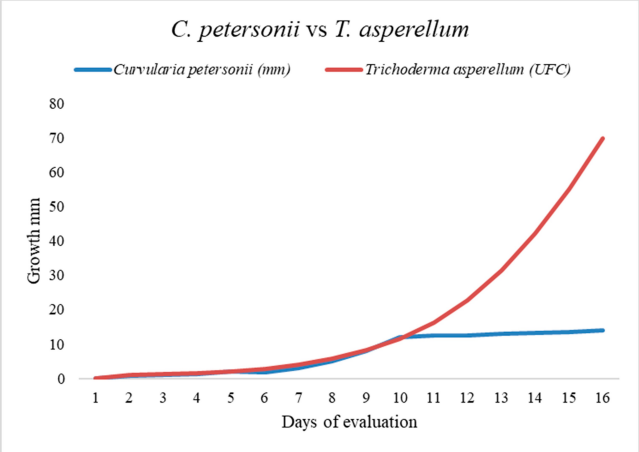


Figure 2. Average of plates showing antagonistic interaction between *C. petersonii* and *T. asperellum*

T. asperellum exhibits exponential and dominant growth, reaching nearly 70 mm at the end of the measurement. Its curve rises very sharply. In contrast, *C. petersonii* undergoes severe growth inhibition. Its curve flattens drastically and remains around 15 mm from day 10 onward, indicating that its growth has stopped or is negligible.

Effect of *C. petersonii* on *T. harzianum*

Figure 3 shows the growth curve of *C. petersonii* (blue line) in confrontation with *T. harzianum* (red line).

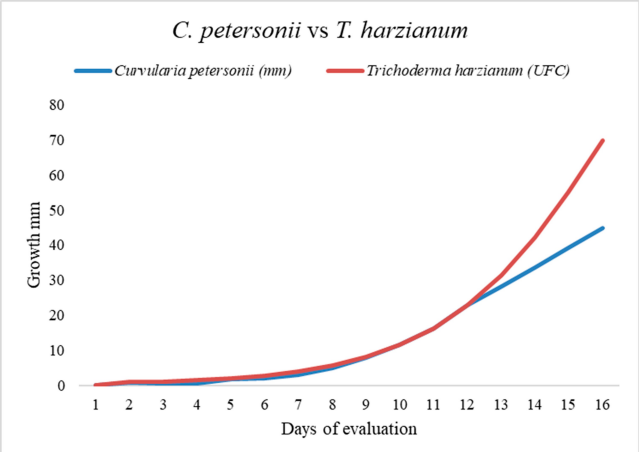


Figure 3. Average of plates showing antagonistic interaction between *C. petersonii* and *T. harzianum*

Both fungi show almost identical growth. The curves overlap or remain very close up to approximately 10 mm. This indicates that they initially grow at a similar rate or that the antagonistic interaction is not yet evident. From 10 mm onward, the curves begin to exhibit exponential growth, diverging at around 25 mm. *T. harzianum* takes the lead and shows faster and accelerated growth, reaching nearly 70 mm at the end of the measurement. *C. petersonii* also continues to grow, but at a significantly lower rate than Trichoderma. Its curve is more linear and less pronounced, reaching around 45 mm at 16 days of evaluation.

Effect of *C. petersonii* on *P. fluorescens*

Figure 4 represents the interaction between the fungus *C. petersonii* (blue line) and the bacterium *P. fluorescens* in a plate confrontation assay. The graph interprets the curve of relative growth/activity of the antagonist.

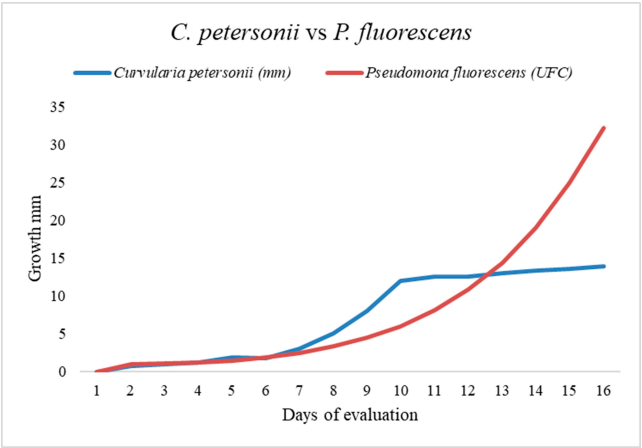


Figure 4. Average of plates showing antagonistic interaction between *C. petersonii* and *P. fluorescens*

Initially, both organisms show low and similar growth or activity, with the curves touching or crossing several times. *C. petersonii* exhibits faster and stronger initial growth than its antagonist, jumping from 2 to 13 mm, around day 10, the growth of *C. petersonii* stops abruptly and stabilizes at approximately 14 mm. This sudden stagnation provides strong evidence of an inhibitory action by the bacterium. *P. fluorescens* then enters an exponential and dominant growth phase, rising rapidly until the end of the measurement.

Effect of *C. petersonii* on *B. subtilis*

Figure 5 shows the interaction between the fungus *C. petersonii* (blue line) and the bacterium *B. subtilis* (red line).

At the beginning, both organisms display low activity, with their growth curves remaining closely aligned until day 6. From day 7, *B. subtilis* gains an advantage and demonstrates markedly accelerated exponential growth. In contrast, *C. petersonii* continues to grow at a slow and constrained pace, reaching only 10 mm by the final measurement on day 16.

DISCUSSION

The figure shows that *T. asperellum* dominates and significantly suppresses the growth of *C. petersonii* under the conditions of this experiment. Species of *Trichoderma* are well-known biological control agents due to their mycoparasitism, which involves the production of enzymes

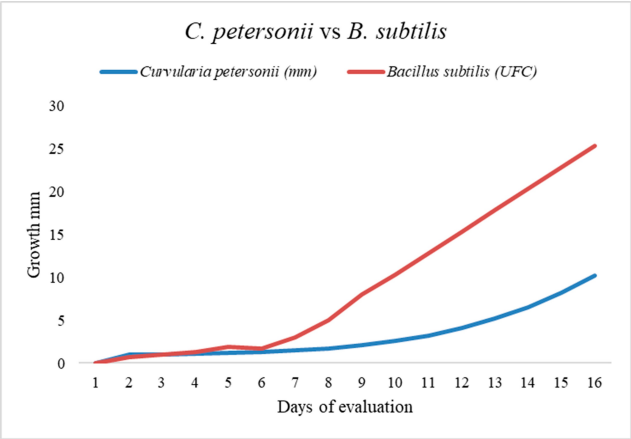


Figure 5. Average of the plates with antagonistic interaction of *C. petersonii* and *B. subtilis*

such as chitinases that degrade the fungal cell wall (17, 18). The rapid growth and substrate colonization confer a competitive advantage to *T. asperellum* over slow-growing pathogens.

Similarly, *T. harzianum* is an effective antagonist against *C. petersonii*, dominating and surpassing its growth. Variation in strain and species of *Trichoderma* may result in different levels of antagonism, ranging from pure competition to complete mycoparasitism (19). This dynamic suggests that competition for nutrients and space is the primary mechanism, possibly complemented by partial or weaker antagonism through secondary metabolites, as indicated by the researcher.

In addition, *P. fluorescens* proves to be a highly effective antagonist against *C. petersonii*. The inhibition pattern of the fungus is similar to that observed with *T. asperellum*, where the growth of *C. petersonii* began to plateau around 13 mm. *P. fluorescens* is a plant growth-promoting rhizobacterium that acts as a biocontrol agent through the production of antifungal secondary metabolites such as pyrrolnitrin and phenazines (20). The sudden cessation of *C. petersonii* growth provides strong evidence of antibacterial or antifungal metabolite action by the bacterium. Nutrient competition may also contribute, but the categorical inhibition points to antibiosis as the main mechanism.

Finally, *B. subtilis* is an effective antagonist against *C. petersonii* under the conditions of this experiment, severely limiting the growth of the phytopathogenic fungus. *B. subtilis* is an important biological control agent due to its ability to secrete cyclic lipopeptides (iturins and fengycins) that act directly on fungal membranes (21). The dominance of *B. subtilis* is likely mediated by antibiotic production.

CONCLUSIONS

All beneficial microorganisms used in the study exhibited an antagonistic effect against *C. petersonii*. The fungus *T. asperellum* showed the greatest inhibitory capacity, with a significant reduction in the pathogen's development rate compared to the control. *T. harzianum* also reduced the growth of *C. petersonii* relative to the control, though to a lesser extent.

The bacteria *P. fluorescens* and *B. subtilis* likewise demonstrated inhibitory activity against the pathogen. Comparative analysis of growth rates in the absence and presence of antagonists suggests that these microorganisms have potential to be employed as successful biological control agents against *C. petersonii*.

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