Inhibiting Effects of *Bacillus subtilis* and *Trichoderma harzianum* on Brown Root Rot Disease Caused by *Phellinus noxius*

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Background

The research focuses on the effectiveness of biocides against plant pathogens. Brown Root Rot, caused by the wood-rotting fungus *Phellinus noxius*, severely damages trees by clogging water and nutrient transport from roots to the trunk, leading to root death and instability. Infections cause wilting, dieback, discoloured or thinning leaves and brown rot with fungal growths on roots and trunk, eventually resulting in dieback and death. The pathogen spreads through airborne spores, infected root contact or pruning/mulch. The focus of the control method is biological agents. *T. harzianum* and *B. subtilis* are two selected agents that have the potential to inhibit the growth of *P. noxius* by releasing hormones and enzymes, triggering the induction of systemic resistance in plants.

Since 2012, the Brown Root Rot Disease (BRRD) outbreak in Hong Kong has resulted in tree removals, injuries, and public safety concerns. Efforts to manage BRRD have included guidelines, experimental treatments, and expert critiques. The establishment of the Urban Forestry Advisory Panel (UFAP) and improved inspection techniques reflect ongoing initiatives to combat BRRD. Despite challenges, Hong Kong continues to address BRRD through tree removals, replanting and enhanced disease detection methods.

Research Objectives

The study aims to examine the inhibiting effects of *Bacillus* species and *Trichoderma* species on BRRD caused by *Phellinus noxius*. It is assumed that there is a negative relationship between the growth rates of biological agents and the pathogen. Commercial biocontrol agents (BCAs) are believed to be stable and easy to activate. They are also designed to be accessible to less skilled workers and farmers.

Methodology

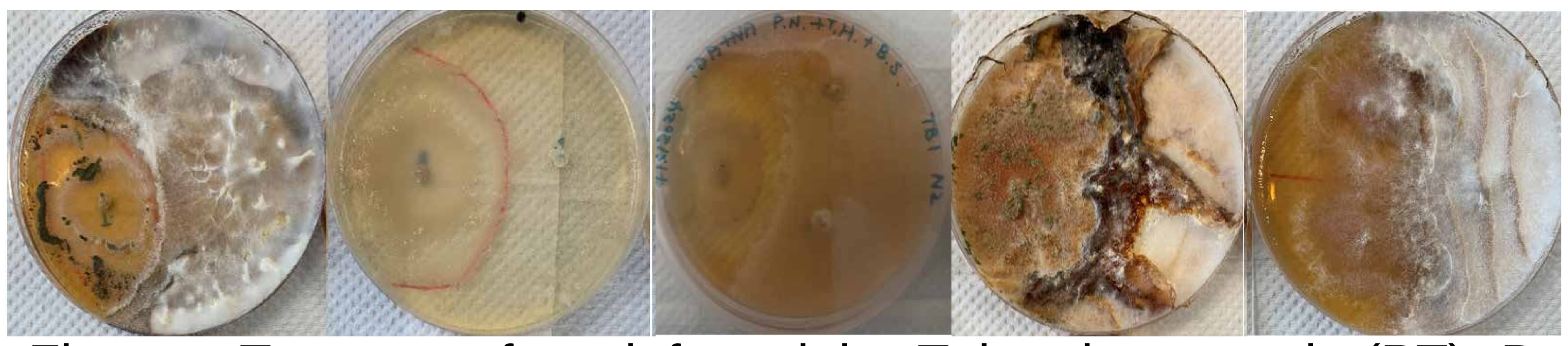
In Hong Kong's Victoria Park, the *M. elengi* (OVT LCSD WCH/38) tree, registered as an Old and Valuable Tree since September 2004, has been invaded by *P. noxius* since May 11, 2022. This unique species stands at 12.5 meters tall, with a crown spread of 14 meters and a trunk diameter of 480 millimetres at 1.3 meters above ground level (DBH). Symptoms of dieback twigs, small leaf size, abnormal leaf colour, and sparse foliage were observed, more prominent on the north and west sides of the crown. While the preliminary diagnosis suggested BRRD infection, further testing using root samples was required to analyse BRRD treatment.

Commercialized microbial biopesticides containing *T. harzianum* and *B. subtilis* were used in the study due to challenges in isolating these agents in Hong Kong. The agents were diluted, plated on agar, and incubated. Morphological characteristics confirming *T. harzianum* and *B. subtilis* presence were observed under a microscope.

After isolating and purifying the cultures, a dual culture experiment was carried out to assess inhibitory activity. The setup, referencing Rahman et al. (2009) and Nysanth et al. (2022), involved placing pathogenic fungal plugs and bioagent plugs at opposite ends of Petri plates, enabling them to interact and compete. In a dual culture experiment, *T. harzianum* and *B. subtilis* were tested against *P. noxius* to assess inhibitory activity. Growth inhibition was measured as the percentage inhibition of radial growth (PIRG). The experiment, conducted over five independent trials with six plates per treatment, followed a completely randomized design.

Findings and Discussion

Mycelium of *P. noxius* can be seen significantly in different treatments while the intensity and severity varies. Each treatment consists of one *P. noxius* plug.



Figures: Treatment from left to right: T. harzianum only (PT), B. subtilis only (PB), T. harzianum and B. subtilis (PTB), 2 T. harzianum (PTT) and 2 B. subtilis (PBB).

From the result of PIRG, the majority showing over 50% of inhibiting effects are PT and PB. Notably, PTT showed the lowest inhibiting percentage, followed by PTB. Half of the PBB samples showed an antagonistic effect. This may indicate the competitive competition of living areas and food in combined treatment compared to a single treatment. It is due to the fact that the control of the study is plug *P. noxius* isolates in the middle of the Petri dish which provides the greatest living area and growth radial length among the study. Post hoc tests support the claims.

This deviation from the standard setup may introduce bias or confounding effects. The central placement could impact the interactions between the BCAs and the pathogen differently than the side placements. Also, the result of PIRG is impacted and the inhibiting percentage is altered.

For improving consistency, consider placing the control variable (*P. noxius*) consistently across all treatments. In a single treatment, position it on one side of the Petri dish. In combined treatments, arrange it in a triangular or three-section layout: Place the two BCAs (*T. harzianum and B. subtilis*) on opposite sides; position the pathogen (*P. noxius*) in the middle section. This arrangement ensures uniformity and facilitates accurate comparisons.

Conclusion

The in-vitro tests conducted in this study did not provide significant evidence that these BCAs are inhibitory when used in combination. The study recorded mycelium distribution, percentage inhibition of radial growth (PIRG), and post hoc test results. Further investigations are needed to understand their combined effects more comprehensively on field trials to validate laboratory findings.

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