Efficacy of biological control against soil-borne pathogens in *Catharanthus roseus*

A biológiai védekezés hatékonysága a *rózsás meténg* (Catharanthus roseus) *talajból fertőző kórokozói ellen*

Lilla Szendrei, Dóra Piroska Borsos, Marietta Petróczy, Gábor Markó, and Annamária Tóth

Department of Plant Pathology, Institute of Plant Protection, Hungarian University of Agriculture and Life Sciences (MATE), Ménesi út 44, Budapest 1118, Hungary

**Correspondence: Toth.Annamaria@uni-mate.hu*

Abstract: The cultivation of Madagascar periwinkle (*Catharanthus roseus* L.) is affected by many pathogens, including soil-borne pathogenic fungi such as *Fusarium* spp., *Phytophthora* spp., and *Pythium* spp. The importance of biological control methods has been increasing due to the ongoing phase-out of chemical pesticides. Biocontrol agents like *Bacillus amyloliquefaciens*, *Trichoderma asperellum*, and *T. atroviride* are increasingly used due to their pathogen suppression capabilities and ability to enhance plant resilience to stress. This study evaluated the *in vitro* efficacy of *T. asperellum* against *F. oxysporum*. Significant inhibition of *F. oxysporum* growth was observed in dual culture confrontation test. Our results highlighted the potential of *T. asperellum* as a biocontrol agent for managing *F. oxysporum* in *C. roseus* cultivation. The application of these agents can reduce the dominance of chemical pesticides and promote sustainable agriculture practices.

Keywords: *Madagascar periwinkleis; Trichoderma asperellum; Fusarium wilt*

Összefoglalás: A rózsás meténg (*Catharanthus roseus* L.) termesztését számos kórokozó befolyásolja, beleértve a talajból fertőző kórokozókat is, mint például a *Fusarium*, *Phytophthora* vagy *Pythium* fajokat. Egyre inkább előtérbe kerül a biológiai növényvédőszerek használata a kémiai növényvédőszerek hatóanyagainak folyamatos kivonása miatt. A kórokozók elleni védekezésben egyre gyakrabban használnak antagonista szervezeteket, mint a *Bacillus amyloliquefaciens*, a *Trichoderma asperellum* és a *T. atroviride.* Ebben a tanulmányban a *T. asperellum in vitro* hatékonyságát vizsgáltuk *F. oxysporum* ellen. A konfrontációs teszt során a *F. oxysporum* növekedését jelentősen gátolta az antagonista gomba. Ez előrevetíti *T. asperellum* használatának a lehetőségét *C. roseus* termesztésében *F. oxysporum* ellen. Alkalmazása csökkentheti a kémiai növényvédő szerektől való függőséget és elősegítheti a fenntartható mezőgazdasági gyakorlatot

Kulcsszavak*: Madagascar periwinkleis; Trichoderma asperellum; fuzáriumos hervadás*

1 Introduction

The cultivation of Madagascar periwinkleis (*Catharanthus rosaeus* L.) is highly affected by many pests and pathogens, particularly soil-borne fungi. These pathogens mainly infect seedlings causing root rot, wilting and eventually the death of seedlings. Mature, but less

vigorous plants can also be attacked, leading to foliage wilting. These symptoms are often caused by plant pathogenic fungi belonging to different genera, including *Fusarium* spp., *Pythium* spp., *Phytophthora* spp., *Alternaria* spp. (Farr et al., 2021). In the case of Madagascar periwinkleis, several plant pathogenic fungi such as *Rhizoctonia solani*, *Fusarium equiseti*, *F. oxysporum* and *F. solani* have been reported to cause wilt (Yasir and Almaliky, 2023). Infected plants showing these symptoms become unsellable, causing serious economic losses for the growers. Possibilities in chemical control against these pathogens is negatively affected by the continuous withdrawal of pesticides. However, biological control methods for soil-borne pathogens have proven to be a promising alternative. Several biocontrol products are currently approved in Hungary, including different strains of *Bacillus amyloliquefaciens*, which have a broad spectrum of activity and can be applied directly in the soil. In addition, *Trichoderma asperellum* (formerly known as *T. harzianum*) and *T. atroviride* are also approved. These mycoparasitic fungi not only suppress plant pathogens but also enhance plant tolerance to both biotic and abiotic stresses (Singh et al., 2018).

The aim of this study was to evaluate the effectiveness of the antagonist organism, *Trichoderma asperellum*, in inhibiting the growth of *Fusarium oxysporum,* and we assumed that the *Trichoderma* species inhibits and parasitizes the tested pathogen in laboratory conditions.

2 Materials and Methods

Experimental Setup

The effectiveness of *Trichoderma asperellum* as an antagonist against *Fusarium oxysporum* plant pathogen was tested using a dual-culture agar confrontation test in Petri dishes. PDA (potato dextrose agar) medium was prepared and poured into sterile Petri dishes (90 mm diameter) under a laminar flow cabinet. Seven to ten-day-old *Fusarium oxysporum* culture from the Department's culture collection (isolated from *Catharanthus roseus,* 2023) were used to cut mycelial discs (7 mm diameter) with sterile tools. These pathogen discs were inoculated on one side of the Petri dishes, 1.5 cm from the edge.

Mycelial discs of the mycoparasitic antagonist organism were prepared similarly and placed on the opposite side of the Petri dishes, 1.5 cm from the edge. in a total of 40 replicates. Control dishes contained only the mycelium of the pathogen, antagonistic, *Trichoderma* was replaced with a sterile PDA disc. All dishes were sealed with Parafilm, labelled, and incubated at room temperature (23±2 °C).

Growth Measurements

Pathogen growth was assessed by measuring the radial growth of colonies at 48-hour intervals, starting 48 hours post-inoculation. This measurement was repeated twice.

Statistical Analysis

The impact of the antagonist was analyzed using a Linear Mixed-Effect Model (LMM) in R (Version 4.1.0, 2019). Colony radius was square root-transformed to approximate a normal distribution. The model included transformed colony radius as the dependent variable, with the presence of antagonist ('Treatment'), measurement days ('Time'), and their interaction as fixed effects. The unique identifier of each Petri dish was included as a random factor.

3 Results

In all cultures, the colonies increased in size, but with different growth dynamics as shown in Table 1. The significant interaction ('Treatment×Time') indicates that the two fungi showed a different growth pattern. The antagonist organism significantly ('Treatment(Treated)') inhibited the growth of *Fusarium oysporum* in the dual cultures, successfully demonstrating the *in vitro* efficacy of *Trichoderma asperellum* as a biocontrol agent against the pathogen (Fig. 1).

Table 1 Effects of the *T. asperellum* treatments and the time on the growth of *F. oxysporum using LMM*.

Predictors	Estimate	Std. Error	df	t value	P value
Intercept	1.72	0.01	48	125.94	<0.001 ***
Treatment(Treated)	-0.39	0.02	48	-25.80	<0.001 ***
Time	0.37	0.01	98	34.53	<0.001 ***
Treatment(Treated)×Time	-0.31	0.01	98	-25.73	<0.001 ***

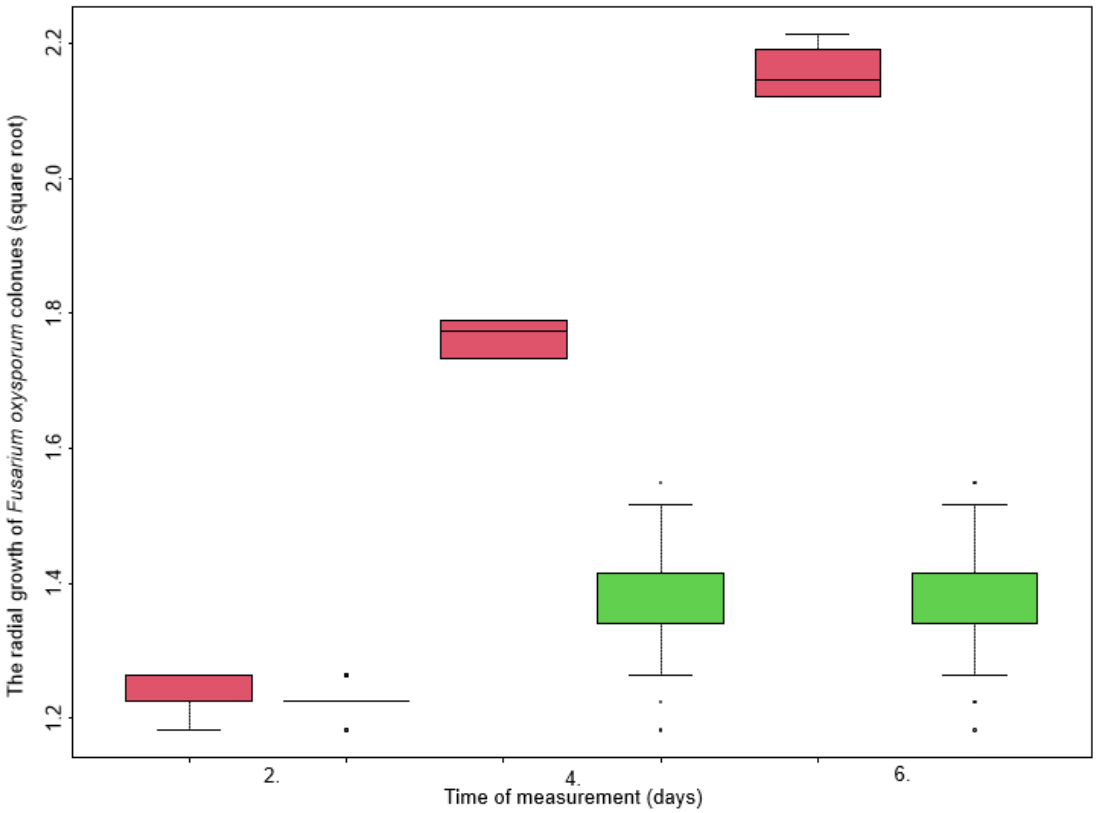

Figure 1 *Whisker's plot shows the effect of antagonistic fungi on the growth of* Fusarium oxysporum *colonies:*

Red indicates control Petri dishes, and green indicates colonies growing with the antagonist. The horizontal line in the diagram represents the median. The boxes indicate the interquartile range. Whiskers indicate 95% confidence intervals.

4 Discussion

The results demonstrate that *Trichoderma asperellum* acts as an effective biocontrol agent against *Fusarium oxysporum in vitro*. The different growth pattern may be the result of the pathogen development in the treated Petri dishes having stopped when the two species interacted. The significant inhibition of pathogen growth in the dual cultures highlights the antagonistic interaction between *Trichoderma asperellum* and *Fusarium oxysporum*.. This inhibition suggests that the antagonist's mechanisms, such as mycoparasitism or competition for space, may play a role in limiting pathogen growth. Similar interactions have been observed in previous studies involving *Fusarium oxysporum* and other *Fusarium* species (Ghanbarzadeh *et al*., 2014; Larran *et al*., 2020).

Trichoderma species are widely distributed in soils and could be effectively used in integrated pest management due to their ability to degrade the hyphal cell wall thereby reducing disease symptoms and limiting the spread of pathogens including *Fusarium* species (Monte, 2001; Woo *et al*., 2013).

These findings support the potential application of *T. asperellum* in managing *F. oxysporum* infection in *Catharanthus roseus* cultivation.

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