# ANTAGONISTIC EFFECT OF AUTOCHTHONOUS ISOLATE *TRICHODERMA HARZIANUM* AGAINST FUSARIUM WILT OF CABBAGE

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(Received 9th Sep 2024; accepted 25th Nov 2024)

**Abstract.** The pathogenic fungus *Fusarium solani* is increasingly causing Fusarium wilt in cabbage cultivation around the world due to climate change and the pathogen's adaptation to high temperatures. Although the use of *Trichoderma harzianum* as a biofungicide is well documented, the evaluation of the antagonistic effect of the autochthonous isolate is of great local importance. The aim of this study is to test the antagonistic effect of *T. harzianum* on the growth of *F. solani* under in vitro and in vivo conditions. The tested hypothesis is that *T. harzianum* will significantly reduce the growth of pathogen in all experiments. The antagonistic potential of the *T. harzianum* against *F. solani* was evaluated by testing non-volatile and volatile production compounds and using a pot experiment on cabbage seedlings. The results demonstrated that *T. harzianum*, by producing non-volatile metabolites, significantly inhibits the *F. solani* mycelium growth by 57.8% while it achieves an inhibition of 30.6% by volatile metabolites with significant changes in pathogen microstructures. The results of pot experiment confirmed a significant increase of infected cabbage seedlings growth area after treatment with *T. harzianum* by 28.3% compared to uninfected plants and by 51.6% compared to infected plants. This study underlines the significant potential of the autochthonous *T. harzianum* isolate as a biocontrol agent for the suppression of Fusarium wilt in cabbage.
**Keywords:** *biological control, Fusarium solani, pot experiment, volatile metabolites, non- volatile metabolites*

## Introduction

Cabbage (*Brassica oleracea* var. *capitata* L.) is an annual or biennial herbaceous plant of the *Brassicaceae* family (Elif et al., 2016; Moreb et al., 2020), cultivated for its leaves, which are rich in nutrients, minerals and fiber (Anil et al., 2018; Khafagi et al., 2020). Red cabbage (*Brassica oleracea* var. *capitata* f. Rubra), characterized by its high content of anthocyanins (Silva et al., 2011), which are important for the food industry (Ghareaghajlou et al., 2021), is of great economic importance. During the cold seasons, cabbage is grown in a temperate climate and in the Mediterranean region with high temperatures (Elif et al., 2016; Moreb et al., 2020). With climate change, cabbage cultivation becomes problematic due to damage caused by pathogens adapted to high temperatures (Jelínek et al., 2019).

During the growing season, cabbage is affected by many fungal diseases, especially Fusarium wilt as the most widespread and destructive plant disease (El-Mougy et al., 2011; Khafagi et al., 2020), which is caused by a fungi of the genus *Fusarium* that are transmitted through the soil and affect a variety of plant species (Khafagi et al., 2020). Symptoms of the disease occur when these pathogens infect the roots and cause clogging of the vascular tissue through the development of mycelia, leading to plant wilting (Khafagi et al., 2020; Martínez-Padrón et al., 2023). Members of the genus


http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_16591674

*Fusarium* are considered a global threat to cabbage cultivation as they are the main source of biotic stress in the plant's rhizosphere (Liu et al., 2020). In recent years, the economically important acomycete fungus *Fusarium solani* (Mart.) Sacc. is causing Fusarium wilt in cabbage due to climate change (Wenxue et al., 2018; Jelínek et al., 2019; Chai et al., 2022). Climate change significantly affects the severity of Fusarium wilt, which has affected cabbage crops worldwide, including cultivars that were once resistant to this disease (Jelínek et al., 2019). It is interesting that cabbage is used in crop rotation because the leaf residues are incorporated into the soil at the end of the growing season to reduce infection caused by *Fusarium* sp. (Gilardi et al., 2016; Prasad and Kumar, 2017). However, as cabbage is becoming an increasingly common host for *F. solani* (Wenxue et al., 2018) the effectiveness of this agrotechnical method is decreasing.

Currently, chemical control is the most effective method to control *Fusarium* sp. but the application of fungicides has negative ecological impacts, leading to ecosystem imbalance and the development of pathogen resistance due to over-application (Bardin et al., 2015; Khafagi et al., 2020; Yao et al., 2023). As cabbage is mostly consumed fresh, it is important to strive for ecological control methods (Moreb et al., 2020; Serhiienko et al., 2023). Biological control use microorganisms with antagonistic activity (biofungicides), which is an efficient, economical and harmless method for plant production (Osorio-Hernández et al., 2016; Harman et al., 2021; Martínez-Padrón et al., 2023; Yao et al., 2023). Biofungicides not only inhibit phytopathogens through direct antagonistic action, but also indirectly improve fertility and soil structure and promote plant growth as environmentally beneficial factors (Khafagi et al., 2020).

*Trichoderma* sp. are widespread fungi, which have been implemented in various crops like biocontrol agents (Anees et al., 2010) due to their rapid growth and metabolites production with antimicrobial properties (Schuster and Schmoll, 2010; Martínez-Padrón et al., 2023) and their effectiveness in controlling a wide range of soil-borne and foliar pathogens including *Fusarium* sp. (Wang et al., 2023b; Martínez-Padrón et al., 2023; Yao et al., 2023). *Trichoderma* sp. use biological control mechanisms, such as competition, antibiosis and mycoparasitism, releasing volatile and non-volatile metabolites to inhibit *Fusarium* sp. growth (Mulatu et al., 2022; Elshahawy and Marrez, 2024). As a result, they promote plant growth and induce systemic plant resistance, leading to disease reduction (Belete et al., 2015; Abbas et al., 2022; Yao et al., 2023) without promoting the development of pathogen resistance (Serhiienko et al., 2023). Similarly, these fungi produce non-volatile metabolites that degrade the cell walls of pathogenic fungi, thereby inhibiting them and reducing the severity of plant diseases (Kubicek et al., 2019; Druzhinina et al., 2018; Yao et al., 2023; Martínez-Padrón et al., 2023). Barakat et al. (2014) report that non-volatile compounds cause a more drastic reduction in mycelial growth of pathogenic fungi as they diffuse into the culture medium and cause mycelial lysis. The situation described above may be accompanied by the envelopment of *Trichoderma* hyphae around the pathogen hyphae (mycoparasitism), whereupon *Trichoderma* sp. invade the host cells and cause degradation. On the other hand, by producing a wide spectrum of volatile metabolites (Siddiquee et al., 2012) *Trichoderma* sp. leads to mycofumigation that can limit and even stop the development of economically important pathogenic fungi (Dubey et al., 2007; Yuan et al., 2012; Sharma and Sharma, 2017; Degani et al., 2021; Elshahawy and Marrez, 2024). These are secondary metabolites such as ethylene, carbon dioxide, hydrogen cyanide, aldehydes and ketones, which play an important role in the control of


http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_16591674

plant pathogens (Vey et al., 2001; Faheem et al., 2010; Prasad and Kumar, 2011). Non-volatile secondary metabolites of *Trichoderma* sp. have been shown to be more effective in suppressing mycelial growth than volatile ones (Barakat et al., 2014).

Among the better-known species, *Trichoderma harzianum* Rifai has different inhibitory effects on 29 species of phytopathogenic fungi from 18 genera, including *Fusarium* sp. (Tian et al., 2018; Yao et al., 2023). Studies confirm that *Trichoderm*a sp. reduces the severity of Fusarium wilt while promoting the growth and development of cabbage (Leta et al., 2023; Wang et al., 2023b). The potential of *Trichoderma* sp. as a biofungicide in the control of *F. solani* in various crops is well documented (Mulatu et al., 2022; Nagendran et al., 2016; Matloob, 2019; Abd-El-Khair et al., 2019), but only a few studies have been conducted in cabbage. Moreover, since the efficacy of antagonistic fungi in control depends on successful colonization of the plants (Griffin et al., 2013) the use of autochthonous isolates is of great local importance. Inoculation of plants with autochthonous fungal isolates that are locally adapted to native plant communities is often not available, and commercially propagated species are not necessarily locally adapted to target plant community conditions (Middleton et al., 2015). This can lead to a disconnection between readily available commercial fungi and plants, reducing the success of plant colonization and the efficacy of the inoculated fungus. Several studies have confirmed that autochthonous fungal species (especially mycorrhizal fungi) are more effective than commercial fungi in plant promotion and pathogen control (Taheri and Bever, 2011; Johnson et al., 2012). However, there are not enough studies to confirm this, which is why detailed researches are needed.

The aim of this study is to test the antagonistic effect of the autochthonous isolate *T. harzianum* on the growth and development of *F. solani* under in vitro and in vivo conditions. The tested hypothesis is that *T. harzianum* will significantly reduce the growth of *F. solani* in all experiments.

## Materials and methods

### *In vitro experiment*

#### *Isolation and identification of fungal isolates*

The autochthonous isolate of *F. solani* was isolated from tomato stem and the autochthonous isolate of *T. harzianum* was isolated from soil. Both isolates were grown on PDA medium and incubated in several replicates in a climate chamber at 24°C in the dark.

The morphological identification of the species *Fusarium* sp. was performed according to Leslie and Summerell (2016) and the species of the genus *Trichoderma*, according to Kubicek and Harman (2002).

Molecular identification of the isolates was performed by conventional PCR (for *Trichoderma* sp. according to Jovičić-Petrović et al., 2014 and for *Fusarium* sp. according to Suga et al., 2000) and sequenced to species level (Macrogen Europe, Netherlands).

#### *Antifungal non-volatile compounds test*

The antagonistic effect of *T. harzianum* against *F. solani* was tested using non-volatile metabolites test (dual culture method) according to the modified method of Coskuntuna et al. (2008).


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Micellar discs of antagonistic and phytopathogenic fungi (Ø 5 mm) were cut with a circular cutter from the edge of 7-day-old fungal colonies. The micellar disc of *T. harzianum* was placed 2 cm from the edge of the Petri dish (Ø 8.5 cm) on the bottom of the previously poured PDA medium. The *F. solani* micellar disc was placed at the same distance on the opposite side of the antagonist disc and incubated for 7 days at 24°C in the dark. Petri dishes containing only a *F. solani* disc were used as control (*Fig. 1*). The experiment was set up in two variants and six repetitions.

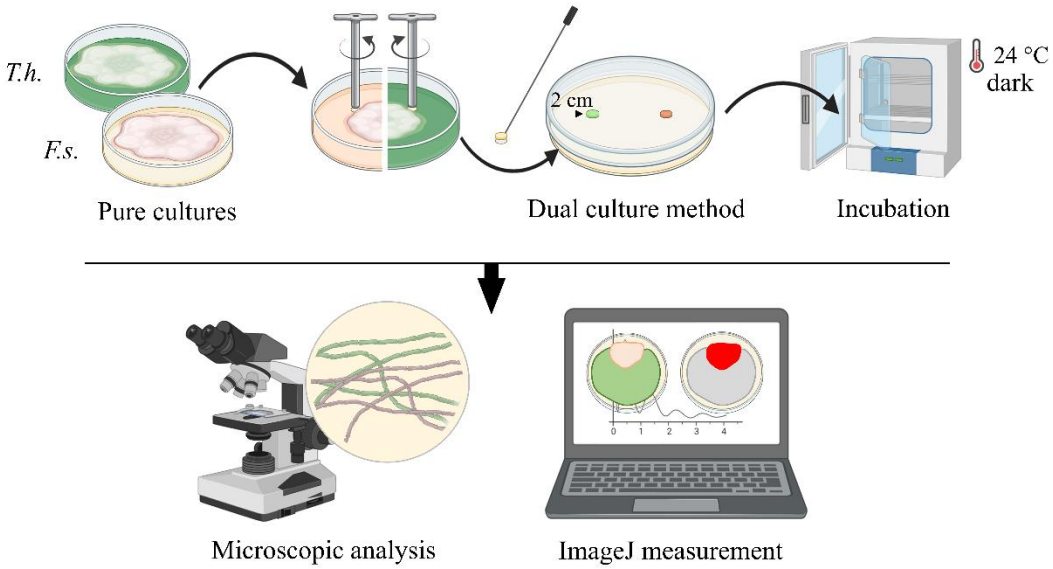


***Figure 1.*** *Schematic representation of in vitro experiment: antifungal non-volatile compounds test (Trichoderma harzianum – T. h.; Fusarium solani – F. s). Created with BioRender.com*

### *Antifungal volatile metabolites test*

Antagonistic effect of volatile metabolites produced by *T. harzianum* isolate was assessed by indirect confrontation method. According to the method described by Ruangwong et al. (2021), a disc of *F. solani* mycelium was cut from a 4-day-old culture and inserted into the middle of the Petri dish bottom while the lid was removed. The bottom of a Petri dish containing PDA was inoculated with *T. harzianum* and covered with a bottom of Petri dish containing a micellar disc of *F. solani*. Petri dishes were closed and double wrapped to avoid the volatile evaporation and incubated for 7 days at 24°C in the dark. Petri dishes which contained only a disc with *F. solani* were served as control. The experiment was set up in two variants and six repetitions.

### *Microscopic analysis*

The analysis of *T. harzianum* antagonistic effect on *F. solani* was carried out using a light microscope and a stereomicroscope according to the modified method of Dourou and La Porta (2023). In order to quantify the antagonistic effect on the microstructures of the pathogen, microscopic preparations were made with pathogen hyphae from the inhibition zone and pathogen hyphae from the control variant. The antagonistic effect was quantified on the basis of the observed and photographed hyphal structural changes.


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*Data analysis*

After 7 days, the test and control Petri dishes were photographed and the photos were processed with the computer program ImageJ (Schneider et al., 2012) according to the modified method of Martinko et al. (2022a).

Based on the obtained mean values of *F. solani* mycelial growth area ($cm^2$), the inhibition index (%) was calculated and the antagonistic effect of *T. harzianum* was quantified.

*Statistical analysis*

The results of *T. harzianum* antagonistic effect are presented as mean values and standard deviations (SD). The difference between the mean values of the test and control groups was determined using the Student t-test in the SPSS statistical program (IBM, version 15.0, Chicago, IL, USA) and was considered statistically significant at $p < 0.05$.

***In vivo experiment***

*Preparation of conidia suspension*

Isolates of *T. harzianum* and *F. solani* were grown on PDA and incubated for 14 days at 24°C in the dark to stimulate sporulation. After 14 days, the conidia were harvested by adding a solution of sterile distilled water (10 ml) and a surfactant (0.1% Tween-80) to remove the surface tension of the water.

The immersed conidia were carefully collected with a sterile rubber brush. The solution was filtered to remove mycelial fragments and the conidial suspension was collected in a sterile bottle for both isolates. Spore concentration was determined by serial dilution and spore counting using a Neubauer hemocytometer under a light microscope.

The final suspensions of the *T. harzianum* isolate ($9.6 \times 10^7$ spores/ml) and the *F. solani* ($3.2 \times 10^7$ spores/ml) were stored at room temperature until use.

*Cabbage seedling cultivation*

Seeds of variety Redma RZ F1 – Hybrid (Kadmo d.o.o., Zagreb, Croatia) were used to grow the red cabbage seedlings. The seeds were sown in July 2024 according to the manufacturer's instructions in the substrate (Klasmann Potgrond P., Euro Brod d.o.o.), which was in containers. The seedlings were used when they had developed three true leaves (BBCH 13). The plants were grown under average conditions: 22°C (night) and 27°C (day), 75% relative humidity with natural daylight, which were recorded every day. During cultivation, the plants were not treated with agrochemicals and were watered once a day.

*Pot experiment*

The experiment was carried out according to the modified method of Khafagi et al. (2020) by immersing the seedling roots in; a suspension of *F. solani* conidia; a suspension of *F. solani* conidia, after which the same seedling roots were immersed in suspension of *T. harzianum* conidia; and a sterile distilled water. Each root immersion was carried out for 60 min (*Fig. 2*).


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After treatment, cabbage seedlings were transplanted into aseptic plastic pots (Ø 10 cm) filled with a mixture of substrate (Klasmann Potgrond P., Euro Brod d.o.o., Zagreb, Croatia) and perlite (Plagron, GeoFlora d.o.o., Zagreb, Croatia) in a ratio 2:1. The experiment was repeated twice, in three variants and 20 replicates. The plants were grown under the average conditions monitored daily: 22°C (night)/27°C (day) and 75% relative humidity in natural daylight.

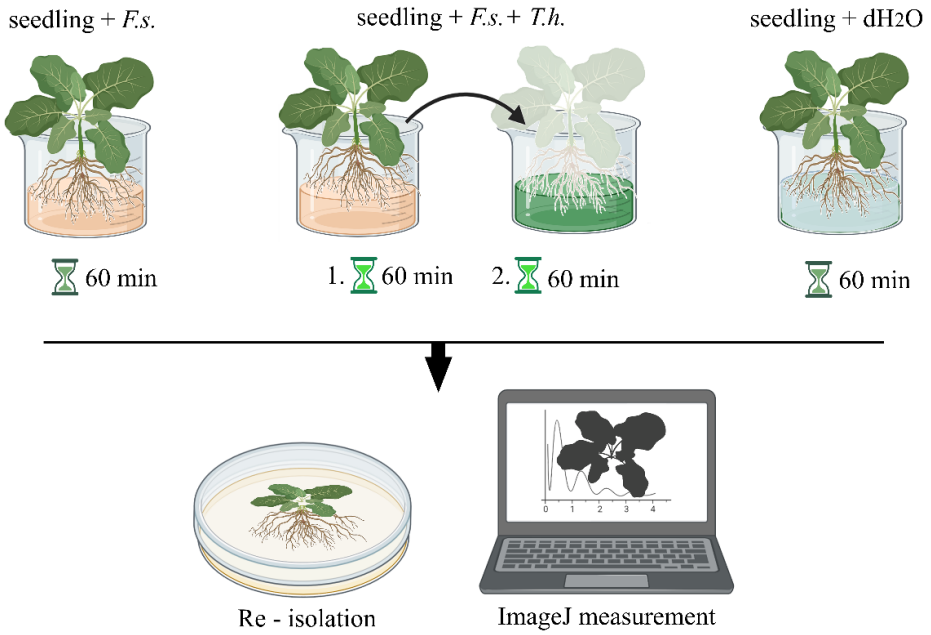


***Figure 2.*** *Schematic representation of in vivo experiment: pot experiment (Trichoderma harzianum – T. h.; Fusarium solani – F. s). Created with BioRender.com*

*Data analysis*

After 15 days, whole cabbage plants (with above- and below-ground parts) were collected, separated from the soil and washed with sterile distilled water.

Each seedling was placed on a white background with a scale and photographed to determine the total growth area of the plant ($cm^2$). According to the modified method of Martinko et al. (2022b), the photographs were processed using the computer program ImageJ (Schneider et al., 2012). The antagonistic effect of *T. harzianum* on *F. solani* was quantified as a percentage of plant growth (%).

*Statistical analysis*

The results of *T. harzianum* antagonistic effect that presented normal distribution were submitted to analysis of variance (ANOVA) for the comparisons between treatments followed by Tukey's honestly significant difference (HSD) test, at 95% confidence ($p < 0.05$) using the SPSS computer program. The results are presented as mean values and standard deviations.

***Re-isolation from seedling samples***

Re-isolation of *F. solani* and *T. harzianum* from the treated cabbage seedlings was performed 15 days after the start of the experiment according to Rees et al. (2022). The


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DOI: http://dx.doi.org/10.15666/aeer/2301_16591674

collected plants were cut into smaller fragments which were surface sterilized with 70% ethanol for 1 min, washed twice with sterile distilled water and placed separately on the PDA surface in Petri dishes. The plant samples were incubated in a climate chamber at 24°C and in the dark.

Growth of *F. solani* and *T. harzianum* was observed after 7 days. Growth of the fungus on at least one part of the plant was considered an indicator of successful inoculation of the seedlings. In order to morphologically confirm the presence of genus *Fusarium* and *Trichoderma* species, a microscopic analysis was carried out according to the morphological determination keys used at the beginning.

## Results

### *Identification of fungal isolates*

Morphological and molecular identification confirmed that the isolates belong to the species *T. harzianum* and *F. solani.*

### *Antifungal non-volatile metabolites tests*

The results of significant *T. harzianum* antagonistic effect in inhibition of *F. solani* mycelia in the non- volatile metabolites test are shown in *Table 1* and *Figure 3.*

### *Antifungal volatile metabolites tests*

The results of the volatile metabolites test show a significant antagonistic effect of *T. harzianum* in inhibition of *F. solani* mycelia are presented in *Table 2*.

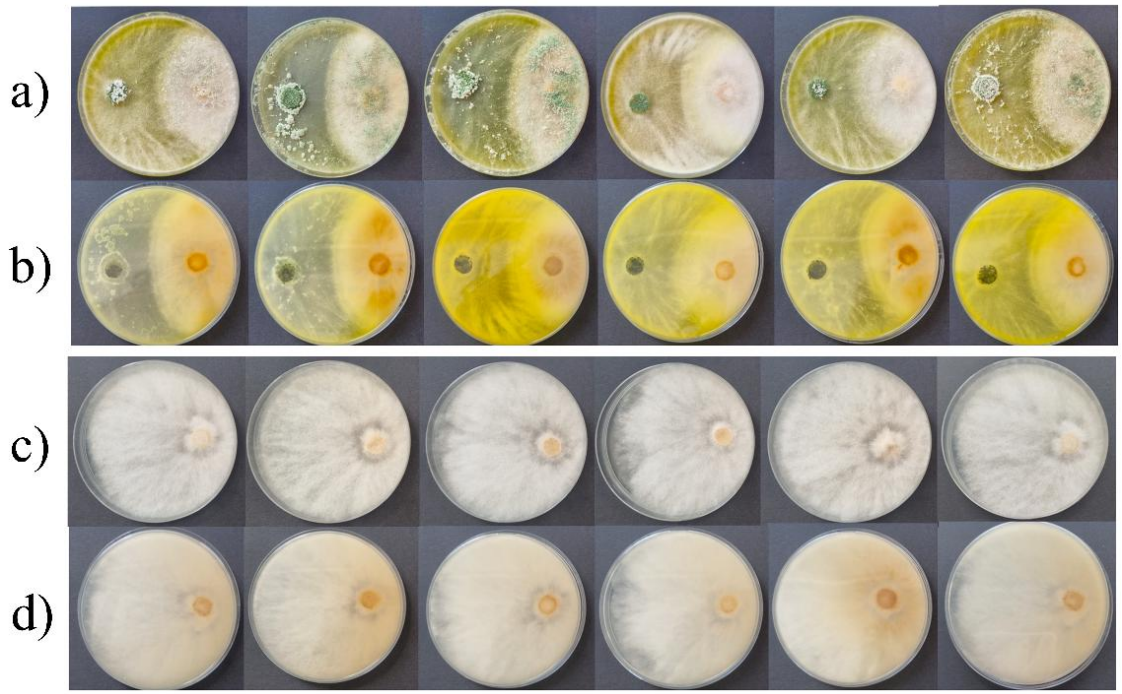


***Figure 3.*** *Growth inhibition of Fusarium solani by Trichoderma harzianum in non- volatile compounds test after 7 days (a, b) compared to the growth of Fusarium solani in the control (c, d) after 7 days; the front (a, c) and the back (b, d). Production of non- volatile metabolites (yellow pigment) by Trichoderma harzianum (b)*


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***Table 1.*** *Antagonistic effect of Trichoderma harzianum on the micellar growth inhibition of Fusarium solani in non- volatile compounds test after 7 days*

| | **Control** | **Test** |
|---|---|---|
| | ***F. solani*** | ***F. solani + T. harzianum*** |
| Mean value ($\bar{x}$) of pathogen growth area ($cm^2$) ± SD | 63.2 | 26.1* |
| I (%) | 58.7 | |

*Significant difference in the mean values of pathogen growth compared to control according to t-test ($p < 0.05$); pathogen inhibition index (I); standard deviation (SD)

***Table 2.*** *Antagonistic effect of Trichoderma harzianum on the micellar growth inhibition of Fusarium solani in volatile compound test after 7 days*

| | **Control** | **Test** |
|---|---|---|
| | ***F. solani*** | ***F. solani + T. harzianum*** |
| Mean value ($\bar{x}$) of pathogen growth area ($cm^2$) ± SD | 42.1 | 29.2* |
| I (%) | 30.6 | |

*Significant difference in the mean values of pathogen growth compared to control according to t-test ($p < 0.05$); pathogen inhibition index (I); standard deviation (SD)

### *Microscopic analysis*

The results of microscopic analysis of the *T. harzianum* antagonistic effect on the microstructures of the pathogen *F. solani* in non- volatile compounds test are shown in *Figure 4.*

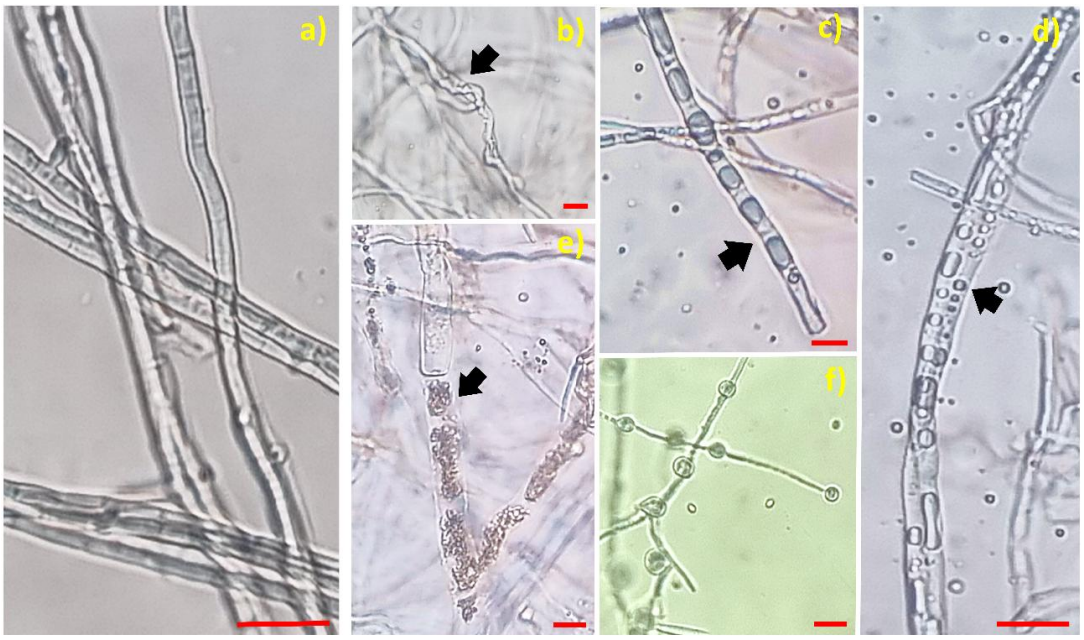


***Figure 4.*** *Microscopic analysis of the Trichoderma harzianum antagonistic effect on the microstructures of the pathogen Fusarium solani in non- volatile compounds test after 7 days; pathogen hyphae in the control (a); mycoparasitism (b); vacuolization of the pathogen hyphae (c, d); coagulation of the cell content of the hyphae due to cell wall degradation (e); increased production of chlamydospores (f); scale bar 10 µm*


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### *Pot experiment*

The results of *T. harzianum* antagonistic effect on red cabbage seedlings infected with *F. solani*, confirm a significant increase in plant growth area compared to the plant growth area treated only with water (*Table 3*).

***Table 3.*** *Antagonistic effect of T. harzianum on growth of red cabbage seedlings infected with Fusarium solani compared to the growth of infected and uninfected seedlings after 15 days*

| | **Variants in experiment** | | |
|---|---|---|---|
| | **Plant + water** | **Plant + *F. solani*** | **Plant + *F. solani* + *T. harzianum*** |
| Mean ($\bar{x}$) of plant growth area ($cm^2$) ± SD | $79.2 \pm 10.9^a$ | $56.9 \pm 11.2^b$ | $110.4 \pm 15.2^c$ |
| Plant growth area (%) | 0 | -28.2 | 28.3 |

Different letters indicate a statistically significant difference between mean values (Tukey test, $p < 0.05$); standard deviation (SD)

## Discussion

The results of the non- volatile metabolites test show a significant antagonistic effect of *T. harzianum* in inhibition of *F. solani* mycelia by 57.8% after 7 days (*Table 1*).

The *T. harzianum* isolate filled half of the PDA surface area in the Petri dish on the third day of evaluation and was in direct contact with the *F. solani* hyphae, showing an intense yellow pigment, indicating the production and release of non- volatile metabolites in the PDA medium (*Fig. 3*). On the other hand, the results of the volatile metabolites test show a significant antagonistic effect of *T. harzianum* in inhibition of *F. solani* mycelia by 30.6% after 7 days (*Table 2*). Microscopic analysis of the microstructures of *F. solani* in non- volatile compound test confirmed the significant *T. harzianum* antagonistic effect based on the observed twisting of the hyphae around the pathogen hyphae (mycoparasitism), the leakage of cell contents due to cell wall degradation of pathogen hyphae, the occurrence of vacuolization and the development of numerous persistent structures of the pathogen (chlamydospores) under unfavorable conditions compared to the control, in which the hyphae of the pathogen are viable and turgid (*Fig. 4*). Microscopic changes of pathogen structures in the volatile compounds test included only shortening of pathogen hyphae (not presented).

The results obtained are in agreement with the investigations of Azevedo et al. (2021), in which the mycelial growth of *F. solani* was stopped in the non – volatile compounds test (dual culture method) after direct contact with the *T. harzianum* hyphae due to mycoparasitism. The degree of inhibition observed ranged from 64–85% depending on the *F. solani* isolate (Azevedo et al., 2021). In a study by (Begum et al., 2015), different *Trichoderma* species (*T. hamatum, T. viride and T. harzianum*) were tested using the dual culture method, with the species *T. harzianum* achieving the highest degree of inhibition of the pathogenic fungus *Penicillium citrinum*. According to Michel-Aceves et al. (2008) *T. harzianum* showed a significantly high inhibition index (78.8%) on *Fusarium* sp. in the dual culture method. The authors mentioned report the appearance of a distinct yellow pigment in the PDA medium and emphasize the ability of *T. harzianum* to produce a wide range of non- volatile metabolites due to pathogen inhibition. Also, Raza et al. (2013) determined that the *T. harzianum* isolate inhibits *Fusarium oxysporum* by 40% through the production of volatile metabolites which is consistent with the results of this research.


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It is known that *Trichoderma* sp. can produce hundreds of antimicrobial metabolites (Begum et al., 2015), which inhibit various phytopathogenic fungi and synergize with enzymes that degrade the cell walls of pathogenic fungi (Michel-Aceves et al., 2008). Manganiello et al. (2018) found that the secondary metabolites produced by *Trichoderma* sp. cause irregular mycelial growth, disruption and degradation of the cell wall of the pathogenic fungus, proving that *Trichoderma* sp. has an antagonistic effect on the pathogenic species *Phytophthora nicotianae*. Bunbury-Blanchette et al. (2018) reported similar results in a dual culture in which *T. harzianum* parasitized *Sclerotinia sclerotiorum*. At the time of direct mycelial contact, the author mentioned the appearance of a yellow pigment in the PDA medium, which was interpreted as a sign of antagonism of *T. harzianum* during the inhibition of the pathogenic fungi.

The microscopic analysis in our study confirmed similar microscopic changes found in the mentioned studies, but it is certainly important to mention the occurrence of vacuolization of hyphae (*Fig. 4c, d*). The results of our study indicate that the disruption of vacuole activity (increase in number and shape) is a consequence of the effect of antifungal agents on the growth and development of the pathogen. Vacuoles look like membrane-enclosed spherical structures, which are smaller in untreated fungi than in fungicide-treated ones (Borjihan et al., 2009) and at the same time serve to detoxify the hyphae from antifungal compounds (Sirikantaramas et al., 2008). Therefore, it is assumed that the observed hyphal vacuolization of the pathogen *F. solani* in the non-volatile compounds test of our study is a consequence of hyphae detoxification from secondary metabolites secreted by the antagonist *T. harzianum* into the PDA medium (yellow pigment). Bunbury-Blanchette et al. (2018) point out that the laboratory methods excludes environmental factors that could affect the practical application of *Trichoderma* species, but is a preliminary screening method that allows observation of the interaction between the antagonist and the pathogen in the laboratory.

The results of *T. harzianum* antagonistic effect on red cabbage seedlings infected with *F. solani*, after 15 days confirm a significant increase in plant growth area by 28.3% compared to the plants growth area treated only with water. The growth area of cabbage seedlings infected with *F. solani* was significantly reduced (28.2%) compared to plants treated with water, which confirmed the infection and pathogenicity of *F. solani*. In addition to a significant reduction in plant surface area, symptoms of wilting, yellowing of leaves and difficulty in water absorption by infected seedlings were observed, which was not observed in the case of untreated plants. The use of *T. harzianum* in this experiment apparently led to a reduction in the *Fusarium* wilt severity caused by *F. solani* infection, through a significant growth increase of infected seedlings treated with *T. harzianum* (51.6%). The obtained results are in accordance with the results of Rabeendran et al. (2000), where the application of *Trichoderma* sp. in the treatment of cabbage seedlings led to a significant increase in leaf area compared to untreated plants. Likewise, Leta et al. (2023) stated that the treatment with *T. harzianum* reduced the severity of *F. oxysporum* infection in cabbage by 31.4% and significantly increased the cabbage growth. In this context, Wang et al. (2023a) emphasize the importance of the secondary metabolites activity of *Trichoderma* sp. that dominate the inhibition of *Fusarium* sp. after colonization of the infected cucumber plant roots. Also, they state that the *Trichoderma* sp. antagonism effect in suppressing *Fusarium* wilt is combination of *Trichoderma* defense mechanism action. The same results are reported by Belete et al. (2015), where isolates of *Trichoderma* sp. significantly increased the height and biomass of bean seedlings compared to bean seedlings inoculated only with


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DOI: http://dx.doi.org/10.15666/aeer/2301_16591674

*F. solani.* Disease reduction through significant growth promotion of cabbage seedlings infected with *F. solani* and treated with *T. harzianum* in this study is probably the result of secondary (non- volatile and volatile) metabolites action in combination with other *T. harzianum* defense mechanisms. Since the *T. harzianum* application in the root zone of seedlings inoculated with *F. solani* leads to a significant growth increase seedlings (by 28.3% compared to uninfected plants, i.e. by 51.6% compared to infected plants), it can be observed that the infection is almost nullified. The antagonistic effect of *T. harzianum*, manifested through the seedling growth promotion, is almost twice as high in infected seedlings, compared to uninfected seedlings. We assume that this situation is a consequence of the activation of induced systemic resistance of cabbage plants caused by *T. harzianum* colonization, which (like the *F. solani* seedlings infection) was confirmed in re- isolation. *Trichoderma* sp. reduce the severity of plant diseases through various mechanisms such as antagonism and mycoparasitism by directly inhibiting the growth of plant pathogens or by inducing systemic plant resistance (Harman et al., 2004a; Fontenelle et al., 2011) which can lead to long-term systemic protection against a variety of pathogens (Hammerschmidt et al., 2001; Métraux, 2001). Harman et al. (2004a) and Brotman et al. (2010) agree that *Trichoderma* isolates are capable of producing such interactions that cause the process of activation of systemic broad-spectrum resistance which begins with the colonization of plant roots (Fontenelle et al., 2011). It often increases root growth and development, plant productivity, resistance to abiotic stress and the uptake and utilization of nutrients (Harman et al., 2004a), especially the most cited species – *T. harzianum*, which acts as a resistance elicitor in various crops (Benítez et al., 2004; Fontenelle et al., 2011).

The fact that successful colonization by *Trichoderma* species leads to the activation of induced systemic plant resistance is supported by recent studies (Singh et al., 2021; Chen et al., 2021; Contreras-Cornejo, 2024) which highlights the importance of successful colonization by *Trichoderma* sp. and the importance of autochthonous isolates that are adapted to the local agroecosystem in contrast to commercial ones which needs to be confirmed in future researches.

## Conclusion

Based on this research, we can conclude that the autochthonous isolate of *T. harzianum* significantly inhibits the growth and development of *F. solani* in laboratory conditions with a significant antagonistic effect on the microstructures where vacuolization, as poorly described change in literature, is a reflection of antibiosis through the production of metabolites. Also, *T. harzianum* significantly reduced the severity of *Fusarium* wilt of cabbage seedlings and promoted the growth of seedlings in vivo, which can be attributed to the successful seedlings colonization by the autochthonous isolate and the presumed stimulation of induced resistance of the plant. All results confirm the hypothesis.

This approach of biocontrol is an ecologically safe way of protecting plants and is particularly relevant for vegetables that are consumed fresh such as cabbage. This research considered the importance of using autochthonous isolates of *Trichoderma* sp. because their antagonistic effect depends on the successful colonization of the plant host. Additional research in in vivo conditions is certainly needed to complete the knowledge about the antagonistic effect of the autochthonous isolate of *T. harzianum* compared to the commercial one in order to verify the assumptions of this research. The


http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_16591674

use of a wide range of biofungicides and bio promotors in agriculture seems to be an important measure for a new model of sustainability and can lead to a reduction of agrochemical and fertilizer inputs into our food chain and the environment. The compatibility of *T. harzianum* (commercial and autochthonous) should first be assessed to guarantee successful inoculum application. The use of isolate *T. harzianum* would be especially useful in cabbage transplanting when it is important to promote the rapid establishment of fungal colonization with plant hosts in order to achieve early and high yields. Also, it is necessary to investigate whether the effectiveness of *T. harzianum* is closely related to the level of inoculum in the substrate, after the application of the inoculum formulation and compare it with the unformulated inoculum. Encapsulation of the inoculum, in order to protect the inoculum from environmental influences, is also something that should be included in future in vivo research under diverse field conditions to confirm the efficacy of the autochthonous *T. harzianum* isolate to explore potential benefits compared to commercial strains before recommending its broader application in real-world farming practices.

**Acknowledgements.** This work was supported by funding from the University of Zagreb Faculty of Agriculture.

**Conflict of interests.** The authors declare no competing interests.

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http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_16591674