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ORIGINAL PAPER

# Significance of Local *Trichoderma* Isolates in Controlling *Pythium ultimum* and *Rhizoctonia solani* on Bean in Egypt

**Nofal, A.M.[1]; Abdel-Ghany, T.M.[2] and Abd-EL-Hamed, W.F.M.[3]**

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## ABSTRACT

Biological control of plant diseases is a safer and more cost-effective technology for controlling serious diseases, besides facilitating and improvement plant development, yield and production. The causative organisms of root-rot and damping-off diseases were isolated from diseased bean roots, these isolates were identified as *Pythium ultimum* 4413AUMC and *Rhizoctonia solani* 6590AUMC. Colony characteristics, growth, mycelium structure, conidiophores, phialides and conidia were used to identify *Trichoderma* species isolated from rhizosphere soils around healthy bean roots collected from 23 locations in Egypt. *Trichoderma* spp. successfully reduced *P. ultimum* mycelial growth, with eight isolates showing total overgrowth on *P. ultimum*. The results demonstrated that all the isolated *Trichoderma* spp. severely affected *R. solani* radial growth, with nine isolates showed full overgrowth. The *Trichoderma* isolate T5 caused total overgrowth (100 percent inhibition) against phytopathogenic fungi and was chosen for species identification as *Trichoderma koningii*. The examination of fungus filtrate showed that *T. koningii* produces the enzyme chitinase which hydrolyzes chitin in fungal infections (0.98 u.mg$^{-1}$ protein) and a large amount of protease enzyme (6.34 u/mg protein). In a scanning electron microscope from interaction zone, the mycelial samples of *R. solani* and *T. koningii* from a dual culture, indicated the mycoparasitic nature of *T. koningii* on *R. solani*. *T. koningii* was found to be a cost-effective bioagent for suppressing the phytopathogenic microorganisms, *P. ultimum* and *R. solani*.

***Key words***. Bean, *Phaseolus vulgaris*, *Pythium ultimum*, *Rhizoctonia solani*, biological control, *Trichoderma koningii*.

***Correspondence: Abdel-Ghany, T.M.**
tabdelghany.201@azhar.edu.eg
**Ashraf M. Nofal**
1- Sustainable Development Department, Environmental Studies and Research Institute, University of Sadat City, Sadat City, Egypt
**Tarek M. Abdel-Ghany**
https://orcid.org/0000-0001-6418-5890
2- Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Cairo, Egypt.
**Walaa F.M. Abd-EL-Hamed**
3- Plant Pathology Research Institute, Agricultural Research Center, 12619, Giza, Egypt.

## INTRODUCTION

Legume crops are essential for human nutrition. Due to their significant commercial worth, widespread production, consumer use and nutritional value (carbohydrates, protein, minerals, and vitamins), *Phaseolus vulgaris* (Bean) is considered the 3$^{rd}$ most important legume crop in the world (Nogueira *et al*., 2021). It has long been a staple food crop in many poor countries, and it is a key source of plant protein in both rural and urban settings. Diseases are a significant constraint on bean production. Based on its geographic location and agro-climatic conditions, Egypt has a major comparative advantage in the production of horticulture commodities, particularly green bean for export. Egypt is the leading exporter of green bean in the Netherlands, with a 25% market share.

The genus *Trichoderma*, as one of the promising bio-control agents, has been described for control biologically of soil-borne phytopathogenic fungi (Eid, 2014; Sarhan *et al*., 2018 and Nofal *et al*., 2021). Different varieties of *Trichoderma* have been used to successfully manage plant diseases in a variety of crops (Elad and Kapat, 1999; Abd El-Moneim *et al*., 2012; Abdel-Ghany *et al*., 2018 and Abdel-Ghany and Bakri, 2019). Biocontrol agents such as *Trichoderma* spp. are reported to be effective, ecofriendly, and inexpensive, negating the negative impacts of pesticides. As a result, these biocontrol agents have recently been identified to work against a variety of critical soil-borne plant pathogens that cause major crop illnesses. Considering the high expense of chemical pesticides and the risks they entail; biological plant disease control looks to be a cost-effective and environmentally benign method that is used all over the world. *Trichoderma* spp. which are commonly isolated from soil and are found in plant root ecosystems, are one of the most significant bioagents (Whipps and Lumsden, 2001; Harman *et al*., 2004; Abdel-Ghany *et al*., 2009 and Sarhan *et al*., 2018). They colonise

plant roots and rhizospheres and inhibit plant infections by a variety of processes, including mycoparasitism, competition, induced systemic resistance and antibiosis production, improvement of plant health through boosting growth of plant and root growth stimulation (Harman *et al*., 2004 and Sarhan *et al*., 2018). *Trichoderma* spp. are an essential biocontrol agent that are used to treat a variety of ailments (Harman *et al*., 2004.). *Trichoderma* spp. are found in soil and root environments and are free –living fungi that aid plant growth (Yedidia *et al*., 2001). In various crop plants, *Trichoderma* spp. are useful in controlling soil/seed-borne fungal infections (Kubicek, 2001 and Eid, 2014). *Trichoderma* spp. have been used to manage soil-borne plant diseases for biological control (Mohamed *et al*., 2020).

*Rhizoctonia solani* thrives in the soil as sclerotia or within sick host material. It can live for years in soil, especially because so many plants are hosts (Kankam *et al*., 2021). When bean seeds are put in fungus-infested soil, the fungus damages the growing seedling's young radicle or hypocotyl. Seedling roots rots and dies in damp settings, while surviving seedlings develop root or collar rots. On pods formed near to the ground, pathogen causes circular rots by splashing inoculum on these pods. Sclerotia germinate in damaged host tissue and survive in the soil (Farr *et al*.,1989).

Pythium root rot is a widespread crop disease caused by species of organisms known as Pythium, sometimes known as water moulds (Whipps and Lumsden, 1991). Pythium damping off, which kills newly emerging seedlings in fields and greenhouses, is a widespread problem. *Pythium* spp. are exceedingly generalist and unspecific in their host range, causing widespread and catastrophic root rot that is sometimes difficult to prevent or control (Jarvis and Colleagues, 1992). The goal of this study was to demonstrate how the effective bioagents, *Trichoderma* isolates that isolated from Egyptian fields, control the soil-borne phytopathogenic microorganisms, *P. ultimum* and *R. solani in vitro.*

## MATERIALS AND METHODS

**The Isolation of *Rhizoctonia solani* and *Pythium ultimum* and their identification:**

From several districts of Menoufia governorate (Kafer-Dawood, Shebin-Elcom) and Behera governorate (Bader), the infected bean roots were collected and cleaned by tap water then washed in sterilezed water. Roots were cut into little pieces by a sterilezed scalpel (1-2 cm). For surface sterilization, the pieces were submerged in 1.0% solution of hypochlorite for three minutes, placed numerous times in sterilized water and dried on sterilized filter papers. The pieces were then put into Petri dishes containing potato dextrose agar (PDA) medium treated by antibacterial agent (L-chloramphenicol 5mg/L and streptomycin sulphate 5mg/L). The plates were incubated at 25°C for five to seven days, they were examined for fungal development. The pure cultures of the isolated fungi were identified according to their, morphological and microscopical characteristics as described by Domsch *et al.* (2007)**.**

***Trichoderma* spp. isolates:**

Samples of soil around healthy bean roots were collected from various localities of Egypt (Table, 1). By using PDA and *Trichoderma* specific media (TSM) via a dilution plate approach *Trichoderma* spp. were isolated from the rhizospheric soil samples (Johnson, 1957). The hyphal tip technique was used to purify all Trichoderma isolates (Tuite, 1996). Periodical transfers on PDA and TSM slants under aseptic conditions were used to keep the isolated *Trichoderma* spp. cultures fresh and viable throughout the investigation.

**Dual culture experiment:**

The antagonistic activity of *Trichoderma* isolates was investigated by using a dual culture method against the tested phytopathogenic fungi (Morton and Stroube, 1955). Inocula of *Trichoderma* spp. and the tested fungi were spaced 6 cm apart. For each treatment, three duplicates were kept and incubated for seven days at 25°C. As a control, two monoculture plates were used. Radial growth of the tested fungus and *Trichoderma* spp. was measured six days after *R. solani* and *P. ultimum* were incubated. Colony diameter of the tested fungi in a dual culture plate was measured and compared to the control (Vincent, 1947). The percentage of radial growth inhibition (percent RGI) was computed using the formula:

$$\text{Radial growth inhibition\%} = (C\text{-}T)/C \times 100$$

**Where:**

**C**= The growth of the tested fungus with the absence of the antagonist

**T**= The growth of the tested pathogen with the antagonist

**Identification of selected *Trichoderma* isolates:**

*Trichoderma* isolates that produced total overgrowth (reached to 100% inhibition) of the

phytopathogenic fungi were chosen for species identification based on colony characteristics, conidia, phialides, conidiophores and mycelium structure (Kubicek and Harman, 2002). Mycology Center, Assiut University, Egypt, validated the identification.

**Filtrate Enzymes assays and antibiotics of selected *Trichoderma* isolates:**

**Cellulase activity:**

A suitable portion (100 µL) of supernatant from the culture and 400 µL of 100mM sodium citrate buffer (pH 5.2) were incubated, containing 1% carboxy methyl cellulose (CMC) (Collmer *et al*., 1988). Glucose which released was quantified using the substance of Dinitrosalicylic acid (DNSA) technique after a 15-minute incubation at 55°C (Sadasivam and Manickam, 1992). In a test tube, a known volume of aliquot was placed, and the last volume of 1.0 mL was adjusted by distilled water. 0.5 mL of DNSA reagent (200mg crystalline phenol, 50mg sodium sulphite,1g of DNSA and 100mL of sodium hydroxide) was added then thoroughly mixed. For 5 minutes, contents were boiled. One mL of sodium potassium tartrate (40 %) was added then chilled while contents of the tubes were still warm. With distilled water, the final volume was adjusted to 5.0 mL and the spectrophotometer was read at 540nm. A reagent blank (1.0 mL distilled water) treating it by the same way as the aforementioned process. Following the aforesaid technique, a known concentration of glucose standard was calibrated, and enzyme activity was expressed as needed.

**Polygalacturonase (PG) activity:**

Supernatant (100 µL) from the culture and 400 µL of 50 mM buffer (pH 5.2) sodium acetate were incubated with 0.25 percent sodium polypectate (Collmer *et al*., 1988). The galacturonic acid released was quantified using the DNSA technique after one hour of incubation at 37°C (Sadasivam and Manickam, 1992).

**Chitinase activity:**

Two hundred µL 0.5 % percent chitin and 400 µL buffer (pH 5.2) of 10 mM sodium acetate buffer plus 100µL supernatant of the culture were combined in a reaction mixture and incubated for 1 hour at 50°C (Boller and Mauch, 1988) at 50°C. Dimethylamino benzaldehyde (DMAB) was used to evaluate the production of sugar N-acetylglucosamine (Reissig *et al*., 1955). An amount from the reaction mixture (0.5 mL) was placed in a test tube, along with buffer of (pH 8.9) of 120mM potassium borate buffer 0.5mL. Then tubes were aggressively cooked in a water bath for 3 min. then cooled. After that, the reagent of DMAB 3 mL (500 mL glacial acetic acid and 5.0 g DMAB were dissolved, containing 12.5 percent v/v of 10 N HCl, held at degree of 20°C as a stock then diluted with 9 volume glacial acetic acid prior to use) were added to all tubes then incubated at 38°C for twenty minutes. After cooling the tubes, the absorbance was measured in a spectrophotometer at 544 nm. Following the foregoing process, the standard of N-acetylglucosamine was produced in borate buffer then tested. N-acetylglucosamine was determined and expressed in the right way.

**β-1, 3 glucanase activity:**

Reaction mixture contained buffer (pH 5.2) of 50 mM sodium acetate and 100 µL of 4% laminarin and 100 µL from supernatant of the culture (Kauffman *et al*., 1987). After incubation at 37ºC for 10 min, released glucose by enzyme β-1,3 glucanase was measured by DNSA method (Sadasivam and Manickam, 1992).

Cellulase, PG, b-1,3 glucanase and chitinase, specific activity measured in Unit.mg-1 protein. However, enzyme quantity required for create one mM of matching the reducing sugar per minute per mL of culture supernatants was defined as unit activity. Non-enzymatic controls were also carried out with boiling enzymes, and the results were subtracted from the enzymatic results.

**Protease activity:**

Reaction mixture containing 500 mL enzyme solution, 500 mL of 0.36 percent casein, plus 2.0 mL buffer (pH 3.6) of 100 mM acetate. The reactions were allowed to run for one hour at 50°C then 3 mL of 5% trichloroacetic acid were added (Malik and Singh, 1980). The blank was treated as if it had been incubated for no time. After centrifuging the reaction mixtures 5000 rpm to ten minutes, the known volume from supernatants (500µl) was utilized to estimate the liberated free amino acids by using technique of the ninhydrin (Lee and Takahashi, 1966). Amount of the protein required for produce one gram of free amino acids per minute per one mL of culture supernatant expressed as Unit.mg-1 protein,

**Xylanase activity:**

Activity of Xylanase enzyme was carried out using the method described by Bailey *et al*. (1992). Oat spelt xylan (Sigma-Aldrich, St Louis, MO, USA) was used as a substrate. The released sugar was measured by Dinitrosalicylic acid (DNS) method using the glucose or xylose as standard (Miller *et al*., 1960).

**β-Glucosidase activities:**

The activities of -glucosidase were measured using the 4-nitrophenyl-D-glucopyranoside as the standard and *p*-nitrophenol as the control (Berghem and Petterson, 1974). The enzyme quantity that liberated substrate at a rate 1 mol per min was defined as one unit (U) of enzyme activity.

**Scanning Electron Microscopy (SEM) examination of prepared specimen:**

SEM was used to observe hyphal interaction between *R. solani* and *Trichoderma koningii*. To obtain sites of hyphal interaction, a PDA plate with a mycelial disc (5mm) cut from leading edge of both *R. solani* and *T. koningii* colonies was injected at a constant distance from the Petri dish's edge. Both of two fungi are growing in the same direction, and their hyphae have merged. By the light microscope, the plate cultures were examined after 6 days of incubation to ensure that they were still in early stages of hyphal interaction. The contact areas were noted then 1cm of the agar blocks were removed to prepare for SEM. The tissue processor model Lynexel from Leica was used to prepare the samples. The mycelial samples which were taken from the interaction zone were fixed by osmium oxide then dehydrated with the series of ethyl alcohol dilutions before finally being dehydrated with acetone substance. Treated samples were subsequently dried by using an EMS 850 critical point dryer, coated with gold using an EMS 550 sputter coater and inspected using a SEM (JEOL100CX-ASID-4D).

**Statistical analysis:**

The Stat graphics Centurium XV program (Statistical software) was used to perform the analysis of variance (ANOVA), and the treatment means were compared by using Fisher's protected L.S.D. test at 5% probability according to the procedure outlined by Snedecor and Cochran (1989).

## RESULTS

**Isolation of fungi associated with bean root rots:**

Microorganisms associated with bean root rots were isolated from rotted samples collected from different localities (Table 1). The isolates were purified and identified according to their morphological characteristics. *Phthium ultimum* and *Rhizoctonia solani* were the most dominant. *P. ultimum* 4413 AUMC and *R. solani* 6590 AUMC were identified at Assiut's Mycology Center, Assiut University.

**Isolation of *Trichoderma* isolates:**

Twenty-three localities in Egypt were sampled for rhizosphere soils (Table 1). From soil samples, *Trichoderma* isolates were isolated using the potato dextrose agar medium and selective medium of *Trichoderma*. Colony characteristics, growth, mycelium structure, conidiophores, phialides, and conidia were used to identify the isolated species. The hyphal tip approach was used to purify all *Trichoderma* spp. *Trichoderma* spp. were preserved on PDA and TSM slants.

**Table1. Sources of Trichoderma isolates**

| No. | Governorates | Locations | Host / habitat | Isolate No. |
|---|---|---|---|---|
| 1 | Matrouh | El-Alamain | Apple | T10 |
| 2 | Kafrelsheikh | Kafrelsheikh | Pigeons manure | T9 |
| 3 | El-Beheira | Wadi-Elnatroun | Grape | T15 |
| 4 | | Wadi-Elnatroun | Grape | T16 |
| 5 | | Wadi-Elnatroun | Grape | T17 |
| 6 | | Housh-Eiasa | Pepper | T18 |
| 7 | | Housh-Eiasa | Maize | T19 |
| 8 | Menoufia | Elkhatatba | Grape | T1 |
| 9 | | Elkhatatba | Tomatoes | T2 |
| 10 | | Elkhatatba | Banana | T3 |
| 11 | | Elkhatatba | Banana | T4 |
| 12 | | Elkhatatba | Banana | T5 |
| 13 | | Sadat City | Oxidation pond | T14 |
| 14 | | Menof City | Tomatoes | T11 |
| 15 | | Menof City | Beans | T20 |
| 16 | | Menof City | Clover | T21 |
| 17 | Giza | Abo-Galleb | Pigeons manure | T7 |
| 18 | | Abo-Galleb | Pigeons manure | T8 |
| 19 | | Abo-Galleb | Pomegranate | T12 |
| 20 | Fayoum | Attsa | Cucumber | T13 |
| 21 | | Attsa | Tomatoes | T22 |
| 22 | Minia | El Minia | Pigeons manure | T6 |
| 23 | Assiut | Assiut | Pomegranate | T23 |

**The antagonistic activity of *Trichoderma* against *P. ultimum*:**

*Trichoderma* spp. effectively inhibited the mycelial growth of *P. ultimum* (Table 2 and Fig. 1). Eight isolates T2, T3, T4, T5, T11, T13, T20 and T22 showed completely overgrowth on *P. ultimum*. Isolate T7 caused the lowest inhibition in mycelial growth (68.11%) of the tested pathogen.

**The antagonistic activity of *Trichoderma* against *R. solani*:**

Results in Table (3) and Fig. (2) show the screening of twenty-three local isolates of *Trichoderma* for their antagonistic potential against *R. solani*. Results show that *Trichoderma* isolates significantly inhibited *R. solani* mycelial growth and the most effective *Trichoderma* isolates were T2, T5, T11, T13, T14, T18, T20, T21 and T22 which showed completely overgrowth. The lowest inhibition (73.31%) was due to isolate T19.

**Table (2): The antagonistic activity of *Trichoderma* isolates against the mycelial growth of *P. ultimum***

| *Trichoderma* isolates | * Growth of mycelium (mm) | Inhibition % |
|---|---|---|
| Control | 90.00a | 0.0 |
| T1 | 20.66e | 77.04 |
| T2 | 0.00h | 100 |
| T3 | 0.00h | 100 |
| T4 | 0.00h | 100 |
| T5 | 0.00h | 100 |
| T6 | 27.33bc | 69.63 |
| T7 | 28.66b | 68.11 |
| T8 | 12.66f | 86.00 |
| T9 | 26.66bc | 70.44 |
| T10 | 26.00bc | 71.11 |
| T11 | 0.00h | 100 |
| T12 | 6.00g | 93.33 |
| T13 | 0.00h | 100 |
| T14 | 24.33dc | 73.00 |
| T15 | 22.33de | 75.22 |
| T16 | 15.00f | 83.33 |
| T17 | 14.00f | 84.44 |
| T18 | 15.66f | 82.56 |
| T19 | 24.33dc | 73.00 |
| T20 | 0.00h | 100 |
| T21 | 21.00de | 76.67 |
| T22 | 0.00h | 100 |
| T23 | 21.00e | 76.67 |
| L.S.D. | 3.32 | - |

* Values are means of 3 replicates, the means the having the same alphabetical letter in the column, using Duncan's multiple range test procedure at p= 0.05 level of significance.

**Table (3): The antagonistic activity of *Trichoderma* isolates against mycelial growth of *R. solani***

| *Trichoderma* isolates | * Growth of mycelium (mm) | Inhibition % |
|---|---|---|
| Control | 87.33a | 0.0 |
| T1 | 16.30gh | 81.33 |
| T2 | 0.00j | 100 |
| T3 | 20.73dc | 76.26 |
| T4 | 19.33fde | 77.89 |
| T5 | 0.00j | 100 |
| T6 | 17.70fge | 79.73 |
| T7 | 18.00fge | 79.38 |
| T8 | 17.00fge | 80.53 |
| T9 | 19.00fde | 78.24 |
| T10 | 15.00h | 82.02 |
| T11 | 0.00j | 100 |
| T12 | 0.00j | 100 |
| T13 | 0.00j | 100 |
| T14 | 0.00j | 100 |
| T15 | 15.33h | 82.47 |
| T16 | 12.33i | 85.91 |
| T17 | 22.00bc | 74.79 |
| T18 | 0.00j | 100 |
| T19 | 23.33b | 73.31 |
| T20 | 0.00j | 100 |
| T21 | 0.00j | 100 |
| T22 | 0.00j | 100 |
| T23 | 18.73fde | 78.58 |
| L.S.D. | 2.25 | - |

* Values are means of 3 replicates, the means the having the same alphabetical letter in the column, using Duncan's multiple range test procedure at p= 0.05 level of significance.

**Identification the selected *Trichoderma* isolates:**

*Trichoderma* isolate T5 caused completely overgrowth (100% inhibition) against both of *P. ultimum* and *R. solani*. This isolate was identified to the species level as *Trichoderma koningii* Oud by Mycological center, Assiut University, Egypt.

**Analysis of the fungal filtrate of the most active *Trichoderma* isolate (*T. koningii*):**

Filtration of the fungal culture of *T. koningii* was carried out with a sterilized bacterial filter under aseptic conditions yielded the isolate T5 filtrate. The enzyme assay and identification of antibiotics produced in culture filtrate were detected in fungal filtrate. The experiment was carried out at the Research labs Unit of the National Research Center (Giza), T5 isolate produced 0.98 u.mg-1 protein as chitinase, an enzyme that hydrolyzes chitin in fungal infections. As demonstrated in Table (4). T5 isolate filtrate produced a high amount of protease enzyme, being 6.34 u/mg protein (5).

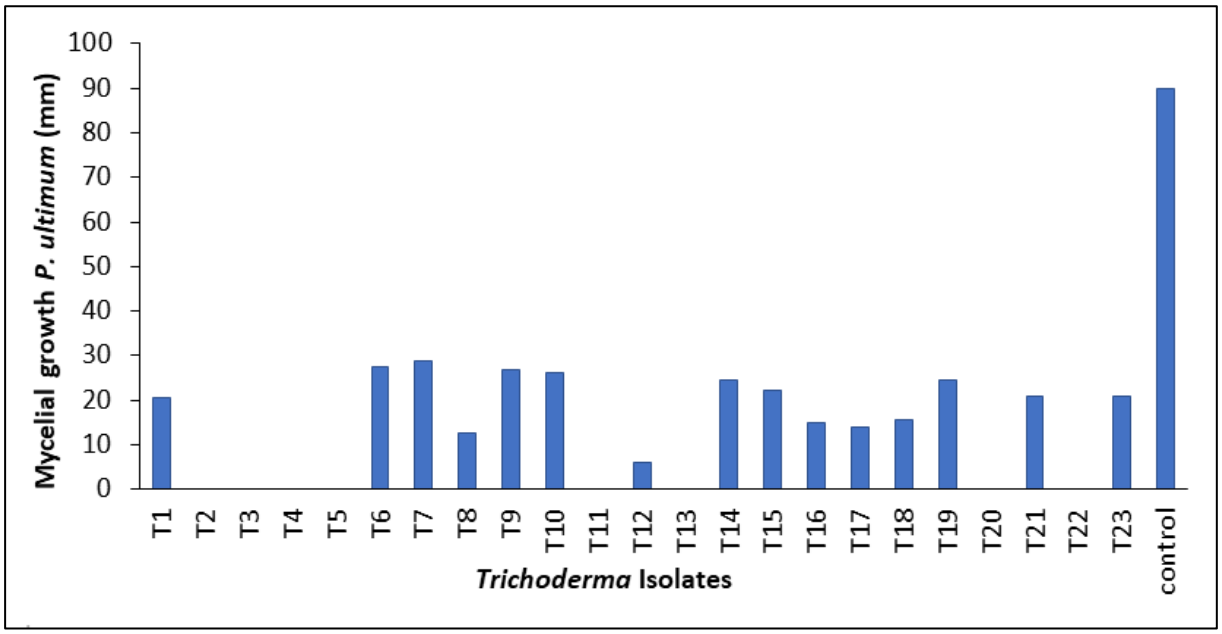


**Fig. (1): Antagonistic activity of *Trichoderma* isolates against the mycelial growth of *P. ultimum*.**

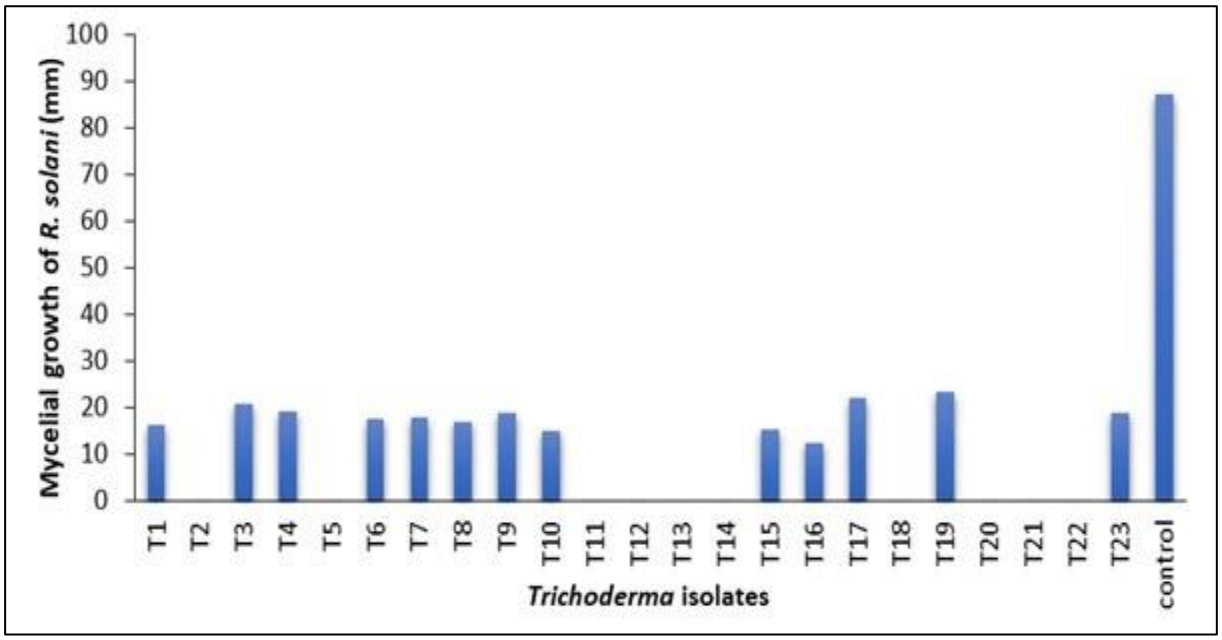


**Fig. (2). The antagonistic activity of *Trichoderma* isolates against mycelial growth of *R. solani***

**Table (4). Analysis of the fungal filtrate of the most active *Trichoderma* isolate *(T. koningii*).**

| No. | Enzyme (U.mg$^{-1}$ protein) | *Trichoderma* isolate T5 |
|---|---|---|
| 1 | Chitinase | 0.98 |
| 2 | β, 1-3-exoglucanse | 2.12 |
| 3 | Protease | 6.34 |
| 4 | Cellulase | 1.92 |
| 5 | Poly galacturonase | 3.42 |
| 6 | β-glucosidase | 1.12 |
| 7 | Xylanase | 3.12 |

**Observations on the mycoparasistic nature of *T. koningii* by SEM on the tested phytopathogenic fungi.**

In a scanning electron microscope, the interaction zones of mycelium samples of *R. solani* and *T. koningii* were examined. The process of mycoparasitism depicted in micrographs by SEM (Fig. 3). The hyphae of *T. koningii* develop parallel with the hyphae of the host (*R. solani*) then adhere to the host's surface, then coiling and form structures called appressoria on the surface of *R. solani* hyphae. Finally, walls of *R. solani* were seen to be lysed.

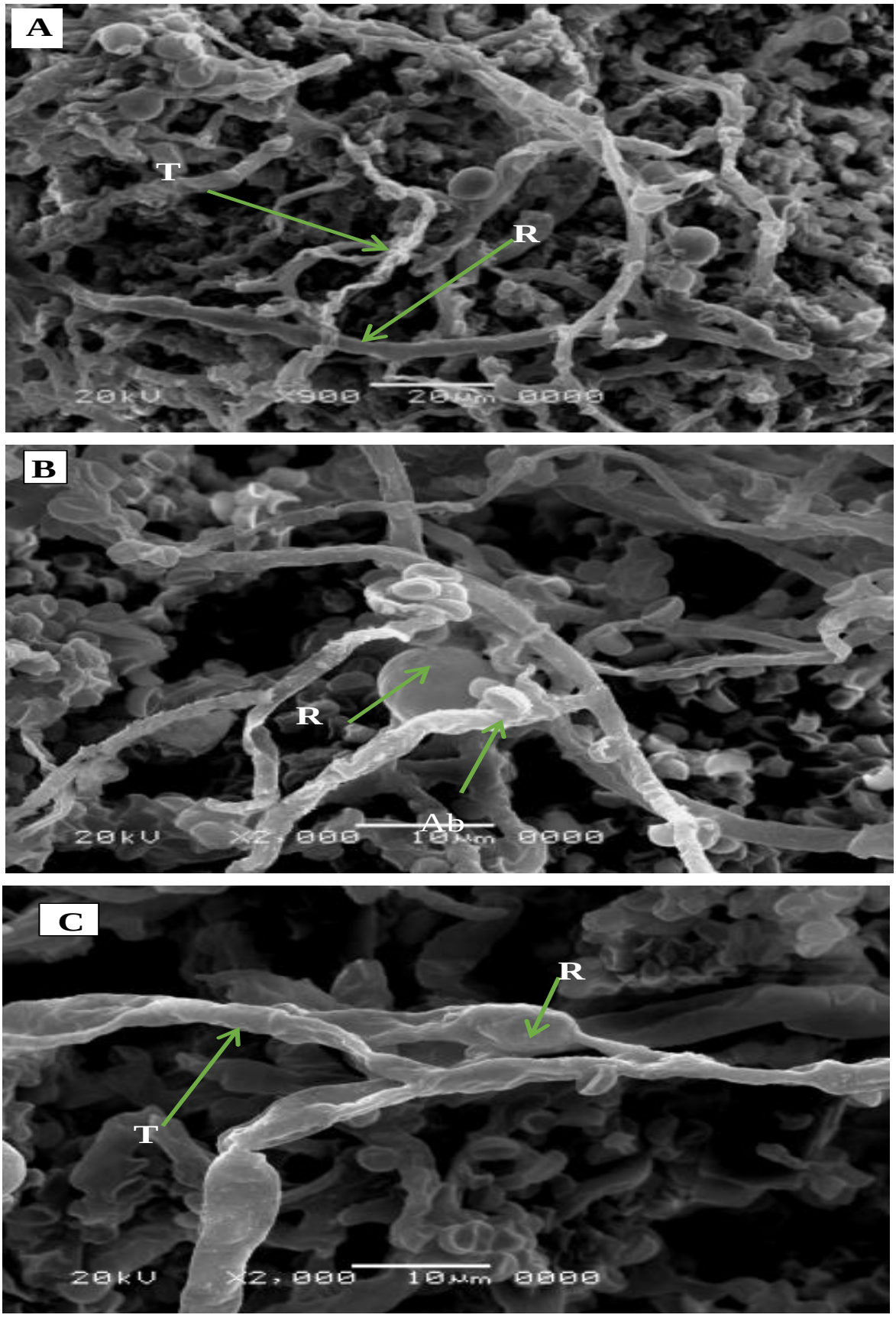


**Fig. (3): Observations on the mycoparasistic nature of *T. koningii* by SEM on the tested phytopathogenic *R. solan*. A, growth of *Trichoderma* hyphae (T) parallel to hyphae of *R. solan* (R) and colied (T) around (R); B, *Trichoderma* hyphae (T) stcking with hyphae of *R. solan* (R), and the *Trichoderma* hyphae formed appressoria like structures (Ab); C, finally hyphal walls of *R. solan* are lysed.**

## DISCUSSION

The goal of this research was to determine if the isolated locally antagonistic *Trichoderma* spp. might be used as bioagents against *P. ultimum* and *R. solani*, which are responsible for bean damping off and root rot diseases. *Trichoderma* spp. are a known antagonists against plant pathogens and has been found to be an effective biological control agent for a variety of soil-borne plant pathogenic fungi (Whipps and Lumsden, 2001). *Trichoderma* spp. are useful for biological control because of the several mechanisms through which it inhibits the growth of other fungi (Harman *et al.*, 2004; Abd El-Moneim *et al*., 2012 and Sarhan *et al*., 2018). *In vitro* tests, it was demonstrated that isolates of *T. koningii* extracted from the soil around healthy bean plants showed significantly antagonistic activity against *P. ultimum* and *R. solani*. *Trichoderma* spp. have long been recognized for their ability to act as biological control agents against phytopathogenic fungi in a variety of crops (Rojo, 2007). *T. koningii, T.viride* and *T. harzianum* protected seedlings of bean plants from *R. solani* infection during germination (Abou-Zeid *et al*., 2003; Abd-El-Khair *et al*., 2010 and Abd El-Moneim *et al*., 2012)**.** Results of the current investigation showed that all *Trichoderma* isolates have antagonistic potential. In dual *in vitro* tests, *Trichoderma koningii* inhibited mycelial growth of *R.solani* and *P. ultimum*. The proportion of plant pathogens were *P. ultimum* (68.11 to 100 percent) and *R. solani* mycelial growth inhibited by *Trichoderma* isolates (73.31 to 100 percent). Some of *Trichoderma* isolates were very suppressive to the growth of the pathogens studied, while others had just a minor effect. *Trichoderma* isolates grow considerably quicker in the medium and reduced the development of the tested fungi *in vitro*. *Trichoderma*'s competing mechanism is based on quick rate of their growth, which limits the space and nutrients for phytopathogenic fungi, potentially inhibiting *P. ultimum* and *R. solani*. The ability of effective biological control agents to grow considerably quicker than the pathogenic fungus inhibits the growth of target organisms, allowing them to compete effectively for space and nutrients (Harman *et al*., 2004). The most prevalent cause of death for microorganisms caused by starvation and the competition for restricted nutrients leads phytopathogenic fungi being biologically controlled. When conidia of phytopathogenic fungi require external nutrients in order to germ tube elongation and process of germination, then competition is successful (Elad, 2000 and Eid, 2014)**.** Mycoparasitism was a second pathogen control strategy that *Trichoderma* demonstrated. Mycoparasitic nature of the selected isolates of *Trichoderma* was demonstrated morphologically by subsequent profuse *Trichoderma* isolates sporulation and their ability to overgrow upon *P. ultimum* and *R. solani* mycelial growth in culture, possibly indicating their ability to directly parasitize on the pathogen. Species of *Trichoderma* compete for nutrients and space with fungal phytopathogens, affecting environmental circumstances, or stimulating plant development, antibiosis and mechanisms of plant defensive, or directly by using mycoparasitism processes (Bentez *et al*., 2004; Abd-El-Khair *et al*., 2010; Sarhan *et al*., 2018 and Nofal *et al*., 2021b)**.** Our results showed that the extracellular enzymes were released from the tested *Trichoderma* isolate (T5). This was in agreement with the results obtained by Thangavelu and Mustaffa (2010) and Sarhan *et al*. (2018). Many reports showed that the biological control of a wide range of plant pathogens such as *P. ultimum* and *R. solani*. by *Trichoderma* spp. the fragments of cell walls of phytopathogenic fungi released by the action of extracellular enzymes during mycoparasitic reaction**.**

## CONCLUSIONS

Plant infection with each of *P. ultimum* and *R. solani* disrupts plant growth and causes serious illnesses. Although numerous crop protection technologies are employed to manage such diseases, they are quite expensive and are deemed dangerous to the environment and to long-term development. As a result, biocontrol is a safer and more cost-effective technique of controlling these serious diseases while also facilitating improved plant development, yield, and production. It seems that *Trichoderma* spp. are the most beneficial, as they provide a higher level of safety with the least amount of environmental damage. Through numerous mechanisms such as mycoparasitism, competition, synthesis of several lytic enzymes, and other antimicrobial properties, this symbiotic fungus demonstrates considerable biocontrol activity against a wide spectrum of plant pathogenic fungi.

### LIST OF ABBREVIATIONS

**PDA:** Potato dextrose agar medium
**TSM:** *Trichoderma* specific media
**C:** Test pathogen growth with the absence of *Trichoderma*

**T:** Test pathogen growth with presence of *Trichoderma*
**CMC:** Carboxy methyl cellulose
**DNSA:** Dinitrosalicylic acid
**PG:** Polygalacturonase
**DMAB:** Dimethylamino benzaldehyde
**LSD:** Least significant difference
**AUMC:** Assiut University Mycology Center

## CONFLICTS OF INTEREST

The author(s) declare no conflict of interest.

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