BIOCONTROL OF PYTHIUM DAMPING-OFF ON PEPPER (CAPSICUM ANNUUM) WITH SELECTED FUNGAL AND RHIZOBACTERIAL AGENTS

Sabrine Mannai, Hayfa Jabnoun-Khiareddine*, Bouzid Nasraoui, Mejda Daami-Remadi

* Higher Agronomic Institute of Chott-Mariem, University of Sousse, Chott-Mariem, Tunisia.

b UR13AGR09-Integrated Horticultural Production in the Tunisian Centre-East, Regional Research Centre on Horticulture and Organic Agriculture, University of Sousse, Chott-Mariem, Tunisia.

c Laboratory Research/Bio-aggressors and Integrated Pest Management in Agriculture (LR/BPIA), National Agronomic Institute of Tunisia, Tunis, University of Carthage, Tunisia.

d National Agronomic Research Institute of Tunisia (INRAT), University of Carthage, Tunis, Tunisia.

ARTICLE INFO

Article history
Received: December 19, 2019
Revised: March 18, 2020
Accepted: March 31, 2020

Keywords
Aggressiveness
biological control
disease severity
pepper

ABSTRACT

Pythium ultimum is a common soilborne pathogen causing serious losses of pepper seedlings in nurseries and few weeks post-planting. Two pepper associated- P. ultimum isolates (P1 and P2) were shown pathogenic to pepper cv. Altar causing post-emergence damping-off with P2 isolate being the most aggressive. Fungal and bacterial antagonists have been evaluated in vitro and in vivo for their ability to suppress P. ultimum. In dual culture assay, Trichoderma harzianum, T. viride and Gliocladium virens inhibited pathogen radial growth by 18.54, 17.52 and 15.24%, respectively, relative to control, while none of the tested bacteria was shown able to significantly inhibit pathogen growth. However, drastic changes in pathogen hyphae expressed as strong lysis, the formation of mycelial cords and mycoparasitism have been observed. Pepper seeds treated with fungal antagonists’ conidial suspensions showed 60, 50 and 60% less pre-emergence damping-off infections, respectively, compared to the positive control. When tested as root dipping, only G. virens resulted in 40% reduced post-emergence damping-off. An improved seedlings fresh weight, by 79.31 and 76%, was respectively induced by G. virens, and T. viride-based treatments while an increment of 27.58, 25.33 and 22.22% was recorded following treatments with G. virens, T. viride and T. harzianum, relative to the positive control. The majority of tested bacterial isolates, applied as a seed treatment, had significantly improved the emergence percentage of inoculated seedlings as compared to control with Burkholderia glathei isolate 35 being the most efficient. When applied as root dipping, reduction of post-emergence damping-off ranged between 40 and 100% with Pseudomonas aureofaciens isolate 314 being the most effective agent. Seedlings treated with P. aureofaciens isolates 314 and 31, Bacillus pumilus (420) showed 35.38 and 28.51% higher heights, respectively. Plant weight was enhanced by 73.06, 61.18, 77.39, 61.8 and 67.93% over control following treatments with P. aureofaciens isolates 314 and 31, Bacillus pumilus 420, P. fluorescens and P. putida 227.

Corresponding Author: Hayfa Jabnoun-Khiareddine
Email: jkhayfa@yahoo.fr
© The Author(s) 2020.

INTRODUCTION

In Tunisia, pepper (Capsicum annuum L.) is an economically important crop coming right after tomato and potato in terms of cropped vegetable areas, of about 20 000 ha (Anonymous, 2019), and with an average production of about 346 000 tons during the last five
years (Anonymous, 2019). It is widely grown in almost all Tunisian regions both on open fields, for season and late season crops, and under plastic greenhouses for the off-season product (Zhani et al., 2012). These crops ensure a continuous supply of pepper market and Tunisia ranking as one of the major of pepper producers and exporters in Africa (STAT, 2013).

However, soilborne fungal and fungal-like diseases are major yield limiting factors in pepper production in Tunisia and worldwide. Among them, damping-off is a serious disease complex which involves germination failure, prevention of seedling emergence after germination, or the rotting and collapse of seedlings at the soil level (Lamichhane et al., 2017). *Pythium* spp. *Fusarium* spp., *Rhizoctonia* spp., and *Phytophthora* spp. are the most frequently important pathogens associated with vegetable crops such as pepper.

Two types of the damping-off disease occur in plants susceptible to *Pythium*, pre-emergence and post-emergence which are economically important worldwide (Whipps and Lumsden, 1991). *Pythium* species responsible for pre-emergence damping-off can attack seeds or seedlings below the soil line before emergence, while post-emergence damping-off symptoms occur when seedlings decay, wilt, and die just after emergence (Lamichhane et al., 2017).

*Pythium* species can cause more than 60% seedling mortality both in nursery and in main field (Manoranjitham et al., 2000). In addition to damping-off disease, *Pythium* spp. can cause wilt in older seedling and mature plants. Yield loss due to these pathogens in different crops has been estimated approximately of multibillion dollar worldwide (van West et al., 2003). Among *Pythium* species, *P. ultimum* is one of the most pathogenic and problematic species that cause seed and seedling losses in nurseries in the first weeks after sowing (Whipps and Lumsden, 1991).

Given the complex nature of Pythium damping-off disease and the numerous factors involved in its occurrence, its management is still very difficult. In fact, due to the soilborne nature of these microorganisms, their wide host range, the prolonged survival of their propagules in the soil, the lack of resistant cultivars and the ability of *Pythium* spp. to develop resistance against recommended pesticides, strategies to control Pythium damping-off diseases are limited (Li et al., 1995).

Continuous efforts have been emphasized on developing effective biocontrol agents for the management of these diseases, which are considered as economically viable alternatives, ecologically sustainable and safe crop protection solutions (Khare and Upadhyay, 2009; Muthukumar et al., 2011).

Considerable research has been done to investigate the potential that offers various microbial agents including filamentous fungi, yeast, bacteria and actinomycetes, for the biocontrol of Pythium damping-off. In the last three decades, several antagonists were used against *Pythium* spp., for reducing their growth and preventing their establishment in the rhizosphere (Howell, 2003). Various fungal agents belonging to *Trichoderma* and *Gliocladium* genera i.e. *Gliocladium virens*, *Trichoderma harzianum* and *T. hamatum* are among the most commonly studied biocontrol agents which effectiveness has been demonstrated against not only *P. ultimum* (Naseby et al., 2000) but also towards several damping-off causative pathogens such as *R. solani* (Lewis and Papavizas, 1987; Mannai et al., 2018). The successful application of these species for the management of damping-off in chili pepper and tomato has been previously reported (Jayaraj et al., 2006; Muthukumar et al., 2011). The combination of the biocontrol agents, *Pythium nunn* and *T. harzianum* isolate T-95 reduced Pythium damping-off disease in cucumber grown under greenhouse conditions (Paulitz et al., 1990). *G. virens* was among the most consistent and effective agents in controlling damping-off of zinnia, cotton, and cabbage seedlings incited by *P. ultimum* (Lumsden, 1989).

Several bacterial species belonging to *Pseudomonas*, *Bacillus*, *Burkholderia* and *Streptomyces* genera have also been used to manage *Pythium*-induced diseases on pepper and many other vegetable crops (Naseby, Pascual, et al., 2001; El-Mohamedy, 2012; Khabbaz and Abbasi, 2014). Numerous studies have demonstrated the potential of different *P. fluorescens* strains as biocontrol agents of *P. ultimum* (Ramamoorthy et al., 2002; Carisse et al., 2003). For instance, *P. fluorescens* isolate Pf1 was effective in reducing the damping-off incidence in tomato and hot pepper crops grown under greenhouse and field conditions (Ramamoorthy et al., 2002). Hultberg et al. (2000) studies recorded the biocontrol potential of specific strains of *P. fluorescens* against damping-off of tomato seedlings. Gravel et al. (2005) showed the efficiency of *P. fluorescens* to suppress tomato damping-off caused by either *P. ultimum* or *P. aphanidermatum* in Rockwool. Two *B. subtilis* strains were shown effective against *P. ultimum* and exhibited
effective control of damping-off on cauliflower (Abdelzaher, 2003).

Presently, in Tunisia, none of the pepper cultivars used in greenhouse or open field crops showed satisfying levels of resistance to Pythium spp. infections and no biocontrol studies have been done for this pathogen. Therefore, the objectives of the present study were: (i) to evaluate the virulence of two P. ultimum isolates involved in pepper damping-off (ii) to select the most effective fungal and bacterial isolates in suppressing P. ultimum mycelial growth and pepper damping-off disease and in improving plant growth.

MATERIALS AND METHODS

Plant Material
Pepper cv. Atlar seeds and seedlings were used in this study. This cultivar was widely used in Tunisia. After surface disinfection with 5% sodium hypochlorite solution for 5 min, three rinsings with sterile distilled water (SDW) and air drying, pepper seeds were sown in 77 cell-plates containing sterilized peat and kept under greenhouse conditions for 30 days. Seedlings were watered as needed.

Pathogen Culture and Inoculum Preparation
Two characterized P. ultimum isolates recovered from pepper seedlings exhibiting typical damping-off symptoms were used in the present study (Table 1). Root samples (1-2 cm in length) were surface-disinfested in sodium hypochlorite solution (3%) for 2 min, cut in small pieces (0.5 cm in length), were rinsed three times with SDW, dried on sterile filter paper and plated onto Potato Dextrose Agar (PDA) medium amended with streptomycin sulphate (100 mg/L) (w/v). Fungal cultures were incubated for 7 days at 25°C. Pythium growing colonies were cleaned up by successive sub-culturing. Pathogenicity of Pythium isolates collected was confirmed on pepper cv. Atlar seedlings fulfilling Koch’s postulates. Before use, Pythium isolates were cultured in the dark for 6 days at 25°C on PDA medium. P. ultimum inoculum was prepared by collecting mycelia and sporangia from five 6-day-old cultures grown on PDA and mixing them in 0.5 L of SDW. The resulting mycelial fragments and sporangia served for substrate inoculation.

Fungal and Bacterial Biocontrol Agents
Trichoderma harzianum (TH), T. viride (TV) and Gliocladium virens (GV) were the three selected biocontrol agents to be used in the current study (Table 2). These fungal agents, originally recovered from Tunisian soils, were selected based on their antifungal potential previously demonstrated against various soilborne plant pathogens such as Verticillium spp., Fusarium spp., and R. solani (Ayed et al., 2006; Jahnoun-Khiareddine et al., 2009; Soumaya et al., 2013).

Table 1. Pythium ultimum isolates used in this study.

| Isolates | Original host | Cultivar | Isolation site |
|----------|---------------|----------|---------------|
| P1       | Capsicum annuum | Beldi    | Chott-Mariem   |
| P2       | C. annuum     | Baklouti | Sahline       |

Table 2. Rhizobacterial isolates used in this study.

| Isolate codes | Bacterial species       | Origin                  |
|---------------|-------------------------|-------------------------|
| Pf            | Pseudomonas fluorescens | Tunisia (a reference bacterium) |
| 263           | Bacillus subtilis       | Tunisia                 |
| 227           | P. putida               | Tunisia                 |
| 31            | P. aureofaciens         | Tunisia                 |
| 420           | B. pumilus              | Missouri                |
| 35            | Burkholderia glathei    | Missouri                |
| 314           | P. aureofaciens         | Missouri                |
| 69            | P. huttiensis           | Missouri                |

To prepare biological treatments, mycelium from 7-day-old cultures grown on PDA medium was scraped off, homogenized with SDW, and then filtered through two layers of muslin. The resulting conidial suspension was adjusted to 10^7 spores/mL. Eight rhizobacterial isolates belonging to three genera, namely Pseudomonas, Bacillus and Burkholderia, were tested in this study (Table 2). They were isolated and identified in previous work.
(Nasraoui et al., 2007). Bacterial stock cultures were maintained at -20°C in Nutrient Agar (NA) amended with 40% glycerol. Before use, bacterial isolates were grown on NA and incubated at 25°C for 48 h. Bacterial cell suspensions used for *in vitro* and *in vivo* bioassays were prepared by scraping bacterial colonies, previously grown in NA for 48 h, in SDW and adjusted to 10⁶ cells/mL.

**Pathogenicity Tests**

*P. ultimum* isolates were challenged to pepper cv. Atlar seedlings to test their ability to cause damping-off disease. Thirty days-old pepper seedlings were transplanted in cell trays filled with sterilized peat mixed with *P. ultimum* inoculum at the rate of 1:3 (v/v). Seedlings transplanted in uninoculated peat were used as an untreated control. The experiment was conducted in a completely randomized design. Five pepper seedlings were used for each pathogen isolate. Trays were then incubated at 26/18°C (day-night temperatures). At five days post-transplanting, damping-off incidence, estimated as the percentage of wilted pepper seedlings, was noted for each *Pythium* isolate. Plant height (cm) and plant fresh weight (g) were also determined.

**In vitro Antagonism Assay**

The ability of fungal and bacterial agents to inhibit *P. ultimum* *in vitro* growth was assessed using the dual culture technique. Agar plugs (6 mm diameter) cut from 7-day-old pathogen cultures and fungal colonies were placed opposite to each other at the peripheries of 9-cm Petri plate containing PDA. Six plates were used per each individual treatment and the experiment was repeated twice. For the screening of bacterial agents, dual confrontation was performed by depositing two agar plugs (6 mm in diameter) carrying the pathogen equidistantly with respect to the tested bacterial agent seeded in the form of a line carried along the axis of the plate using a sterile rod soaked with the bacterial suspension. Control plates were challenged with pathogen plugs and the bacterial suspension was replaced by SDW. Three plates were used per individual treatment and the experiment was repeated twice. All cultures were incubated at 25 °C for 2 days. The diameter of the pathogen colony was measured, together with macroscopic and microscopic observations made to characterize the *in vitro* pathogen-antagonist interactions more focused on hyphal alterations. Percentage growth inhibition (%) of the pathogen was calculated according to the following formula: Growth inhibition % = [(dc-dt)/ dc] × 100, Where dc = Colony diameter in control plates; dt = Colony diameter in treated plates.

**In vivo Biocontrol Trials**

Two biocontrol trials were carried out to evaluate the ability of the tested fungal and bacterial agents in reducing pre- and post-emergence damping-off, incited by *P. ultimum*.

**Evaluation of Pre-Emergence Damping-Off Suppression Ability**

Ten pepper cv. Atlar seeds were soaked for 10 min in each antagonist suspension prepared as previously described. Treated seeds were sown in cell trays filled with sterilized peat mixed with the inoculum of the virulent *P. ultimum* isolate (P2) at a rate of 1:3 (v/v). Trays were then kept at room temperature (25-30 °C). Pre-emergence damping-off percentage was noted 15 days post-treatment, based on the number of non-emerged seeds in relation to the number of total sown seeds as follow:

Pre-emergence damping %= germination in untreated control–germination in treatment/ germination in untreated control.

**Evaluation of Post-Emergence Damping-Off Suppression Ability**

Pepper cv. Altar seedlings (30-day-old) were treated by root soaking in the antagonist suspension for 30 min. Then, treated seedlings were transplanted in cell trays filled with peat previously infected with *P. ultimum* isolate P2 inoculum at the rate of 1:3 (v/v). Control seedlings were root soaked in SDW and transplanted in pathogen-inoculated and pathogen-free substrates (for positive and negative controls, respectively). All treated seedlings were incubated under growth chamber conditions (at 27-30/15-18°C day-night temperatures). Five repetitions were used per each individual treatment. Three parameters were recorded five days post-transplanting: (i) plant height, (ii) plant fresh weight and (iii) post-emergence damping-off percentage. Post-emergence damping-off (%) was calculated as follow: Post-emergence damping-off (%) = number of infected seedlings in untreated control–number of infected
seedlings in treatment/ number of infected seedlings in the untreated control.

**Statistical Analysis**

Results from the current study were subjected to one-way analysis of variance and means separations were carried out using the Student-Newman-Keuls (SNK) test at \( P \leq 0.05 \). ANOVA was performed using the Statistical Package Social Sciences (SPSS) software version 20.0. Experiments were conducted according to a completely randomized design for *in vitro* (6 replicates), pre-emergence damping-off (10 replicates) and post-emergence damping-off trials (5 seedlings per individual treatment).

**RESULTS**

![Figure 1](image1.png)

Figure 1. Comparison between a pepper cv. Altar seedling inoculated with *Pythium ultimum* P2 isolate (at right) and an uninoculated control (NIC) one (at left).

Table 3. Effects of *Pythium ultimum* isolates on damping-off incidence and some growth parameters of pepper cv. Altar noted five days post-inoculation.

| Treatments | Damping-off incidence (%) | Plant weight (g) | Plant height (cm) |
|------------|---------------------------|------------------|------------------|
| NIC        | 0.00 ±0.00 a               | 0.60±0.04 a      | 4.40±0.07 a      |
| P1         | 40.00±0.00 b               | 0.52±0.12 a      | 4.16±0.19 a      |
| P2         | 100.00±0.00 c              | 0.20±0.03 b      | 3.40±0.23 b      |

Within each column, values followed by the same letter are not significantly different according to SNK test at \( P \leq 0.05 \).

*Pythium ultimum* Biocontrol Using Fungal and Bacterial Bio-Agents

*In vitro* Biocontrol Activity Displayed by Fungal Agents

*P. ultimum* mycelial growth, noted after six days of incubation at 25 °C, varied significantly \( (P \leq 0.05) \) depending on fungal treatments tested. *T. harzianum*, *T. viride*, and *G. virens* reduced pathogen radial growth by 18.54, 17.52, and 15.24%, respectively, relative to the untreated control (Figure 2). Moreover, these antagonists grew and sporulated abundantly, invaded *P. ultimum* colonies and reach their opposite edge after six days of dual culture at 25 °C (Figure 3).

![Figure 2](image2.png)

Figure 2. Radial growth inhibition of *Pythium ultimum* noted after two days of dual culture with fungal antagonists as compared to control.

**Pathogenicity of *Pythium ultimum* Isolates**

At five days post-transplanting in *Pythium*-inoculated peat, all pepper cv. Altar seedlings exhibited typical damping-off symptoms with varying incidence depending on the isolate used for inoculation whereas the uninoculated control (NIC) seedlings remained symptomless. P2 isolate induced complete death of all pepper seedlings (Figure 1) compared to only 40% noted on P1-inoculated seedlings.

As given in Table 3, only P2 isolate significantly \( (P \leq 0.05) \) reduced plant weight and height by 66.66 and 22.72%, respectively, compared to pathogen-free control.
Microscopic observations made at the contact zone between *P. ultimum* and the biocontrol agents colonies revealed the formation of mycelium cords (Figure 4a) and the coiling of *T. harzianum*, *T. viride* and *G. virens* mycelia around pathogen mycelium (Figure 4b).

**In vitro Biocontrol Activity Displayed by Rhizobacterial Agents**

*P. ultimum* radial growth, noted after two days of incubation at 25 °C, did not vary significantly depending on tested bacterial treatments as compared to control. However, microscopic observations made at the contact zone between the tested rhizobacteria and *P. ultimum* showed hyphal lysis (Figure 5a) and formation of mycelial cords as a stress response to these biological treatments (Figure 5b).

![Figure 3](image1.png)

**Figure 3.** Antagonistic potential of *Trichoderma harzianum* (TH), *Gliocladium virens* (GV) and *T. viride* (TV) dual cultured with *Pythium ultimum*, noted two days after incubation at 25 °C, as compared to pathogen-inoculated and untreated control.

![Figure 4](image2.png)

**Figure 4.** Hyphal interactions at the confrontation zone between *Pythium ultimum* and *Trichoderma* spp.: (a) Mycelial cords of *P. ultimum* (c) treated with *T. harzianum*; (b) Coiling (co) of *T. viride* hyphae around pathogen mycelium (×400).

![Figure 5](image3.png)

**Figure 5.** *Pythium ultimum* mycelium lysis (a) and formation of mycelial cords (b) noted at the confrontation zone with of *Burkholderia glathei* 35 (a) and *Pseudomonas aureofaciens* 31 (b). L: Mycelial lysis; C: Mycelial cords (×400).
Biocontrol of Pepper *Pythium* Damping-Off Using Fungal Antagonists

**Suppression of Pre-Emergence Damping-Off**

The treatment of pepper cv. Altar seeds with the tested fungal antagonists improved the percentage of seedling emergence noted after 15 days of incubation, compared to the pathogen-inoculated and untreated control. *P. ultimum* pre-emergence damping-off was suppressed by 75, 62.50 and 75%, with *G. virens, T. viride* and *T. harzianum* based treatments, respectively, as compared to pathogen-inoculated and untreated control (Figure 6).

![Figure 6. Pre-emergence damping-off (%) of pepper cv. Altar seedlings inoculated with *Pythium ultimum* and treated with fungal antagonists, noted 15 days post-inoculation, as compared to pathogen-inoculated and untreated control. Bars sharing the same letter are not significantly different according to SNK test (*P* ≤ 0.05). IC: Inoculated with *Pythium ultimum* and untreated control; GV: Inoculated and treated with *Gliocladium virens; TV: Inoculated and treated with *Trichoderma viride; TH: Inoculated and treated with *T. harzianum.*](image)

**Suppression of Post-Emergence Damping-Off**

Pepper cv. Altar seedlings treatment with *G. virens* resulted in 40% reduced infection compared to the pathogen-inoculated and untreated control, while the other fungal treatments did not significantly suppress post-emergence damping-off, 5 days after inoculation (Table 5). Growth parameters noted on pepper cv. Altar seedlings 5 days post-transplanting, differed significantly (*P* ≤ 0.05) upon tested treatments (Table 5). *G. virens-* and *T. viride*-based treatments significantly increased plant fresh weight of *P. ultimum*-inoculated plants, by 79.31 and 76%, respectively, compared to pathogen-inoculated and untreated control (Figure 7). In addition, height noted of inoculated and treated seedlings were significantly compared to that of the uninoculated and untreated ones (Table 5).

![Figure 7. Comparison between pepper cv. Altar seedlings inoculated with *Pythium ultimum* and treated with *Gliocladium virens* (GV), pathogen-inoculated (IC), and uninoculated and untreated (NIC) controls, noted 5 days post-treatment.](image)
Plant height varied significantly (P ≤ 0.05) depending on tested treatments. All tested biological treatments had significantly improved this parameter by 27.58% (for G. virens), 25.33% (for T. viride) and 22.22% (for T. harzianum) over pathogen-inoculated and untreated control. It is also to note that the height of G. virens-treated, and pathogen-inoculated seedlings was improved by 3.9% over the uninoculated and untreated control ones (Table 5).

**Biocontrol of Pepper Pythium Damping-Off Using Rhizobacterial Agents**

**Suppression of Pre-Emergence Damping-Off**

At 15 days post-inoculation, all tested rhizobacterial isolates, excepting B. subtilis 263, P. fluorescens and P. putida 227, had improved the emergence percentage of P. ultimum-inoculated seedlings as compared to pathogen-inoculated and untreated control. The recorded increment reached 50% following treatment with Burkholderia glathei 35 (Figure 8).

**Suppression of Post-Emergence Damping-Off**

All tested bacterial treatments reduced post-emergence damping-off on pepper cv. Altar seedlings already infected with P. ultimum as compared to pathogen-inoculated and untreated control. The recorded reduction ranged from 100% for seedlings treated with P. aureofaciens 314 to 40% for those treated with Burkholderia glathei 35 and P. buttiiensis 69. Compared to the reference strain, P. fluorescens (Pf), P. aureofaciens 314 and B. pumilus 420 were found to be more effective in suppressing pepper P. ultimum damping-off (Table 6). As shown in Table 6, plant height, noted 5 days post-treatment, varied significantly upon bacterial treatments tested. Plant height, noted on pepper seedlings treated with P. aureofaciens 314 and B. pumilus 420, was improved by 35.38 and 28.51%, respectively, relative to positive control whereas those treated with the remaining isolates showed similar heights as both controls (Table 6).

---

Table 5. Damping-off incidence and growth parameters noted 5 days post-treatment on pepper cv. Altar seedlings inoculated with Pythium ultimum and treated with different fungal antagonists as compared to controls.

| Treatments | Damping-off incidence (%) | Plant weight (g) | Plant height (cm) |
|------------|---------------------------|-----------------|------------------|
| NIC        | 0.00±0.00 a               | 0.61±0.02 a     | 4.46±0.18 a      |
| IC         | 50.00±0.00 b              | 0.12±0.02 b     | 3.36±0.19 b      |
| GV         | 10.00±0.00 a              | 0.58±0.02 a     | 4.64±0.12 a      |
| TV         | 40.00±0.00 b              | 0.50±0.03 a     | 4.50±0.12 a      |
| TH         | 50.00±0.00 b              | 0.21±0.05 b     | 4.32±0.27 a      |

*Within each column, values followed by the same letter are not significantly different according to SNK test (P ≤ 0.05).

NIC: Uninoculated control; IC: Inoculated with Pythium ultimum and untreated control; GV: Inoculated and treated with Gliocladium virens; TV: Inoculated and treated with Trichoderma viride; TH: Inoculated and treated with T. harzianum.

Table 6. Damping-off incidence and growth parameters noted on pepper cv. Altar seedlings inoculated with Pythium ultimum and treated with different rhizobacterial antagonists as compared to controls noted 5 days post-treatment.

| Treatment | Damping-off incidence (%) | Plant weight (g) | Plant height (cm) |
|-----------|---------------------------|-----------------|------------------|
| NIC       | 0.00±0.00 a               | 0.61±0.02 a     | 4.46±0.18 abc    |
| IC        | 50.00±0.00 d              | 0.12±0.02 e     | 3.36±0.19 c      |
| 314       | 0.00±0.00 a               | 0.44±0.04 bc    | 5.20±0.21 a      |
| 35        | 30.00±0.00 c              | 0.20±0.01 de    | 3.54±0.12 bc     |
| 31        | 20.00±0.00 bc             | 0.30±0.04 cd    | 4.06±0.21 abc    |
| 420       | 10.00±0.00 ab             | 0.52±0.03 ab    | 4.70±0.12 ab     |
| 69        | 30.00±0.00 c              | 0.19±0.03 de    | 4.28±0.16 abc    |
| 263       | 20.00±0.00 bc             | 0.08±0.01 e     | 3.32±0.10 c      |
| Pf        | 20.00±0.00 bc             | 0.29±0.04 cd    | 4.28±0.40 abc    |
| 227       | 0.00±0.00 a               | 0.37±0.04 cd    | 4.14±0.21 abc    |

*Within each column, values followed by the same letter are not significantly different according to SNK test (P ≤ 0.05).

NIC: Uninoculated control; IC: Inoculated with Pythium ultimum and untreated control; 227: Inoculated and treated with Pseudomonas putida 227; 420: Inoculated and treated with Bacillus pumilus 420; 69: Inoculated and treated with P. buttiiensis 69; 31 and 314: Inoculated and treated with P. aureofaciens 31 and 314; 35: Inoculated and treated with Burkholderia glathei 35; 263: Inoculated and treated with B. subtilis 263; Pf: Inoculated and treated with P. fluorescens.
As for their effects on pepper plant weight, analysis of variance revealed the presence of a highly significant difference \((P \leq 0.01)\) between tested bacterial treatments. Seedlings inoculated with \(P. \ ultimum\) and treated with \(P. \ aureofaciens\) 314, \(P. \ aureofaciens\) 31, \(Bacillus\ pumilus\) 420, \(P. \ fluorescens\) and \(P. \ putida\) 227 showed 73.06, 61.18, 77.39, 61.8 and 67.93% higher fresh weights, respectively, relative to pathogen-inoculated and untreated control (Table 6). As illustrated in Figure 9, \(P. \ aureofaciens\) 314 was able to enhance plant growth and to reduce the post-emergence damping-off relative to \(P. \ ultimum\)-inoculated and untreated control.

Figure 8. Pre-emergence damping-off of pepper cv. Atlar seedlings inoculated with \(Pythium\ ultimum\) and treated with different rhizobacterial isolates, noted 15 days post-inoculation, as compared to inoculated control. Bars sharing the same letter are not significantly different according to SNK test \((P \leq 0.05)\).

IC: Inoculated with \(Pythium\ ultimum\) and untreated control; 227: Inoculated and treated with \(Pseudomonas\ putida\) 227; 420: Inoculated and treated with \(Bacillus\ pumilus\) 420; 69: Inoculated and treated with \(P. \ huttiensis\) 69; 31 and 314: Inoculated and treated with \(P. \ aureofaciens\) 31 and 314; 35: Inoculated and treated with \(Burkholderia\ glathei\) 35; 263: Inoculated and treated with \(B. \ subtilis\) 263; Pf: Inoculated and treated with \(P. \ fluorescens\).

**DISCUSSION**

In the current investigation, two \(P. \ ultimum\) isolates were shown pathogenic to pepper cv. Altar causing post-emergence damping-off. P2 isolate was found to be the most virulent and able to decrease plant weight and height by 66.44% and 22.72% respectively, as compared to the uninoculated control. These findings are also in agreement with previous studies reporting the pathogenicity of \(P. \ ultimum\) on pepper (Ramamoorthy *et al.*, 2002) as well as on different crops like bean (Rossmann *et al.*, 2017), tomato (Rafin and Tirilly, 1995), pea (Naseby, Way, *et al.*, 2001), cabbage (Tojo *et al.*, 2005), broccoli (El-Mohamedy, 2012), and sorghum (Idris *et al.*, 2008). The rapid germination of \(Pythium\) sporangia exposed to root exudates or volatiles from seeds (Osburn, 1989) and its direct infection, makes very difficult pathogen control (Whipps and Lumsden, 1991). In last three decades, the use of fungal and bacterial biocontrol agents has offered a potential and viable solution to control damping-off. Increasing attention has been paid to biological control through the use of antagonistic fungi belonging to *Trichoderma* and *Gliocladium* genera and different bacterial species affiliated to *Bacillus* and *Pseudomonas* genera (El-Mohamedy, 2012; Khabbaz and Abbasi, 2014; Gravel *et al.*, 2005).

Results from the present study showed that \(Pythium\ ultimum\) radial growth was inhibited, after two days of dual culture, with *T. harzianum*, *T. viride* and *G. virens*. Although this inhibition did not exceed 18%, the hyphal *in vitro* interactions between \(P. \ ultimum\) and tested fungal antagonists resulted in severe alterations of pathogen hyphae. The tested antagonists showed mycoparasitic abilities towards \(P. \ ultimum\) which was demonstrated at the confrontation zone. *Trichoderma* species have developed various mechanisms for attacking other fungi including mycoparasitism (Haran *et al.*, 1996), production of inhibitory compounds (Sivasithamparam and Ghisalberti, 1998), competition...
for space and nutrients (Elad et al., 1999), inactivation of the pathogen’s enzymes. Harman et al. (1980) have also suggested that mycoparasitism is the main mechanism involved in Pythium damping-off control when seeds were coated with Trichoderma hamatum. Our results are also in concordance with those of Daami-Remadi (2001), Kerkeni et al. (2007), El-Katatny et al. (2006), and El-Mohamedy (2012), showing the efficacy of these antagonistic species against P. ultimum mycelial growth. It is also interesting to note that treating pepper cv. Atlar seeds with G. virens, T. viride and T. harzianum had reduced the incidence of the pre-emergence damping-off disease incited by P. ultimum by 60, 50, and 60%, respectively, as compared to pathogen-inoculated and untreated control. When these treatments were applied as root soaking, only G. virens resulted in 40% reduced post-emergence damping-off. An improved pepper seedlings growth (height and fresh weight) was noted using these biological treatments as compared to the pathogen-inoculated and untreated control. These results are in agreement with numerous previous studies. T. viride is effective in controlling Pythium cucumber damping-off (Kerkeni et al., 2007). Besides, Harman (2000) reported that T. viride could colonize roots and promote plant growth. In addition, dipping roots of broccoli seedlings in water suspensions of T. harzianum and T. viride and mixing soil with the same suspensions of biocontrol agents during transplanting is efficient in reducing Pythium root disease (El-Mohamedy, 2012). Moreover, Trichoderma species, added to the soil or applied as seed treatments, are generally considered to be aggressive competitors by growing very fast along with the developing root system of the treated plants and rapidly colonizing substrates to exclude pathogens (Papavizas, 1985; Howell, 2003). Furthermore, Green et al. (2001) explained the efficient biological control using T. harzianum by its ability to compete with P. ultimum for substrates from the seed coat and wounded or infected root tissues. Recently, Elshahawy and El-Mohamedy (2019) reported that in the greenhouse experiment, the combined inoculation of five Trichoderma isolates suppressed damping-off induced by P. aphanidermatum and increased the survival of tomato plants by 74.5%. The mechanism of T. harzianum involved in the control of maize seedling disease caused by P. ultimum, investigated by proteome technique, revealed the capacity of T. harzianum strain T22 to not only promote seedling growth but also to induce the plant resistance through protein accumulation (Chen et al., 2005). Also, biological control of damping-off of seeds and seedlings has been successfully accomplished to various degrees using the antagonistic fungus G. virens (Whipps and Lumsden, 1991).

In the current investigation, eight antagonistic bacteria were also tested for their ability to control the target pathogen. Results from the in vitro assay showed that even though no significant effect was noted against P. ultimum radial growth, microscopic observations made at the contact zone between the confronted agents revealed a strong alteration in pathogen hyphae mainly expressed as mycelium lysis and formation of mycelial cords. Contrarily, Bacillus subtilis and Pseudomonas fluorescens isolated from the rhizospheric soil of healthy broccoli plants could completely inhibit P. ultimum growth on PDA medium (El-Mohamedy, 2012). Besides, Idris et al. (2008) demonstrated that B. cereus, B. subtilis, B. pumilus, and P. fluorescens isolated from the rhizosphere of sorghum and grasses are able to suppress P. ultimum in vitro growth.

In the current study, all rhizobacterial agents tested as a seed treatment, excepting three isolates, had improved the emergence percentage of P. ultimum-inoculated and treated seedlings compared to pathogen-inoculated and untreated ones. However, when tested as root dipping, the reduction of the incidence of post-emergence damping-off ranged between 40 and 100% with P. aureofaciens 314 being the most efficient. Our results are in concordance with many previous studies showing that the use of B. subtilis and P. fluorescens, as soil mixing or root dipping treatments, might be used for controlling Pythium root rot on many crops (El-Mohamedy, 2012). Moreover, Khabbaz and Abbasi (2014) found through growth-room assays that P. fluorescens and B. subtilis are able to suppress Pythium damping-off and root rot of cucumber seedlings. Both pre- and post-planting application of these bacteria to an infested peat mix significantly increased percentage of healthy seedlings by 100-290%, and decreased damping-off and root rot severity by 27-50%. In addition, Naseby, Way, et al. (2001) showed that Pseudomonas strains decreased the number of lesions as well as root and soil Pythium populations. Other works also demonstrated the effectiveness of P. putida, P. aureofaciens and P. aeruginosa in suppressing Pythium damping-off in tomato (Buysens et al., 1996). Parke (1990) also pointed
out that Burkholderia cepacia suppressed P. ultimum infections in pea in growth chamber experiments. Pythium root rot of wheat, attributed to P. ultimum and P. irregularare, is successfully controlled using Bacillus sp. strain L324-92 (Kim et al., 1997). Idris et al. (2008) also showed the potential of B. cereus, B. subtilis, B. pumilus and P. fluorescens to efficiently control P. ultimum infection in sorghum probably due to their ability to produce antibiotic substances and siderophores as well as through the induction of systemic resistance. In addition, Georgakopoulos et al. (2002) found out that Pseudomonas spp. strains are the best candidates for controlling sugar beet damping-off incited by P. ultimum. In fact, P. corrugata, P. fluorescens, P. marginalis, P. putida, P. resinovorans, P. syringae, and P. viridiflava had significantly reduced the rate of decayed seeds due to P. ultimum infection. Among these microorganisms, P. corrugata, P. fluorescens, P. marginalis, P. syringae, and P. viridiflava had also significantly increased the percentage of emerged tomato seedlings (Gravel et al., 2005). Besides, B. subtilis was ranked as an effective microorganism due to its ability to suppress root rot of cauliflower seedlings caused by P. ultimum var. ultimum (Abdelzaher, 2003).

In the present study, the rhizobacterial isolates P. aureofaciens 314, P. aureofaciens 31, B. pumilus 420, P. fluorescens and P. putida 227 showed growth promoting potential by increasing the seedling fresh weight by 73.06, 61.18, 77.39, 61.8 and 67.93%, respectively, over pathogen-inoculated and untreated control. Furthermore, P. aureofaciens 314 and B. pumilus 420 improved seedling height by 35.38 and 28.51%, respectively, as compared to control. These findings are in agreement with previous studies. Pseudomonas strains tested by Naseby, Way, et al. (2001) are also shown able to increase pea shoot and root weights as compared to Pythium-inoculated control. Furthermore, pre- and post-planting application of P. fluorescens and B. subtilis to an infested peat mix significantly had also increased cucumber plant fresh weights by 113-184% over control (Khabbaz and Abbasi, 2014).

These inhibitory and growth-promoting abilities may be achieved through various mechanisms of action. P. fluorescens can inhibit the germination of Pythium oospores, its growth, and subsequently the infection process (Ellis et al., 1999). The mechanism by which P. fluorescens exerts its antagonism against Pythium sp. has been extensively studied and appears to involve the production of a variety of antibiotic compounds (Howell, 1980), competition (Ellis et al., 1999) and induced host resistance (Benhamou et al., 1996; Ramamoorthy et al., 2002).

CONCLUSION

Pythium damping-off of pepper is a serious soilborne disease in Tunisia. As an eco-friendly alternative to chemical fungicide, the management of this disease through biocontrol agents is possible and results from the present study are promising. Some of the tested fungal and bacterial agents tested, were effective in suppressing P. ultimum mycelial growth and pepper damping-off disease and in improving plant growth.

ACKNOWLEDGEMENTS

This work was funded by the Ministry of Higher Education and Scientific Research in Tunisia through the budget assigned to UR13AGR09-Integrated Horticultural Production in the Tunisian Centre-East, The Regional Research Centre on Horticulture and Organic Agriculture of Chott-Mariam, University of Sousse, Tunisia.

REFERENCES

Abdelzaher, H. M. A. 2003. Biological control of root rot of cauliflower (caused by Pythium ultimum var. ultimum) using selected antagonistic rhizospheric strains of Bacillus subtilis. New Zealand Journal of Crop and Horticultural Science, 31: 209-20.

Anonymous. 2019. GIL: Interprofessional grouping of vegetables Ministry of Agriculture and Hydraulic Resources and Fisheries. Tunis, Tunisia.

Ayed, F., M. Daami-Remadi, H. Jabnoun-Khiareddine and M. E. Mahjoub. 2006. Potato vascular Fusarium wilt in Tunisia: Incidence and biocontrol by Trichoderma spp. Plant Pathology Journal, 5: 92-98.

Benhamou, N., R. R. Belanger and T. C. Paulitz. 1996. Pre-inoculation of Ri T-DNA-transformed pea roots with Pseudomonas fluorescens inhibits colonization by Pythium ultimum Trow: An ultrastructural and cytochemical study. Planta, 199: 105-17.

Buysens, S., K. Heungens, J. Poppe and M. Hofte. 1996. Involvement of pyochelin and pyoverdin in suppression of Pythium-induced damping-off of tomato by Pseudomonas aeruginosa 7NSK2. Applied and environmental microbiology, 62: 865-
Carisse, O., J. Bernier and N. Benhamou. 2003. Selection of biological agents from composts for control of damping-off of cucumber caused by *Pythium ultimum*. Canadian Journal of Plant Pathology, 25: 258-67.

Chen, J., G. E. Harman, A. Comis and G.-W. Cheng. 2005. Proteins related to the biocontrol of *Pythium* damping-off in maize with *Trichoderma harzianum* Rifai. Journal of Integrative Plant Biology, 47: 988-97.

Daami-Remadi, M. 2001. Antagonistic activity of *Trichoderma harzianum* against *Pythium aphanidermatum* and *Pythium ultimum* pathogens causing potato leak. Annales de l’INRAT, 74: 167-86.

El-Katatny, M. H., H. M. A. Abdelzaher and M. A. Shoukamy. 2006. Antagonistic actions of *Pythium oligandrum* and *Trichoderma harzianum* against phytopathogenic fungi (*Fusarium oxysporum* and *Pythium ultimum* var. *ultimum*). Archives Of Phytopathology And Plant Protection, 39: 289-301.

El-Mohamedy, R. S. R. 2012. Biological control of *Pythium* root rot of broccoli plants under greenhouse conditions. Journal of Agricultural Technology, 8: 1017-28.

Elad, Y., D. R. David, T. Levi, A. Kapat, B. Kirshner, E. Guvrin and A. Levine. 1999. *Trichoderma harzianum* T-39 mechanisms of biocontrol of foliar pathogens. In: H. Lyr, P. E. Russell, H. W. Dehne and H. D. Sisler (eds.), Modern Fungicides and Antifungal Compounds II Intercept, Andover, Hants: UK.

Ellis, R. J., T. M. Timms-Wilson, J. E. Beringer, D. Rhodes, A. Renwick, L. Stevenson and M. J. Bailey. 1999. Ecological basis for biocontrol of damping-off disease by *Pseudomonas fluorescens* 54/96. Journal of Applied Microbiology, 87: 454-63.

Elshahawy, I. E. and R. S. El-Mohamedy. 2019. Biological control of *Pythium* damping-off and root-rot diseases of tomato using *Trichoderma* isolates employed alone or in combination. Journal of Plant Pathology, 101: 597-608.

Georgakopoulos, D. G., P. Fiddaman, C. Leifert and N. E. Malathrakis. 2002. Biological control of cucumber and sugar beet damping-off caused by *Pythium ultimum* with bacterial and fungal antagonists. Journal of Applied Microbiology, 92: 1078-86.

Gravel, V., C. Martinez, H. Antoun and R. J. Tweddell. 2005. Antagonist microorganisms with the ability to control *Pythium* damping-off of tomato seeds in rockwool. Biocontrol, 50: 771-86.

Green, H., N. Heiberg, K. Lebjølle and D. F. Jensen. 2001. The use of a GUS transformant of *Trichoderma harzianum*, strain T3a, to study metabolic activity in the spermosphere and rhizosphere related to biocontrol of *Pythium* damping-off and root rot. European journal of plant pathology, 107: 349-59.

Haran, S., H. Schickler and I. Chet. 1996. Molecular mechanisms of lytic enzymes involved in the biocontrol activity of *Trichoderma harzianum*. Microbiology, 142: 2321-31.

Harman, G. E. 2000. Myths and dogmas of biocontrol changes in perceptions derived from research on *Trichoderma harzianum* T-22. Plant Disease, 84: 377-93.

Howell, C. R. 1980. Suppression of *Pythium ultimum*-induced damping-off of cotton seedlings by *Pseudomonas fluorescens* and its antibiotic, pyoluteorin. Phytopathology, 70: 712-15.

Howell, C. R. 2003. Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: The history and evolution of current concepts. Plant Disease, 87: 4-10.

Hultberg, M., B. Alsanius and P. Sundin. 2000. In-vivo and in-vitro interactions between *Pseudomonas fluorescens* and *Pythium ultimum* in the suppression of damping-off in tomato seedlings. Biological Control, 19: 1-8.

Idris, H. A., N. Labuschagne and L. Korsten. 2008. Suppression of *Pythium ultimum* root rot of sorghum by rhizobacterial isolates from Ethiopia and South Africa. Biological Control, 45: 72-84.

Jabnoun-Khiareddine, H., M. Daami-Remadi, F. Ayed and M. El Mahjoub. 2009. Biocontrol of tomato *Verticillium* wilt by using indigenous *Gloecadium* spp. and *Penicillium* sp. isolates. Dynamic Soil, Dynamic Plant, 3: 70-79.

Jayaraj, J., N. V. Radhakrishnan and R. Velazhahan. 2006. Development of formulations of *Trichoderma harzianum* strain M1 for control of damping-off of tomato caused by *Pythium aphanidermatum*. Archives Of Phytopathology And Plant Protection, 39: 1-8.

Kerkeni, A., M. Daami-Remadi, N. Tarchoun and M. B.
Khedher. 2007. In-vitro and in-vivo suppression of *Pythium ultimum* the causal agent of the cucumber damping-off by some compost fungi. Asian Journal of Agricultural Research, 1: 50-58.

Khabbaz, S. E. and P. A. Abbasi. 2014. Isolation, characterization, and formulation of antagonistic bacteria for the management of seedlings damping-off and root rot disease of cucumber. Canadian Journal of Microbiology, 60: 25-33.

Khare, A. and R. S. Upadhyay. 2009. Induction of mutant strains of *Trichoderma viride* 1433 for biocontrol of *Pythium aphanidermatum*. Environmental Biology and Conservation, 14: 21-27.

Kim, D.-S., R. J. Cook and D. M. Weller. 1997. *Bacillus* sp. L324-92 for biological control of three root diseases of wheat grown with reduced tillage. Phytopathology, 87: 551-58.

Lamichhane, J. R., C. Dürr, A. A. Schwanck, M.-H. Robin, J.-P. Sarthou, V. Cellier, A. Messéan and J.-N. Aubertot. 2017. Integrated management of damping-off diseases. A review. Agronomy for Sustainable Development, 37: 25.

Lewis, J. A. and G. C. Papavizas. 1987. Reduction of inoculum of *Rhizoctonia solani* in soil by germings of *Trichoderma hamatum*. Soil Biology and Biochemistry, 19: 195-201.

Li, Z., S. R. M. Pinson, M. A. Marchetti, J. W. Stansel and W. D. Park. 1995. Characterization of quantitative trait loci (QTLs) in cultivated rice contributing to field resistance to sheath blight (*Rhizoctonia solani*). Theoretical and Applied Genetics, 91: 382-88.

Lumsden, R. D. 1989. Biological control of damping-off caused by *Pythium ultimum* and *Rhizoctonia solani* with *Gliocladium virens* in soilless mix. Phytopathology, 79: 361-66.

Mannai, S., J. Khiareddine H, N. B and D. Remadi M. 2018. *Rhizoctonia* root rot of pepper (*Capsicum annuum*): Comparative pathogenicity of causal agent and biocontrol attempt using fungal and bacterial agents. Journal of Plant Pathology & Microbiology, 09: 431-36.

Manoranjitham, S. K., V. Prakasham, K. Rajappan and G. Amutha. 2000. Control of chilli damping-off using bioagents. Journal of Mycology and Plant Pathology, 30: 225-28.

Muthukumar, A., A. Eswaran and K. Sanjeevkumars. 2011. Exploitation of *Trichoderma* species on the growth of *Pythium aphanidermatum* in Chilli. Brazilian Journal of Microbiology, 42: 1598-607.

Naseby, D. C., J. A. Pascual and J. M. Lynch. 2001. Effect of biocontrol strains of *Trichoderma* on plant growth, *Pythium ultimum* populations, soil microbial communities and soil enzyme activities. Journal of Applied Microbiology, 88: 161-69.

Naseby, D. C., J. A. Way, N. J. Bainton and J. M. Lynch. 2001. Biocontrol of *Pythium* in the pea rhizosphere by antifungal metabolite producing and non-producing *Pseudomonas* strains. Journal of Applied Microbiology, 90: 421-29.

Nasraoui, B., M. R. Hajlaoui, S. Gargouri and R. J. Kremer. 2007. Biological control of wheat take-all disease. II. rapid screening for selection of bacteria suppressive *Gaeumannomyces graminis* var. *tritici* in laboratory with greenhouse and field confirmation trials. Tunisian Journal of Plant Protection, 2: 35-46.

Osburn, R. M. 1989. Dynamics of sugar beet seed colonization by *Pythium ultimum* and *Pseudomonas* species: Effects on seed rot and damping-off. Phytopathology, 79: 709-16.

Papavizas, G. C. 1985. *Trichoderma* and *Gliocladium*: Biology, ecology, and potential for biocontrol. Annual Review of Phytopathology, 23: 23-54.

Parke, J. L. 1990. Population dynamics of *Pseudomonas cepacia* in the pea spermosphere in relation to biocontrol of *Pythium*. Phytopathology, 80: 1307-11.

Paulitz, T. C., J. S. Ahmad and R. Baker. 1990. Integration of *Pythium nunn* and *Trichoderma harzianum* isolate T-95 for the biological control of *Pythium* damping-off of cucumber. Plant and Soil, 121: 243-50.

Rafin, C. and Y. Tirilly. 1995. Characteristics and pathogenicity of *Pythium* spp. associated with root rot of tomatoes in soilless culture in Brittany, France. Plant Pathology, 44: 779-85.

Ramamoorthy, V., T. Raguchander and R. Samiyappan. 2002. Enhancing resistance of tomato and hot pepper to *Pythium* diseases by seed treatment with fluorescent pseudomonads. European journal of plant pathology, 108: 429-41.

Rossman, D. R., A. Rojas, J. L. Jacobs, C. Mukankusi, J. D. Kelly and M. I. Chilvers. 2017. Pathogenicity and virulence of soilborne oomycetes on *Phaseolus vulgaris*. Plant Disease, 101: 1851-59.

Sivasithamparam, K. and F. L. Ghisalberti. 1998.
Secondary metabolism in Trichoderma and Gliocladium. In: C.P. Kubicek and G E Harman (eds.), Trichoderma and Gliocladium Taylor and Francis Ltd: London, UK.

Soumaya, A. B. S., R. Haouala, H. Jabnoun-Khiareddine and M. Daami-Remadi. 2013. Evaluation de l’activité antifongique de Trichoderma spp., Gliocladium spp. et Aspergillus spp. contre Rhizoctonia solani par double culture et test de leurs filtrats de culture. Microbiologie et Hygiène Alimentaire, 25: 3-8.

STAT, F. 2013. Food and Agriculture Organization Statistics. Tunisia.

Tojo, M., T. Shigematsu, H. Morita, Y. Li, T. Matsumoto and S. T. Ohki. 2005. Pythium rot of Chinese cabbage (Brassica rapa L. subsp. pekinensis) caused by Pythium aphanidermatum. Journal of General Plant Pathology, 71: 384-86.

van West, P., A. A. Appiah and N. A. R. Gow. 2003. Advances in research on oomycete root pathogens. Physiological and Molecular Plant Pathology, 62: 99-113.

Whipps, J. M. and R. D. Lumsden. 1991. Biological control of Pythium species. Biocontrol Science and Technology, 1: 75-90.

Zhani, K., B. F. Mariem, M. Fardaous and H. Cherif. 2012. Impact of salt stress (NaCl) on growth, chlorophyll content and fluorescence of Tunisian cultivars of chili pepper (Capsicum frutescens L.). Journal of Stress Physiology & Biochemistry, 8: 236-52.

CONFLICT OF INTEREST
The authors declare that they have no conflicts of interest.

AUTHORS CONTRIBUTIONS
All the authors contributed equally to this work.

Publisher’s note: EScience Press remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license and indicate if changes were made. The images or other third-party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.