Evaluation of *Trichoderma* and *Bacillus* Species for the Management of Watermelon Charcoal Rot and Plant Growth Promotion

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https://dx.doi.org/10.4314/tjpp.v20i2.4 (*Tunisia*)

ABSTRACT

Mannai, S., Ben Salem, I., and Boughalleb-M'Hamdi, N. 2025. Evaluation of *Trichoderma* and *Bacillus* species for the management of watermelon charcoal rot and plant growth promotion. Tunisian Journal of Plant Protection 20 (2): 69-83.

Macrophomina phaseolina responsible for watermelon charcoal rot, is a soilborne pathogen spread worldwide and in Tunisia. This study aimed to control this important disease using eco-friendly treatments. Two fungal and one bacterial antagonists were tested. Dual culture trials showed the efficacy of *Trichoderma harzianum*, *Trichoderma viride* and *Bacillus subtilis* to reduce *M. phaseolina* mycelial growth by 36.45% to 53.67% for MP4 and MP1 isolates confronted to *B. subtilis*. In the in vivo tests, the use of *T. harzianum* and *B. subtilis* and their combination showed that the preventive application is more effective than the simultaneous and the curative treatments. In fact, the preventive application of *T. harzianum* and *B. subtilis* and their combination reduced the disease severity index by 37.5%, 91.75% and 25%, respectively, compared to the inoculated control. Preventive application of *B. subtilis* significantly enhanced root volume, root length, and shoot length by 81.81%, 67.41%, and 73.07%, respectively, compared to the inoculated control. The application of *B. subtilis, T. harzianum* and their combination simultaneously with inoculation significantly increased the length of plants by 99%, 90.85% and 34.11%, respectively, compared to the inoculated control. Our findings indicate that *T. harzianum* and *B. subtilis* can be effectively employed as preventive soil treatments to suppress charcoal rot and promote watermelon growth.

Keywords: Bacillus subtilis, biological control, charcoal rot, *Macrophomina phaseolina*, *Trichoderma* spp., watermelon

Macrophomina phaseolina is a soilborne pathogen affecting several plants worldwide (Islam et al. 2012, Manici et al. 1995, Marquez et al. 2021). Diseases caused by *M. phaseolina* are frequently identified as charcoal rot due to the formation of black microsclerotia in the infected part of plants (Pratt et al. 1998). *M. phaseolina* induces the disease using different enzymes like amylases, phosphatidases, pectinases, hemicellulases and proteases, (Islam et al. 2012, Marquez

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Accepted for publication 11 October 2025

et al. 2021), and toxic metabolites such as asperlin, phomenone, and phaseolinone (Abbas et al. 2020, Mahato et al. 1987, Marquez et al. 2021).

M. phaseolina has been reported as the causal agent of charcoal rot disease and declining in cucurbits grown worldwide (Boughalleb-M'Hamdi et al. 2017, Cohen et al. 2016 , Egel et al. 2020, Negreiros et al. 2019, Wu et al. 2022). On watermelon plants, this pathogen caused numerous small, black sclerotia on the roots, premature yellowing of the top leaves followed by leaf drop (Boughalleb-M'Hamdi et al. 2017). Furthermore, *M. phaseolina* is recognized as a primary etiological agent of root rot and vine decline, with the potential to cause yield losses up to 40% in watermelon cultivation (Alves et al. 2025, Cohen et al. 2016, Gomes-Silva et al. 2018, Porto et al. 2019, Wu et al. 2022).

Strategies to control charcoal rot disease are limited due to the large host range and the long survival of *M. phaseolina* microsclerotia in the soil. Several systemic fungicides like carbendazim, thiophanate methyl, hexaconazole, tebuconazole, difenoconazole, azoxystrobin and non-systemic fungicides like mancozeb, chlorothalonil, and captan were evaluated against *M. phaseolina* at different concentrations (Lokesh et al. 2020, Parmar et al. 2017). However, no chemical product has been registered to control the charcoal rot (Marquez et al. 2021).

Several studies have been accentuated on testing different antagonistic microorganisms for the management of this disease, which are ecologically safe solutions (Khare et al. 2010, Muthukumar et al. 2011). *Trichoderma* and *Bacillus* are the most used antagonists to control different plant diseases (Devi et al. 2022, Mannai and Boughalleb-M'Hamdi 2022, Mannai et al. 2018, Podbielska et al. 2020).

Trichoderma species isolated from different types of soils are efficient against several phytopathogens such as *M. phaseolina* (Bastakotiet al. 2017, Shahid et al. 2014). These microorganisms have different antagonistic mechanisms like competition for nutrients, production of antibiotics and mycoparasitism. Furthermore, some species are also able to increase the plant growth (Chowdappa et al. 2013, Martinez-Medina et al. 2016, Zaim et al. 2018).

Replacing agrochemicals with Plant Growth-Promoting Rhizobacteria (PGPR) represents a sustainable and safe strategy to secure crop production, as these microorganisms enhance both plant growth and health (Bhat et al. 2020). The genus *Bacillus* is frequently used as a biocontrol agent (Devi et al. 2022, Simonetti et al. 2015, Torres et al. 2016).

However, conventional methods for controlling plant diseases are limited by serious drawbacks, including risks to human health and environmental contamination from chemical inputs. Therefore, alternative eco-friendly approaches, such as biological control, are increasingly required (Rojo et al. 2007).

The objectives of the present study were (i) to assess the in vitro antifungal activity of *T. harzianum*, *T. viride*, and *B. subtilis* against *M. phaseolina* isolates associated with watermelon charcoal rot, and (ii) to investigate the efficacy of preventive, simultaneous, and curative applications of *T. harzianum* and *B. subtilis*, individually and in combination, in mitigating disease severity and enhancing the growth of watermelon seedlings.

MATERIALS AND METHODS

Pathogen culture and inoculum preparation.

Four *M. phaseolina* isolates (MP1, MP2, MP3, and MP4) obtained from watermelon seedlings exhibiting symptoms of charcoal rot disease and collected from Chott-Mariem region in Tunisia were used in this study. Isolates identification was achieved through morphological characterization, focusing on colony morphology and microsclerotia features, following the criteria described by Beas-Fernández et al. (2006) and Mayek-Pérez et al. (1997). To prepare the inoculum for the in vivo tests, mycelia of *M. Phaseolina* isolate MP1was harvested from five Petri plates containing one-week-old cultures grown on Potato-Dextrose-Agar (PDA) and subsequently homogenized in 0.5 liter of Sterile Distilled Water (SDW) using an electric mixer. The resulting pathogen inoculum was then used to inoculate watermelon seedlings by watering them with 50 ml/plant (Cohen et al. 2016).

Culture conditions and inoculum preparation of biocontrol agents for in vivo evaluation.

Two isolates of *Trichoderma (T. harzianum* and *T. viride*) and one isolate of *B. subtilis*, naturally associated to watermelon seedlings, were tested in this investigation.

The identification of *Trichoderma* isolates was conducted following a 7-day incubation period for each colony on PDA medium at a temperature of 28°C and based on macro- and micro-morphological traits of the colony, conidiophores, phialides and conidia (Shah and Afiya2019, Siddiquee 2017). The *B. subtilis* isolate was identified through both morphological, biochemical and genetic analyses, as detailed in Furuya et al. (2011).

For the in vivo assay, the concentration of conidial suspension obtained from *T. harzianum* cultures grown on PDA was adjusted to 10^7 CFU/mL by a Malassez slide. *B. subtilis* isolate was incubated at 25°C for two days on Nutrient Agar (NA). Then, cell suspension was prepared by scraping bacterial colony, adjusted to 10^6 CFU/ml by dilution in SDW. Then, 50 ml of each inoculum was used per plant for individual treatment. However, for the combination method (*T. harzianum* + *B. subtilis*), 25 ml of each inoculum was used (Boughalleb-M’Hamdi et al. 2018).

in vitro assessment of the antifungal activity of biocontrol agents against *Macrophomina phaseolina.*

The antifungal activities of the tested antagonists against *M. phaseolina* mycelial growth were determined according to Mannai and Boughalleb-M’Hamdi (2023) method. Agar plugs (6 mm in diameter) of the pathogen culture were positioned on the opposite side of the antagonists plugs. Before use, *B. subtilis* was grown on NA for 48 h and *T. harzianum* and *T. viride* were cultivated on PDA medium for 7 days, at 25°C. The antagonist plugs were replaced with agar plugs for the control plates. Six plates were used per individual treatment, and the experiment was repeated twice in time. All the plates were incubated at 25°C for four days. Then, *M. phaseolina* mycelial growth inhibition was determined according to following formula (Hmouni et al. 1996):

$$I\ (\%) = (1 - T/C) \times 100$$

where, I: mycelial growth inhibition, T: the radius of pathogen colony in treated plates, C: the radius of pathogen colony in control plates.

Effect of *T. harzianum* and *B. subtilis* on watermelon charcoal rot disease incidence.

Based on the in vitro results, *T. harzianum* proved to be a more effective antagonist against *M. phaselina* than *T. viride,* and was therefore selected, together with *B. subtilis,* for the in vivo trials, applied both individually and in combination. Thirty days old watermelon plants cv. Crimson seedlings were used for the in vivo trials. Theses ones were transplanted in plastic pots (17 cm diameter) containing a mixture of peat and vermiculite (v/v) used as soil substrate.

Four types of treatments were carried out for individual antagonists and their combination: preventive, simultaneous treatment and two curative treatments. The preventive treatment consists of watering the watermelon seedlings with 50 ml/plant of the suspension of each tested antagonist (10^7 CFU/mL for *T. harzianum* and 10^6 CFU/mL of *B. subtilis*) 15 days before inoculation. The simultaneous treatment consists of watering seedlings with 50 ml/plant of the spore/cell suspension of each antagonist, just after inoculation with pathogen (at the same day) also applied out by seedling watering. The first curative treatment consists of the plant inoculation and subsequently the treatment with antagonists (50 ml/plant) 15 days after inoculation. The treatment was carried out by watering seedlings around their collars. The second curative treatment consists of inoculating the plants with the pathogen followed by two treatments with the antagonists with an interval of 15 days one month after inoculation with *M. phaseolina*. This treatment was also carried out by watering the seedlings around their collars (Hibar et al. 2006). Inoculated untreated and uninoculated untreated controls were used. A completely randomized factorial design with two factors (antagonist and treatment types) was followed in the current assay. Each treatment was subjected to three replicates and the experiment was repeated twice.

Sixty days after the preventive treatment with antagonists, six parameters were noted: the charcoal rot index rated onto 0–6 scale according to Zanella et al. (2020) with some modifications (0 = symptomless; 1 = 1-3% yellowing of the basal leaves; 2 = 4-10% yellowing with slight discoloration of the roots; 3 = 11-25% yellowing and wilting of the apical part; 4 = 26-50% yellowing and necrosis of leaves and wilting and crown root rot; 5 = 51-75%: the leaves are dried out with blackening of the roots; 6 = 76-100%: complete drying out and rotting).

Based on this rating scale, the disease incidence was calculated using the formula of Wheeler (1969) in each treatment:

Disease incidence (%) = (S*100)/T × M

with S: Sum of all numerical ratings; T: Total observed plant; M: Maximum disease grade.

In this essay, the vegetative and root length, its fresh weight, dry weight, and the volume are also measured. The root volume (cm^3) was estimated by the immersion method as described by Musick et al. (1965).

Statistical analyses.

Statistical analyses (ANOVA) were conducted for both in vitro and in vivo assays using a completely randomized factorial design. For the in vitro assays, the fixed factors were the antagonists and pathogen isolates, whereas for the in vivo assays they were the antagonists and treatment timing. Each treatment was replicated six times in vitro and three times in vivo. Data were analyzed using SPSS version 23, and mean comparisons were performed with the

Student-Newman-Keuls (SNK) test at a least significance level of $p \leq 0.05$.

RESULTS

In vitro effect of antagonists on *M. phaseolina* growth.

Statistical analyses of the in vitro biocontrol essay (dual culture method) revealed a highly significant interaction between the two tested factors (antagonists and pathogen isolates; $p \leq 0.05$) as well as a highly significant main effect of the antagonists. Indeed, the antagonist reacted differently towards all the isolates after four days of incubation at 25°C (Table 1). Inhibition rates ranged from 36.45% to 53.67% for MP4 and MP1 isolates when challenged with *B. subtilis*. This antagonist exhibited the highest efficacy against MP1, but was less effective against MP3 and MP4. In contrast, *T. harzianum* and *T. viride* showed greater inhibitory activity than *B. subtilis*, with inhibition rates of 51.02% and 49.84% against MP3, and 51.58% and 47.80% against MP4, respectively (Table 1). Fig. 1 illustrates the comparative effects of the different antagonists on the four *M. phaseolina* isolates.

Table 1. Inhibition rate of *Macrophomina phaseolina* mycelial growth by the tested antagonists (%), recorded after four days of incubation at 25°C

Antagonists/Pathogen isolates	MP1	MP2	MP3	MP4	*p*-value
Trichoderma harzianum	48.38a±1.55^{aA}	48.97±0.56^{aA}	51.02±0.87^{aA}	51.58±3.43^{aA}	≥ 0.05
Trichoderma viride	47.80±1.29^{aA}	51.33±5.28^{aA}	49.84±2.19^{aA}	47.80±2.68^{aA}	≥ 0.05
Bacillus subtilis	53.67±5.21^{aA}	40.73±8.98^{bA}	38.76±2.69^{bB}	36.45±1.41^{bB}	≤ 0.05
***p*-value**	≥ 0.05	≥ 0.05	≤ 0.05	≤ 0.05	-

* Within each row, values followed by different lowercase letters differ significantly according to the SNK test at $p \leq 0.05$.

** Within each column, values followed by different uppercase letters differ significantly according to the SNK test at $p \leq 0.05$.

MP1, MP2, MP3, and MP4: *M. phaseolina* isolates used in this study.

In vivo effect of *T. harzianum* and *B. subtilis* on watermelon charcoal rot disease incidence.

Analysis of variance of the charcoal rot disease index calculated 45 days after inoculation of watermelon plants, revealed a highly significant interaction, between the two factors (treatment timing x used antagonists) as well as a highly significant effect for each factor analyzed alone. In fact, preventive treatment has been shown to be more effective than the other three treatments. The preventive application (15 days before inoculation) of *T. harzianum* and *B. subtilis* as well as their combination significantly reduced disease severity index by 37.5%, 91.75%, and 25%, respectively, compared to the inoculated control. However, no significant reduction in disease severity index was found for both antagonists and their combination when they were applied simultaneously, once or twice after plant inoculation (Fig. 2).

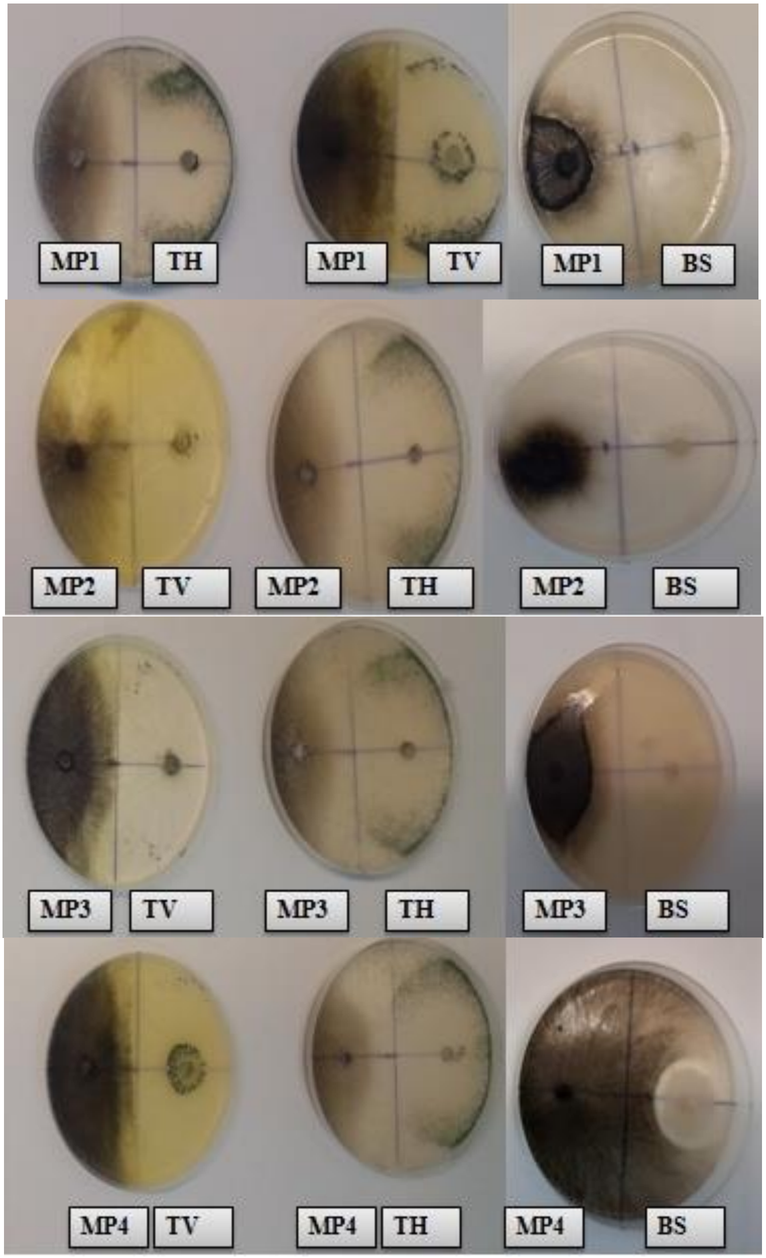

Fig. 1. Confrontations of four isolates of *Macrophomina phaseolina* isolates with the three antagonists after 4 days of incubation at 25°C.
TV: *T. viride*; TH: *T. harzianum*; BS: *B. subtilis*; MP1, MP2, MP3, and MP4: Isolates of *M. phaseolina*

The disease incidence ranged from 33.34% for plants treated preventively with *B. subtilis* to 100% for plants with only one curative treatment by *B. subtilis* and the combination between the antagonists. The timing treatment with *T. harzianum* did not affect this parameter (83.34%). However, the preventive application of *B. subtilis* is the most effective treatment followed by its simultaneous application (Fig. 2).

In vivo effect of antagonists on growth parameters.

Regarding vegetative growth, variance analysis revealed that both factors (treatment timing and used antagonists), considered independently and in interaction, have had a highly significant effect ($p \leq 0.05$). In fact, preventive and simultaneous treatments were found to be more effective than the curative treatments. The preventive application of *T. harzianum* and *B. subtilis* significantly improved this parameter by 37.82% and 73.07%, respectively. Their simultaneous application significantly increased the vegetative growth by 99% and 90.85%, respectively. *B. subtilis* was the most effective with lengths comparable to those of the healthy control. On the other hand, the curative applications were not effective (Fig. 3).

Variance analysis for the root length parameter revealed a highly significant interaction between treatment timing and antagonists, as well as significant individual effects of each factor ($p \leq 0.05$). Preventive application of *B. subtilis* (15 days before inoculation) was the only effective treatment, increasing root length by 67.41% compared to the inoculated control. In contrast, simultaneous or curative applications of the antagonists, alone or in combination, showed no significant effect on this parameter (Fig. 3).

Statistical analysis revealed a highly significant difference in the effectiveness of the antagonists ($p \leq 0.05$), as well as a significant interaction between the two studied factors (treatment timing and type of antagonist) on root volume. Notably, none of the treatments improved this parameter, except for the preventive application of *B. subtilis*, which resulted in an 81.81% increase compared to the inoculated control (Fig. 3).

A highly significant effect of the antagonists and of the timing of their application ($p \leq 0.05$), as well as a highly significant interaction between these two factors ($p \leq 0.05$), was observed on root fresh weight. The preventive treatment was the most effective. Specifically, the preventive and simultaneous applications of *B. subtilis* increased root fresh weight by 185.96% and 84.21%, respectively, compared to the inoculated control. Similarly, the preventive and both curative applications of the two antagonists significantly enhanced this parameter by 107% and 119.30%, respectively (Fig. 3)

A significant interaction between the two factors (treatment timing * antagonist type) and a highly significant effect ($p \leq 0.05$) of antagonists had been proved on the root dry weight. The preventive and simultaneous applications of *B. subtilis* improved this parameter by 109.52% and 142.85%, respectively. The curative applications of the antagonist's combination significantly improved the plants roots dry weight by 90.47% (Fig. 3).

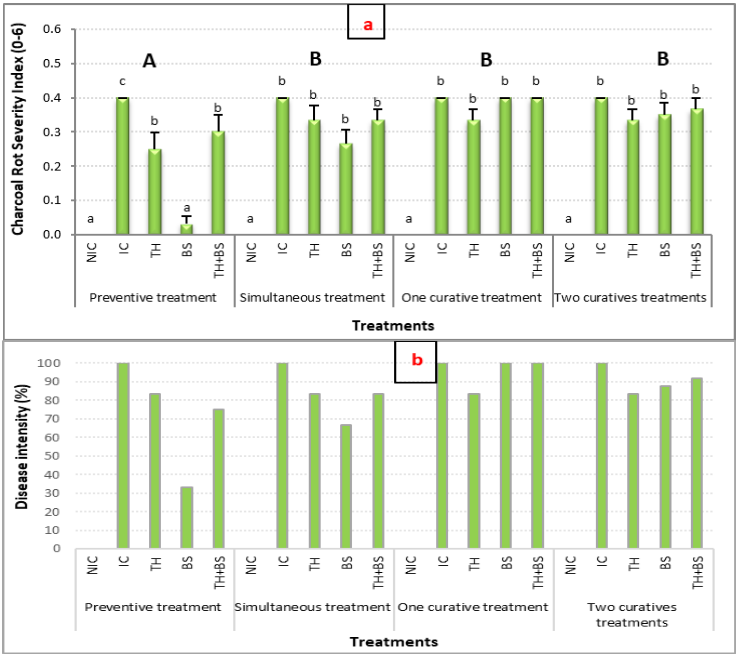

Fig. 2. Disease incidence on watermelon plants (cv. Crimson) treated with different antagonists compared to control, 45 days after inoculation with *Macrophomina phaseolina* isolate MP1. For each treatment type (preventive, simultaneous, curative treated once, curative treated twice), bars sharing the same lowercase letter do not differ significantly according to the SNK test ($p \leq 0.05$).

The capital letters (A, B and C) presented the statistical comparison between the treatment timings regardless of the used antagonist, according to the SNK test at $p \leq 0.05$. NIC: Uninoculated control, IC: Inoculated and untreated control, TH: *Trichoderma harzianum*, BS: *Bacillus subtilis*, TH+BS: Combination between *T. harzianum* and *B. subtilis* used.

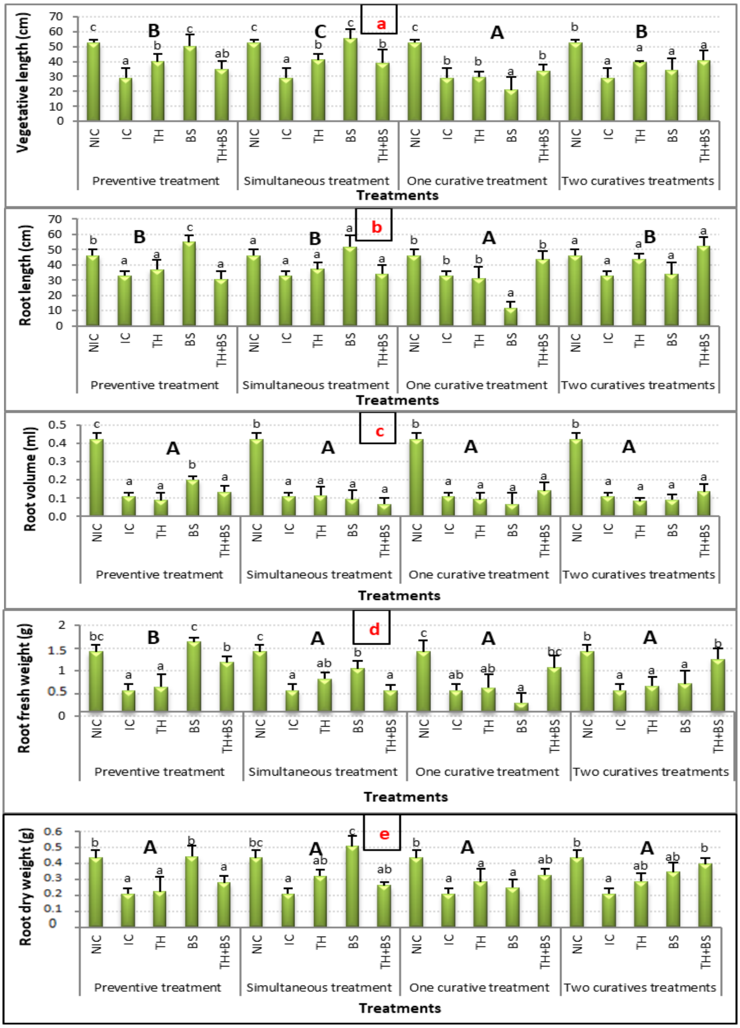

Fig. 3. Vegetative length (a), root length (b), root volume (c), root fresh weight (d), and root dry weight (e) of watermelon plants (cv. Crimson) treated with different antagonists compared to control, 45 days after inoculation with *Macrophomina phaseolina* isolate MP1. For each treatment type (preventive, simultaneous, curative treated once, curative treated twice), bars sharing the same lowercase letter do not differ significantly according to the SNK test ($p \leq 0.05$).
The capital letters (A, B and C) presented the statistical comparison between the treatment timings regardless of the used antagonist, according to the SNK test at $p \leq 0.05$. NIC: Uninoculated control, IC: Inoculated and untreated control, TH: *Trichoderma harzianum*, BS: *Bacillus subtilis*, TH+BS: Combination between *T. harzianum* and *B. subtilis* used.

DISCUSSION

Biocontrol agents like *B. subtilis, T. viride,* and *T. harzianum* are used in the management strategies to control cucurbits charcoal rot. Different products made from *Bacillus* spp. and *Trichoderma* spp. are known to suppress *M. phaseolina* growth under specific conditions (Gacitua et al. 2009).

The present study has shown the effectiveness of *B. subtilis, T. harzianum* and *T. viride* to reduce *M. phaseolina* mycelial growth. Several studies have focused on the effectiveness of these agents in limiting the development of several plant pathogens. In fact, Boughalleb-M'Hamdi et al. (2018) demonstrated the antagonistic efficacy of different *T. harzianum* and *T. viride* isolates against different soilborne phytopathogenic fungi infecting melon and watermelon (*Fusarium oxysporum* f. sp. *niveum*, *Fusarium solani* f. sp. *cucurbitae, F. oxysporum* f. sp. *melonis*, and *M. phaseolina*). In addition, the mycelial growth of five *M. phaseolina* isolates decreased in the presence of *T. harzianum* (between 38.74 and 52.42%) and *T. viride* (between 33.27 and 42.43%) (Boughalleb-M'Hamdi et al. 2018). Besides, the mycelial growth of 14 isolates of *M. phaseolina* was significantly reduced by *T. harzianum, T. viride* and *B. subtilis* isolated from rhizosphere of groundnut, with an inhibition rate (%) varing from 65.64 to 81.25, 63.33 to 81.62 and 38.46 to 62.50, respectively (Kumar et al. 2015). Several other studies have shown that *B. subtilis* has an important inhibitory activity against several plant pathogens such as *Macrophomina, Pythium, Phytopythium,* and *Fusarium* spp. (Devi et al. 2022, Mannai and Boughalleb- M'Hamdi 2022, 2023, Singh et al. 2008). In fact, *B. subtilis* cell suspensions produced an inhibitory activity greater than 50% against three strains of *M. phaseolina* (Torres et al. 2016). Besides, among five screened *B. subtilis* strains, the M-4 *B. subtilis* strain showed a high antagonistic activity in vitro against *M. phaseolina* (81.5%) (Chauhan et al. 2022). The strain *B. subtilis* BGS-10 showed higher mycelial inhibition (61%) against *M. phaseolina* associated to *Gloriosa superba* root rot (Dhanabalan et al. 2024).

T. harzianum has been shown to be effective in vivo to reduce the charcoal rot severity when used preventively. These results are similar to those of Boughalleb-M'Hamdi et al. (2018) who revealed that the preventive application of *T. harzianum* reduced the charcoal rot severity. Besides, *T. harzianum* is able to decrease the charcoal rot incidence by 37-74% on melon plants grown in fields infested with *M. phaseolina*. The *Trichoderma* genus has long been characterized by its ability to act as biological control agent against plant pathogens. The primary biological control mechanisms are food competition, mycoparasitism, and antibiosis (Ghildyal and Pandey 2008, Umamaheswari et al. 2009). In addition, *Trichoderma* species generate compounds that have antimicrobial properties and various enzymes, including pectinases, glucanases, proteases, and chitinases, which break down the cell walls of harmful fungal pathogens (Tchameni et al. 2020, Verma et al. 2007). The in vivo antagonism test in our study also demonstrated the efficiency of *B. subtilis* in reducing disease incidence when applied preventively. These findings are consistent with those of Singh et al. (2008), who reported the effectiveness of *B. subtilis* in suppressing root rot symptoms in *Pinus roxburghii* caused by *M. phaseolina*. Moreover, several studies have recognized *Bacillus* spp. as potent antagonists against *M. phaseolina* (Muhammad and Amusa 2003, Pal et al.

2001, Simonetti et al. 2015). The inhibitory effect of *B. subtilis* is partly explained by its ability to produce lytic enzymes, chitinase and b-1,3-glucanase, which degrade the mycelium and the cell wall component of *M. phaseolina* (Singh et al. 2008). On the other hand, the preventive application of the combined antagonists *T. harzianum* and *B. subtilis* also reduced disease incidence. However, the effectiveness of this combination was lower than that of *B. subtilis* applied alone and comparable to that of *T. harzianum* used individually. This reduced efficacy could be attributed to a possible incompatibility or antagonistic interaction between the *B. subtilis* strain and the *T. harzianum* isolate. This incompatibility was previously reported by Thilagavathi et al. (2007), who tested the biocontrol agents *T. viride* and *B. subtilis* individually and in combination against root rot of green gram caused by *M. phaseolina*. Their results showed that *T. viride* strains were not compatible with *B. subtilis*.

The present study showed also that the tested antagonists improved the plants growth. In fact, *T. Harzianum* and *B. subtilis* improved the growth (length of the vegetative part; root length and volume) following its preventive and simultaneous applications. The simultaneous treatment using the combination increased the length of the plants. These results are in agreement in part with those of Singh et al. (2008) who showed *B. subtilis* effectiveness in increasing the dry weights of *Pinus roxburghii* plant roots and vegetative parts. In addition, previous study proved that the treatment of melon seeds with *T. harzianum* improved the plant fruit yield by 61% compared to plants from non-treated seeds grown in soils naturally infested with *M. phaseolina* (Elad et al. 1986 in Rhouma et al. 2021).

Based on our results, *B. subtilis* could be employed as an individual preventive treatment to control the charcoal rot disease while promoting watermelon plant growth and development.

RESUME

Mannai S., Ben Salem I. et Boughalleb-M'Hamdi N. 2025. Evaluation d'espèces de *Trichoderma* et de *Bacillus* pour la gestion de la pourriture charbonneuse de la pastèque et la stimulation de la croissance des plantes. Tunisian Journal of Plant Protection 20 (2): 69-83.

Macrophomina phaseolina, responsable de la pourriture charbonneuse de la pastèque, est un pathogène tellurique répandu dans le monde et en Tunisie. La présente étude visait à contrôler cette importante maladie en utilisant des traitements respectueux de l'environnement. Deux antagonistes fongiques et un antagoniste bactérien ont été testés. Un essai de confrontation directe sur milieu de culture a montré l'efficacité de *Trichoderma harzianum*, *Trichoderma viride* et *Bacillus subtilis* à réduire la croissance mycélienne de *M. phaseolina* de 36,45% à 53,67% pour les isolats MP4 et MP1 confrontés avec *B. subtilis*. Le test in vivo moyennant *T. harzianum* et *B. subtilis* appliqué individuellement ou en combinaison a montré que le traitement préventif est plus efficace que celui appliqué simultanément ou curativement. En effet, l'application préventive de *T. harzianum* et *B. subtilis* et leur combinaison ont réduit l'indice de la maladie de 37,5%, 91,75% et 25%, respectivement. L'application préventive de *B. subtilis* a augmenté significativement le volume et la longueur des racines et la longueur de la partie végétative des plantes de 81,81%, 67,41% et 73,07% respectivement, par rapport au témoin inoculé. En revanche, l'application de *B. subtilis*, *T. harzianum* et leur combinaison simultanément à l'inoculation a augmenté d’une manière significative la longueur des plantes de 99%, 90,85% et 34,11%, respectivement, par rapport au témoin inoculé. Sur la base de nos résultats, *T. harzianum*, *B. subtilis* et leur combinaison pourraient être utilisés comme traitement préventif du sol pour contrôler la pourriture charbonneuse et augmenter la croissance des plants de pastèque.

Mots clés: *Bacillus subtilis*, lutte biologique, *Macrophomina phaseolina*, pourriture charbonneuse, pastèque, *Trichoderma* spp.

ملخص

مناعي، صبرين وابتسام بن سالم ونعيمة بوغلاب-محمدي. 2025. تقييم أنواع من *Trichoderma* و *Bacillus* لإدارة التعفن الفحمي للبطيخ الأحمر و تعزيز نمو النباتات.

Tunisian Journal of Plant Protection 20 (2): 69-83.

الفطر *Macrophomina phaseolina* هو المسبب لمرض التعفن الفحمي للبطيخ الأحمر والذي ينتقل عن طريق التربة وهو منتشر في جميع أنحاء العالم وفي تونس. تهدف هذه الدراسة إلى مكافحة هذا المرض المهم باستخدام علاجات صديقة للبيئة. تم اختبار اثنين من المضادات الفطرية ومضاد واحد بكتيري. أظهرت التجارب المخبرية *فعالية* *Trichoderma harzianum* و *Trichoderma viride* و *Bacillus subtilis* في تثبيط نمو الفطر *M. phaseolina* بنسبة 36.45% إلى 53.67% للعزلات MP4 وMP1 عند المواجهة مع *B. subtilis* وقد أظهرت الاختبارات على النبتة أن استعمال *T. harzianum* أو *B. subtilis* أو مزيجهما، أن العلاج الوقائي كان أكثر فعالية من العلاجات المتزامنة او المتأخرة. في الواقع، أدى التطبيق الوقائي للفطر *T. harzianum* والبكتيريا *B. subtilis* ومزيجهما إلى تقليل مؤشر شدة الإصابة بنسبة ٪ و91.75% و25%، على التوالي. أدى التطبيق الوقائي للبكتيريا *B. subtilis* إلى زيادة في حجم وطول الجذور وطول الجزء الخضري من النباتات بنسبة 81.81% و67.41% و73.07%، على التوالي، مقارنة بالشاهد، في حين أدى استعمال الفطر *T. harzianum* والبكتيريا *B. subtilis* ومزيجهما في وقت واحد مع الإعداء إلى زيادة في طول النباتات بنسبة 99% و90.85% و34.11%، على التوالي، مقارنة بالشاهد. وبناءً على نتائجنا، يمكن استخدام الفطر *T. harzianum* والبكتيريا *B. subtilis* ومزيجهما كعلاج وقائي للتربة للسيطرة على التعفن الفحمي و زيادة نمو نبات البطيخ الأحمر.

كلمات مفتاحية: بطيخ الأحمر، تعفن فحمي، مكافحة بيولوجية، *Bacillus subtilis*، *Macrophomina phaseolina*، *Trichoderma* spp.

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