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Rhizoctoniasolani-green beans pathosystem uncover bio control efficacy of *Trichoderma* spp.

Abstract

Rhizoctoniasolani on green beans has been chosen as one of the best pathosytems to evaluate root rot diseases as well as to determine the effectiveness of potential of the biocontrol agent *Trichoderma* species (ssp.). In this study we investigate the effective use ofthe pathosystem model; *Rhizoctoniasolani*-green beans to reveal bio control efficacy of three *Trichoderma* spp.; *T. afro-harzianum*, *T. reesei* and *T. guizouhense*, isolated from Moroccan soils. In greenhouse conditions, root-dipping approach was involved in revealing bio control potential of local*Trichoderma* spp. by suppressing root diseases in *Rhizoctonia*-green beans pathosystem. Interestingly, *T. reesei* (T9i12) a breaking cellulose succeeded in suppressing disease incidence in root units (DI-RU) = 0.0% in green bean cultivars infected with *Rhizoctoniasolani.*

Keywords: trichoderma, antagonistic activity, pathosystem, biocontrol potential

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Introduction

Rhizoctoniasolani is a soil borne pathogen which is difficult to control because of its sclerotia conserved structure mostly persistent in soil and crop residue and their broad susceptible hosts range. This pathogen may infest post-emerged seedlings after sowing or when transplanting susceptible cultivars. In different place in the world including European, Scandinavian and North African counties, detection and increase of population of virulent *Rhizoctonia* species (spp). and susceptibility level of cultivars have been determined like that of *R. solani* in potatoes[1,2] and in common beans.[3,4] Free pathogen soil-less mix, fumigants control and dry steam treatment program are used to avoid root rots caused bysoil-borne pathogens contamination in greenhouse including *Rhizoctonia*. However, environmental and health issues attributed to fumigants toxicity has given moving forces to the emergence of bio control beside resistance breeding programs. Therefore, bio control agents (BCA) are more and more applied in integrated plant protection strategies in most of crops-systems.[5] Different pathogen-host combinations were chosen as pathosystem models to evaluate biocontrol efficacy in diseases suppression for a BCA to be introduced in crop system. For instance, application of *Trichoderma* spp. controlled diseases caused by *Rhizoctonia*in different greenhouse experiments and improved growth and yield parameters of variable cultivars.[6,7] MantovanelloLucon et al.,[8] applied simultaneously rice seeds colonized with *Trichoderma* and wheat seeds infected with *Rhizoctonia* to inoculate cucumber roots' seedlings. MantovanelloLucon and co-workers found that *Trichoderma* heals cucumber seedlings damping off in greenhouse conditions. Brewer et al.,[9] studied biocontrol efficacy of *T. virens* and *T.harzianum* amongst other organisms against *Rhizoctonia* damping off on potato in greenhouse conditions. They found that both antagonists reduce stem cankers and black scurf on potatoes tubers. This research work focuses on uncovering *Trichoderma* efficacy to control root diseases in *Rhizoctonia*-green beans pathosystem in greenhouse conditions.

Material and methods

Obtaining fungi for test pathogen

Rhizoctonia was isolated from infected roots of olive trees diagnosed with damping off diseases caused by *Rhizoctoniasolani*. *R. solani* was isolated from tap roots previously disinfected for one minute in 10% sodium hypochlorite, baited in Potatoes Dextrose Agar (PDA) culture and incubated in 25°C for mycelium to grow. To obtain pure culture *Rhizoctonia* was sub-cultured in a fresh PDA plates. *Rhizoctonia* cultures were grown and maintained by subsequent multiplication on PDA (PDA, Difco).

Obtaining Trichoderma isolates

Three *Trichoderma* isolates were used in this study to evaluate their biocontrol performance in vivo against *Rhizoctoniasolani*. These isolates were identified in a previous work at the species level as *T. afro-harzianum* (T8A4), *T. guizouhense* (T4) and *T. reesei* (T9i12) respectively.[10]

Experimental design for trichodermaroot dips treatments in greenhouse conditions

Trichoderma species were tested for their bio control efficacy in *Rhizoctonia*-green beans. Green beans pathosystem were grown for three to four weeks on 77 peat trays. Seedlings of different cultivars were then transplanted into pots after their inoculation with fungi. Pots where seedlings were transplanted are filled with sterile substrate at 3:1 weight/weight (w/w) peat to sand ratio. Experimental design was organized in four randomized complete blocs with four replicates in each experimental unit. That is, four pots were used in each experimental unit. Four treatments were tested; T1 was designed for treatment of cultivars inoculated with *Trichoderma afro-harzianum* (T814) and a pathogen, T2 was designed for treatment of cultivars inoculated with *Trichodermaguizouhense* (T4) and a pathogen

and T3 was designed for treatment of cultivars inoculated with *Trichodermareesei* (T9i12) and a pathogen. TC designed for treatment of cultivars inoculated with *Trichoderma afro-harzianum* extracted from a commercial product to be compared with other *Trichoderma* isolates. Two controls were used in this experiment; Tm1 was designed for healthy cultivars (negative controls), Tm2 was designed for cultivars inoculated with pathogen only (positive control).

Inoculum preparation

Rhizoctonia inoculums was prepared using infected corn seeds with *Rhizoctonia* mycelium. Inoculums type and methods are adopted by Cardoso and Echandi[11] with some modifications. In fact, erlen flasks containing 50 corn seeds and distilled water were sterilized at 120°C for 1 hour in autoclave and sterilization procedure was repeated after 24 hours. Excess of water was discarded and each flask was inoculated with 4 big square pieces of a growing culture of *R. solani* on PDA. Infected corn seeds were incubated at 24°C for 25 days and each flask were shaken every 4 days to allow uniform seed colonization by *Rhizoctoniasolani*.[11]

Root-dipping of green beans seedlings with Trichoderma spp and artificial infection with Rhizoctonia

Roots of seedlings were dipped with fungal suspensions at transplanting. Green beans' roots were respectively dipped first into a suspension of 2.10^6 and 10^8 conidia ml^{-1} of different *Trichoderma* spp. for 15 to 20 minutes (min).[9] Pathogens inoculation of beans' roots was performed using two *Rhizoctonia* infected corn seeds per pot as inoculum. Corn seeds were placed near to beans' roots at the moment of transplanting.

Disease evaluation

Diseases assessment was estimated by measuring Disease Incidence (DI) of the pathogen as reported by previous studies.[12] Disease Incidence (DI) was measured as the percentage of number of plant units showing typical symptoms of each related pathogen. Therefore, DI percentage was calculated as shown in the equation (2) given by Benson and Baker (1974):

$$Disease\ Incidence\left(DI\right) = \frac{number\ of\ infected\ plants}{total\ number\ of\ plants} * 100\left(2\right)$$

Disease incidence was measured in above ground units of the plant (DI-AU) and root units (DI-RU) three months after *Trichoderma* spp. treatments. In this study we evaluate disease incidence at two level measuring disease incidence in aerial plant units (DI-AU) and root units (DI-RU) at the end of each experiment. Symptoms and signs recovered from roots, crown, stem and leaves were recorded. In addition, plant parameters like roots dry weight (RDW) and plant dry weight (PDW), plant height (PH), leaves surface area (LSA) and green bean pods number (Pods N°) were also measured.[13,14]

Statistical analysis

Diseases incidence measurement data were subjected to variance analysis for reliable estimation of the amount of disease. Greenhouse experimentation out puts; DI, root and plant dry weight, leaves surface area and green bean pods number were suggested to analysis of variance to determine differences between four treatments (T1, T2, T3, TC) and to compare with the controls Tm1 and Tm2.

Results

Suppression of Rhizoctoniasolani by Trichoderma root dips treatments in green beans

It can be inferred from DI-AU and DI-RU results that *Rhizoctonia* disease can be efficiently controlled by *Trichoderma* spp. treatments. Significant decrease of disease response in root unit has been observed in different in *Trichoderma* spp. root dipped green beans cultivars (p=0.000). Disease incidence recorded in commercial *Trichoderma* in TC and *T. afro-harzianum* in T1 treatments and found to not exceed 30% incidence in green beans roots. While, root disease incidence in *T. reesei* (T2) treatment was DI-RU= 0.0% similarly compared to Tm1 as indicated in figure 1.Specific symptoms were observed in positive controls Tm2 investigated in pathogenicity test of *Rhizoctonia*. Disease caused by *Rhizoctonia* was assessed by root and stem rots and lesions, red dashes on the surface of green beans stem designated as cankers in the infected green beans. Lesions were not seen in any of treated green beans cultivars. Root dry weight recorded in green beans treated with *T. reesei* (T2) was RDW=4.3 grams (g) and was fairly compared to RDW of healthy green beans in Tm1 with RDW= 4.1g. T1 and TC treatments showed lower root weight with RDW=1.0 g and 1.2 g respectively as shown in figure 2. Green beans in T2 and Tm1 contain the highest numbers of pods which is above 30, then, T1, TC and T3 had between 20 to 25 pods, where, Tm2 contains only 15 pods. RDW and PH data suggest that *Trichoderma* spp. treatments efficiently maintain and improve plant fitness even when infected with *Rhizoctonia*. In fact, the highest the RDW and PH of green beans, the lowest disease responses in aerial and roots units of green beans and the symptomless plants expression were recorded. In the present study, *T. reesei* in T2 treatment successfully suppressed *Rhizoctonia* disease responses (see Figure 3). In fact, *T. reesei* has succeeded in suppressing stem cankers in green beans. Also, other treatment; *T. afro-harzianum*in T1 treatment, *T. guizouhense*in T3 and commercial *Trichoderma* in TC have noticeably reduced stem cankers.

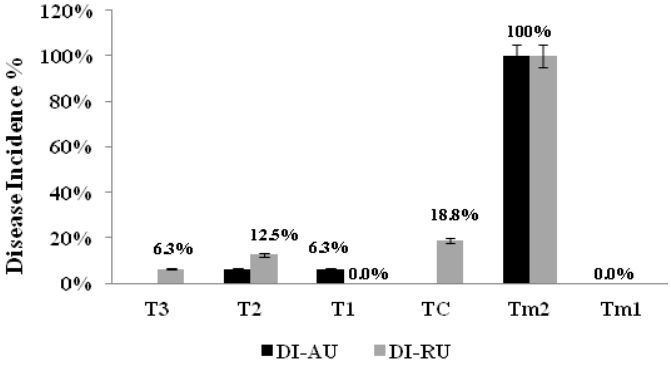

Figure 1 Disease incidence in (%) in Rhizoctoniasolani-green beans pathosystem and bio control effect of *Trichoderma* spp.; *T. afro-harzianum* in treatment T1, *T. reesei* in treatment T2, *T. guizouhense* in treatment T3 and TC Trichoderma extracted from commercial product used as reference.

Discussion

Trichoderma root-dips using local*Trichoderma* spp. have reduced considerably *Rhizoctonia* disease responses in different cultivars. *T. afro-harzianum*and *T. reesei* gave promising results on reducing symptoms caused by this pathogen. Brewer et al.,[9] have demonstrated the efficiency of *T. harzianum* and *T. virens* in suppressing disease

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response caused by *Rhizoctoniasolani* in potatoes. Their results showed less stem cankers and black scurf in potatoes tubers when treated with *T. harzianum*. Interestingly, *Trichodermareesei* in T2 treatment was able to suppress *Rhizoctoniasolani* disease response on green beans. It was able to suppress stem cankers and lesions and roots rots in beans cultivars. *Trichodermareesei* have been reported to serve as a workhorse for industrial cellulosic enzymes production. However, it's important to mention that *Trichodermareesei* under strain code: QM 6a has been employed as an antagonist against *Rhizoctoniasolani*.[15] *Trichoderma* treatments effect on disease suppression was well correlated with their effect on plant development parameters like plant height and root dry weight of green beans. Therefore, it may be inferred that different *Trichoderma* treatments reliably affect green beans growth. It seems that root-dipping revealed to be effective carrier of *Trichoderma* spores towards site of infection in roots. That is, *Trichoderma* efficacy on root diseases suppression demonstrated the good establishment of the antagonists on the site of inoculation. Root-dipping approach has been investigated in our previous study using *Verticillium*-eggplant pathosystem and showed the power of this treatment to reveal *Trichoderma* bio control performance.[16–18] It can be inferred that root-dipping applied at seedling transplantation can effectively be used as root drench treatment to control root disease of *Rhizoctoniasolani* in green beans in greenhouse.

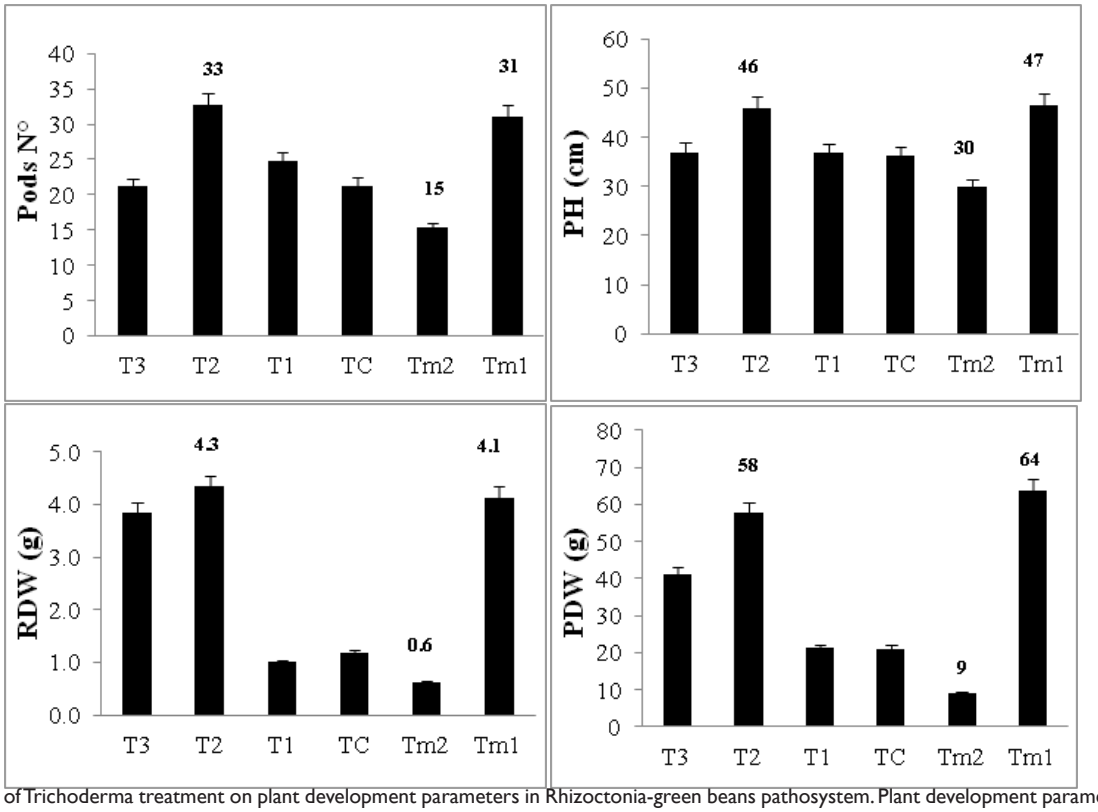

Figure 2 Effect of Trichoderma treatment on plant development parameters in Rhizoctonia-green beans pathosystem. Plant development parameters measured were; Pods Number (Pod N°), Plant Height (PH), Root Dry Weight (RDW) and Plant Dry Weight (PDW).

Figure 3 *T. reesei* in treatment T2 suppress cankers lesions in stem and rots in roots compared to controls Tm2.

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None.

Discloser statement

No potential conflict of interest was reported by the authors.

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