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Biological control of white mold (*Sclerotinia sclerotiorum*) in lettuce using Brazilian *Trichoderma* spp. strains

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Abstract

Widely consumed by the Brazilian, lettuce has a cultivated area of 35,000 ha. Among the diseases that might infect this crop, white mold causes major concerns for producers. Mold is caused by the fungus *Sclerotinia sclerotiorum* (Lib.) de Bar. It can lead to losses of up to 100% in lettuce. The objectives of this study were assessment of antagonistic effect of *Trichoderma* spp. isolates, grown and prepared on rice grain, on white mold of lettuce (*S. sclerotiorum*). The assay was conducted using 12 *Trichoderma* spp. isolates, four of which came from at least a year of storage at 4ºC, four from areas with a history of the disease and four from areas without a history of the disease. Both fungi were grown on wet rice grains and only Trichoderma strains was dried and ground to be used in the next assay. The experiment was completely randomized in a factorial 12x2 design (*Trichoderma* spp. × substrate inoculated or not with *S. sclerotiorum*) and control plants without any of the fungi. The percentage of survived plants was analyzed using AUDPC, number of leaves, stem diameter, length of root system, fresh and dry weight of shoot and root, and total dry matter. The results showed that all *Trichoderma* spp. were capable of lettuce growth promotion in the presence and absence of *S. sclerotiorum*. The isolates that showed the best biocontrol of *S. sclerotiorum* were TC1.15 and WM-13. To promote growth, the best isolates were UFSMT15.1 and WM-13, suggesting that the latter presents desirable characteristics for biocontrol, including excellent feasibility for large-scale production, good antagonistic activity to *S. sclerotiorum* and the ability to stimulate growth promotion in lettuce.

Keywords: Biocontrol; *Lactuca sativa* L.; White mold.

Introduction

Lettuce is widely consumed by the Brazilian population, at a rate of 36 g per day on average, mostly in salads (Sala and Costa, 2012). It is a very delicate herbaceous plant, with a small stem that supports the leaves, which are large and grow in a rosette. In addition to various color shades of green or purple depending on the cultivar, the leaves may be curly or smooth, and these are the characteristics that determine consumer preference.

Among the diseases that infect lettuce, white mold is of major concern to producers. It is caused by the fungus *Sclerotinia sclerotiorum* (Lib.) de Bary, belong to the order Helotiales and to the family Sclerotiniaceae, white mold is a non-specific ascomycete and its occurrence has been reported in almost all countries of the world, infecting more than 500 species (Saharan and Mehta, 2008). Under high humidity conditions, the disease is characterized by the formation of a white mycelium with a cotton aspect. In crops characterized by low aeration and light penetration, white mold is even more aggressive.

The fungus survives in soil and crop debris by means of sclerotia, a mycelial structure that is compact, dark, dormant and resistant to desiccation. These structures have sizes of 2 to 20 mm in length and may be remained viable in soils for up to 10 years (Lopes, 2010) and can be infective for a long period of time, depending on weather conditions.

Disease management using crop rotation is almost impossible, due to the persistence of resistance structures for long periods and their wide host range. Therefore, biological control is successfully employed in lettuce, using fungi from the *Trichoderma* genus as a control agent. This has reduced white mold incidence by 50%, under controlled greenhouse conditions (Chitrampalam, 2008).

The ability of *Trichoderma* spp. to inhabit cultivated soils is proven due to their inverse relationship with the incidence

of soil diseases (Ethur et al., 2008; Shaw et al., 2016). Recent studies indicate that isolates from soil with different uses have different behaviors for controlling *S. sclerotiorum* and *Sclerotinia minor* Jagger (Ibarra-Medina et al., 2010).

In Brazil, the first report of application of this genus was in 1950, when Forster (1950) described the inactivation of tobacco mosaic virus (TMV) by filtering *Trichoderma* spp. More recently, Liu et al. (2008) studied diversity of *Trichoderma* spp. in soils of different production systems. They found that soils under organic practices are more effective in suppressing *Sclerotium rolfsii* Sacc. than those undergoing conventional practices.

Trichoderma strains has been used in many crops, including commercial brands recommended to soybean (Zang et al., 2016), cucumber (Yedidia et al., 2001), maize (Resende et al., 2004), onion (McLean et al., 2012), tomato (Cotxarrera et al., 2002; Ethur et al., 2008; Liu et al., 2008). There is few information available about the efficiency of *Thrichoderma* strains from different sources, mainly to control mold affecting lettuce. Also, the time of storage of these strains might have influence on efficiency of *Trichoderma* biocontrol in field assays. Thus, the objectives of this study were to assess effect of *Trichoderma* spp. spores. The spores were produced on inoculated rice at storage condition then used to assess their effects on white mold severity of lettuce under field condition. We also verified the capacity of *Trichoderma* spp. obtained from storage and from areas with and without a history of white mold on biocontrol of *S. sclerotiorum*; and their action on promoting growth of lettuce plant.

Results and discussion

The stored isolates showed less capacity of colonization of rice grains when compared to other groups, suggesting that year of storage can negatively influence development of this substrate (Table 1). In addition, isolates from the WM- group obtained better values, indicating better growth of isolates obtained from areas without history of white mold. An improved ability for colonization was observed in WM-13 and WM-24 isolates after 15 days, indicating their advantage for large-scale production, since they showed good colonization ability in substrate.

The isolates showed CFU g^{-1} values, at which they do not influence the biocontrol of disease (McLean et al., 2012). Using 1.8 x 10^6 to 1.9 x 10^8 CFU g^{-1}, the authors did not achieve significant changes in biological control of *Sclerotinia* spp. The values found in this assay, ranged from 1.6 x 10^6 to 7.57 x 10^7 CFU g^{-1}, standing among the threshold proposed by McLean et al. (2012), in which concentrations of at least 10^5 CFU g^{-1} are required for *T. atroviride* to control *S. cerviporum* of onion. Ousley et al. (1994) suggest that biocontrol activity of *Trichoderma* spp. has an effect at concentration of approximately 10^5 CFU g^{-1}, while higher values do not promote significant gains. Recent data suggest that the performance of *Trichoderma* spp. can be influenced much more by the time it remains in contact with the pathogen, than by the pressure of the disease itself (McLean et al., 2012).

Due to the highly favorable environmental conditions in the greenhouse, the disease was evident at the beginning of the first week after planting, with development of the white mycelium characteristic of the fungus, causing damping-off in newly transplanted seedlings. the percentage of survived plants was 50%, when inoculation was done only with *S. sclerotiorum*, being statistically lower than control plants and plants, in which *Trichoderma* spp. were applied (Table 2).

All *Trichoderma* spp. isolates reduced the disease severity compared to the *S. sclerotiorum* control (no *Trichoderma treatment*). In the stored isolates group, ETSR20 showed the highest scores for disease severity since the first week of evaluation, indicating that it is not a good inhibitor of *S. sclerotiorum*. All other strains had similar scores to each other at the end of the fifth week, suggesting that they performed similar activities in reducing symptoms (Figure 1A). Considering the WM+ group isolates, only WM+21 was able to act efficiently in disease biocontrol (Figure 1B), because until the end of crop cycle it maintained low scores for severity, repressing advancement of symptoms. WM+12 showed the worst results in this group, providing a linear progression of the disease, although all isolates belonged to the species *T. asperellum*, evidencing that they act in different ways in the control of *S. sclerotiorum*.

Among the isolates from the WM- group (Figure 1C), WM-24 was the best, obtaining scores similar to WM+21 from the previous group, so both were considered to be the most effective in reducing *S. sclerotiorum* severity. In contrast to previous results, in which isolates from the same species showed different behavior, similar behavior in different species was observed among WM- isolates. Thus, WM-23 (*T. koningiopsi*) achieved the same scores as WM-14 (*T. asperellum*).

Several factors are known that influence *Sclerotinia* spp. severity in lettuce, including crop development stage, air temperature and soil moisture (Saharan and Mehta, 2008). In regard to survival, our results are in contrary with those found by Chitrampalam et al. (2008), where number of survived plants inoculated only with *Trichoderma* spp., *Gliocladium* spp. and *Bacillus* spp. were generally lower than control treatment. The control treatment (only treated with *S. sclerotiorum*) produced the largest number of dead plants and the highest scores for the severity of the disease, suggesting that rice grains colonized with *S. sclerotiorum* were able to manifest symptoms in culture. Therefore, the control plants showed highest AUDPC (Table 2). The mycelium produced by the fungus infects the lower leaves and stem tissues and may cause lettuce decline in one week (Saharan and Mehta, 2008).

The AUDPC is inversely proportional to the degree of *Trichoderma* spp. biocontrol; therefore, strains having very low AUDPC could be the first option on the *S. sclerotiorum* control, contrasting those with high AUDPC. Based on the present study, we observed that none of Trichoderma strain was statistically equal to the control. This suggests that all the *Trichoderma* ssp. were capable of reducing the white mold somehow.

Accordingly, WM+ was the best group because the four strains presented the lower mean values of AUDPC. This behavior probably is caused due the time of convivence of *Trichoderma* ssp. with *S. sclerotiorum* at the field where it was collected, while groups WM- and stored where quite similar with WM-24 and UFMST17 presenting the lower mean values of AUDPC. In relation to white mold severity, Hoyos-Carvajal et al. (2009) indicated a high metabolic diversity

Table 1. Colony forming unit (CFU) of *Trichoderma* spp. produced in rice grains. Santa Maria, RS. 2012

Isolates	CFU g^{-1}
UFSMT15.1[2]	1.90 x 10^6 e[1]
UFSMT17	7.14 x 10^6 c
TC1.15	4.65 x 10^6 d
ETSR20	1.60 x 10^6 e
WM+11[3]	5.10 x 10^6 d
WM+12	1.60 x 10^7 b
WM+21	1.30 x 10^7 b
WM+22	9.55 x 10^6 c
WM-13	7.57 x 10^7 a
WM-14	1.98 x 10^6 e
WM-23	2.45 x 10^6 e
WM-24	2.75 x 10^7 a
CV(%)	23.26

[1]Means followed by the same letter do not differ statistically according to the Scott-Knott test (P≤ 0.05). [2]*Trichoderma aureoviride* Rifai (UFSMT17); *Trichoderma koningiopsis* Samuels, Suárez & Evans (UFSMT15.1, WM-13 and WM-23); *T. harzianum* (ETSR20); and *Trichoderma asperellum* Samuels, Lieckf & Nirenberg (TC1.15, WM+11, WM+12, WM+21, WM+22, WM-14 and WM-24). [3]Isolates coming from areas with a history of white mold (WM+) and four without history (WM-) of the disease.

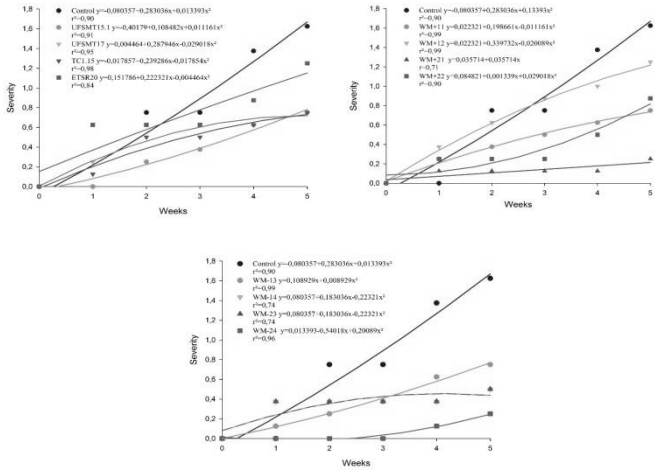

Fig 1. Scores for white mold severity in lettuce infested with *Sclerotinia sclerotiorum* and biocontrol by *Trichoderma* spp. based on the Cotxarrera (2002) scale. **(A)** Isolates coming from storage in the mycology collection at UFSM **(B)** Isolates coming from areas with a history of white mold (WM+); **(C)** Isolates coming from area without history (WM-) of the disease. Santa Maria, RS. 2012

Table 2. Percentage of surviving lettuce plants (cv. Regina) and the area under the disease progress curve (AUDPC), inoculated with *Sclerotinia sclerotiorum* and *Trichoderma* spp. that were stored and from areas with and without history of disease, respectively. Santa Maria, RS. 2012

Trichoderma spp.	Survivors (%)		AUDPC
	Control	*S. sclerotiorum*	*S. sclerotiorum*
Control	100 aA[1]	50.0 bB	3733 a
UFSMT15.1[2]	100 aA	100 aA	840 c
UFSMT17	100 aA	87.5 aA	2341 b
TC1.15	100 aA	87.5 aA	856 c
ETSR20	100 aA	87.5 aA	2333 c
WM+11[3]	100 aA	87.5 aA	1011 c
WM+12	100 aA	100 aA	1356 c
WM+21	100 aA	100 aA	933 c
WM+22	100 aA	87.5 aA	1423 c
WM-13	100 aA	87.5 aA	1672 c
WM-14	100 aA	100 aA	1711 c
WM-23	100 aA	87.5 aA	2765 b
WM-24	100 aA	100 aA	408 c
CV (%)	22.59		46.93

[1]Means followed by the same lowercase letters in column and uppercase letters in rows do not differ statistically according to the Scott-Knott test (P≤ 0.05). [2]*Trichoderma aureoviride* Rifai (UFSMT17); *Trichoderma koningiopsis* Samuels, Suárez & Evans (UFSMT15.1, WM-13 and WM-23); *T. harzianum* (ETSR20); and *Trichoderma asperellum* Samuels, Lieckf & Nirenberg (TC1.15, WM+11, WM+12, WM+21, WM+22, WM-14 and WM-24). [3]Isolates coming from areas with a history of white mold (WM+) and four without history (WM-) of the disease.

Table 3. Number of leaves, stem diameter and length of root system (LRS) for lettuce plants cv. Regina, inoculated with *Sclerotinia sclerotiorum* and *Trichoderma* spp. from storage and from areas with and without history of disease. Santa Maria, RS. 2012

	Nº of leaves		Stem diameter (mm)		LRS (cm)	
Trichoderma spp.	Control	*S. sclerotiorum*	Control	*S. sclerotiorum*	Control	*S. sclerotiorum*
Control	27.57 bA[1]	30.50 aA	10.20 aB	13.10 aA	20.87 bA	19.25 bA
UFSMT15.1[2]	32.83 aA	32.28 aA	12.88 aA	12.48 aA	24.67 aA	22.61 aA
UFSMT17	30.85 bA	31.33 aA	11.21 aA	11.67 aA	24.01 aA	22.60 aA
TC1.15	30.62 bB	34.50 aA	11.81 aA	12.28 aA	19.08 bA	19.73 bA
ETSR20	32.28 aA	31.50 aA	11.87 aA	11.12 aA	20.25 bA	22.66 aA
WM+11[3]	30.71 bA	32.37 aA	11.43 aA	12.44 aA	20.20 bB	24.80 aA
WM+12	28.28 bB	33.14 aA	11.34 aA	12.66 aA	22.87 aA	20.64 bA
WM+21	29.43 bB	33.71 aA	11.71 aA	12.90 aA	22.18 aA	21.63 aA
WM+22	30.85 bA	30.33 aA	10.79 aA	10.77 aA	22.02 aA	19.20 bA
WM-13	33.86 aA	35.50 aA	12.83 aA	13.21 aA	23.64 aA	20.88 bA
WM-14	30.87 bA	31.43 aA	11.46 aA	12.05 aA	20.87 bA	19.15 bA
WM-23	29.57 bA	30.67 aA	11.21 aA	11.73 aA	22.54 aA	20.53 bA
WM-24	30.43 bA	32.71 aA	11.03 aA	12.74 aA	22.71 aA	19.38 bA
CV (%)	9.97		14.52		14.48	

[1]Means followed by the same lowercase letters in column and uppercase letters in rows do not differ statistically according to the Scott-Knott test (P≤ 0.05). [2]*Trichoderma aureoviride* Rifai (UFSMT17); *Trichoderma koningiopsis* Samuels, Suárez & Evans (UFSMT15.1, WM-13 and WM-23); *T. harzianum* (ETSR20); and *Trichoderma asperellum* Samuels, Lieckf & Nirenberg (TC1.15, WM+11, WM+12, WM+21, WM+22, WM-14 and WM-24). [3]Isolates coming from areas with a history of white mold (WM+) and four without history (WM-) of the disease.

Table 4. Fresh weight of shoot (FWS) and of root (FWR), dry matter of shoot (DMS) and of root (DMR) and total dry matter (TDM) for lettuce plants cv. Regina, inoculated with *Sclerotinia sclerotiorum* and *Trichoderma* spp. from storage and from areas with and without history of disease. Santa Maria, RS. 2012

	FWS (g)		FWR (g)		DMS (g)		DMR (g)		TDM (g)	
Trichoderma spp.	Control	*S. sclerotiorum*	Control	*S. sclerotiorum*	Control	*S. sclerotiorum*	Control	*S. sclerotiorum*	Control	*S. sclerotiorum*
Control	60.47 bA[1]	84.45 aA	4.34 aB	10.06 aA	4.23 bB	7.42 aA	0.32 aB	0.62 aA	4.55 bB	8.05 aA
UFSMT15.1[2]	95.01 aA	71.93 aA	7.85 aA	7.04 bA	6.46 aA	5.30 aA	0.51 aA	0.46 aA	6.97 aA	5.77 bA
UFSMT17	75.66 bA	82.15 aA	7.28 aA	6.67 bA	4.57 bA	4.86 aA	0.44 aA	0.45 aA	5.01 bA	5.32 bA
TC1.15	81.75 aA	94.46 aA	5.10 aA	7.83 bA	5.70 aA	6.78 aA	0.41 aA	0.59 aA	6.11 aA	7.37 aA
ETSR20	84.56 aA	73.50 aA	5.92 aA	6.78 bA	5.68 aA	5.79 aA	0.42 aA	0.35 aA	6.10 aA	6.14 bA
WM+11[3]	77.02 bA	85.95 aA	5.48 aB	9.31 aA	4.64 bA	5.53 aA	0.34 aB	0.69 aA	4.99 bA	6.22 bA
WM+12	60.44 bB	97.90 aA	6.83 aA	7.43 bA	4.23 bA	5.62 aA	0.41 aA	0.51 aA	4.64 bA	6.13 bA
WM+21	88.52 aA	97.75 aA	6.52 aA	7.95 bA	4.65 bA	5.55 aA	0.36 aA	0.49 aA	5.01 bA	6.05 bA
WM+22	67.10 bA	71.81 aA	5.34 aA	5.24 bA	5.01 bA	4.68 aA	0.56 aA	0.29 aB	5.58 bA	4.97 bA
WM-13	97.42 aB	109.40 aA	8.50 aA	9.70 aA	5.59 aA	6.27 aA	0.62 aA	0.62 aA	6.21 aA	6.89 aA
WM-14	77.60 bA	92.72 aA	4.96 aA	6.94 bA	4.73 bA	5.98 aA	0.32 aA	0.45 aA	5.05 bA	6.43 bA
WM-23	69.05 bA	79.01 aA	7.41 aA	5.99 bA	4.26 bA	4.67 aA	0.49 aA	0.41 aA	4.75 bA	5.09 bA
WM-24	70.81 bA	91.35 aA	6.48 aA	6.34 bA	4.78 bA	6.03 aA	0.43 aA	0.49 aA	5.21 bA	6.52 bA
CV (%)	26.68		37.59		25.62		48.94		25.13	

[1]Means followed by the same lowercase letters in columns and uppercase letters in rows do not differ statistically according to the Scott-Knott test (P≤ 0.05). [2]*Trichoderma aureoviride* Rifai (UFSMT17); *Trichoderma koningiopsis* Samuels, Suárez & Evans (UFSMT15.1, WM-13 and WM-23); *T. harzianum* (ETSR20); and *Trichoderma asperellum* Samuels, Lieckf & Nirenberg (TC1.15, WM+11, WM+12, WM+21, WM+22, WM-14 and WM-24). 3Isolates coming from areas with a history of white mold (WM+) and four without history (WM-) of the diseas

in isolates of *T. asperellum* obtained from tropical regions. According to Dennis and Webster (1971), isolates of the same species which are morphologically similar, may differ physiologically and act differently in the biocontrol of plant pathogens. Therefore, the physiological characteristics of isolates are more important than morphology of species itself.

The *Trichoderma* spp. used in biocontrol assays were capable of promoting growth gains in the presence and absence of *S. sclerotiorum* in lettuce. For the variable number of leaves (Table 3), the best isolates tested in the absence of *S. sclerotiorum* were UFSMT15.1, ETSR20 and WM-13, while the others were statistically equal to control. In the presence of the pathogen, the isolates showed no significant difference, although TC1.15 and WM-13 excelled numerically.

Data for stem diameter showed that the blank control plant was statistically lower than the control cultivated in substrate infested with *S. sclerotiorum*. In the absence of the pathogen, approximately 64% of isolates of *Trichoderma* spp. acted in assisting to root system development, with increases of up to 17.5% or 3.64 cm in length compared to *Trichoderma* spp. control.

Similar to data on the number of leaves, fresh weight showed a statistically significant result only in the absence of the pathogen (Table 4). UFSMT15.1, TC1.15, ETSR20, WM+21 and WM-13 again stood out, aiding growth.

Data on fresh matter of the shoot corroborate those presented by Duarte et al. (2012), who found a maximum of 90.3 g plant^{-1} using organic fertilizers in lettuce, whereas WM-13 and UFSMT15.1 exhibited 97.42 and 91.01 g plant^{-1}, respectively, in the absence of the pathogen. In the presence of *S. sclerotiorum*, the isolate WM-13 was also able to

promote increased fresh weight when compared to control, even though it did not differ from it.

When plants were harvested this did not allow the total removal of secondary roots that were strongly adhered to the substrate, probably causing an experimental error. Consequently, the results for fresh and dry weight of the root system showed high coefficients of variation. In the presence of pathogen, the control plants showed the best results for fresh weight of the root system, with only two isolates, WM+11 and WM-13, equaled the control.

Like fresh weight, the results for dry matter of shoots presented statistical differences compared to control treatment, but only in the absence of *S. sclerotiorum*. The best results were observed by UFSMT15.1, TC1.15, ETSR20 and WM-13, which showed 6.46, 5.70, 5.68, and 5.59 g, respectively.

We did not find significant differences between the control and lettuce plants treated with the antagonist in the absence of the pathogen for dry matter of root system.

Nevertheless, it is clear that the plants treated with *Trichoderma* spp. are numerically higher than the control treatment. Even in the absence of the pathogen, it was found that isolate WM+11 and the control were significantly lower than their counterparts in the presence of *S. sclerotiorum*. The data for root dry weight in the presence of the pathogen showed no significant differences.

For total dry matter, the results were significant in the absence and in the presence of the plant pathogenic fungus, verifying that only UFSMT15.1, TC1.15, ETSR20 and WM-13 isolates promoted real gains in dry matter, ranging from 34.06 to 53.18%, and statistically outperforming the control plants. However, in the presence of pathogen, the control obtained the highest values, and only TC1.15 and WM-13 were able to match it statistically.

Given these results, the best isolates for biocontrol of *S. sclerotiorum* were TC1.15 and WM-13. However, for growth promotion the best isolates were UFSMT15.1 and WM-13. This suggests that WM-13 has desirable characteristics as a biocontrol agent as well excellent feasibility for large-scale production, good antagonistic activity to *S. sclerotiorum* and ability to stimulate growth promotion in lettuce.

Yedidia et al. (2001) suggest that the effect on growth promotion caused by *T. harzianum* present in soil rhizosphere is due to an increase in root area provided, allowing the plant to exploit a larger volume of soil and consequently more nutrient sources. This may have allowed the ETSR20 isolate to achieve one of the best results when used in the presence of *S. sclerotiorum*, because of its greater release of root exudates, allowing antagonistic agents to use them for development. According to Shaw et al. (2016), the white mold biocontrol is facilitated by the synthesis and exudation of *Trichoderma* spp. compound. Bal and Altintas (2008) also emphasized that while root exudates cause inhibition in onion, on lettuce they are slightly favorable to *T. harzianum*.

While in treatments without *S. sclerotiorum*, all isolates of *Trichoderma* spp. were numerically higher than control plants, there may be evidence that strains from the genus *Trichoderma* act by promoting gains in fresh weight in lettuce roots. Louzada et al. (2009) reported that the antagonist directly acts on pathogen control, presenting indirect action on plant productivity and greater exploitation of soil through the root system.

In dry matter of the shoots, our values are higher than those found by Duarte et al. (2012), which showed around 4.86 g plant^{-1}. However, in the presence of the pathogen, all isolates of *Trichoderma* spp. were numerically lower than control. For the root system, the results were in agreement with Resende et al. (2004), who reported dry matter accumulation in roots receives benefit from application of antagonistic fungus.

Pinto et al. (2011), worked on different cultivars of lettuce hydroponically and found that cv. Regina grew better in the presence of *P. aphanidermatum*, showing higher averages of root weight. We observed a similar phenomenon in this trial, as plants infested with only *S. sclerotiorum* were statistically superior to control plants.

The results shown here are in agreement with Ousley et al. (1994), who found that only one isolate of *T. harzianum* had significantly higher results (5%) to the control plants for growth (total dry mass) in lettuce.

Material and Methods

Plant material

We used a Brazilian lettuce cultivar called Regina, widely accepted on local market by its excellent quality. We sowed the lettuce seeds in trays with 128 cells containing commercial growth substrate. Three to five seeds were deposited in each cell and at seven days thinning took place, leaving only one seedling per cell. Trays received daily irrigation. At 30 days, when seedlings had three pairs of true leaves, they were ready to be used in the biocontrol assay. The lettuce seedlings were produced in a protected greenhouse at the Plant Protection Department, belong to the Federal University of Santa Maria (UFSM) – RS, Brazil, on the period from June to November of 2012.

Trichoderma treatments

We used 12 *Trichoderma* spp. isolates, four (UFSMT15.1, UFSMT17, TC1.15, ETSR20) from storage in the mycology collection at UFSM obtained from the Dr. Elocy Minussi Laboratory of Phytopathology at the Center of Rural Sciences. These strains were kept for at least one year refrigerated at 4ºC, before to be used on the assays. More four strains (WM+11, WM+12, WM+21, WM+22) were obtained from areas with a history of white mold (WM+) disease and the other four (WM-13, WM-23, WM-14, WM-24) coming from areas without history (WM-) of the disease. The first number indicates a different farm and the second number indicates the spot at the farm where the isolates where obtained. Those farms were organic and without pesticide use. It means we had three distinct groups of *Trichoderma* spp. isolates: stored, WM+ and WM-, each one possessing four different strains of the fungi.

The strains belong to the groups WM+ and WM- were isolated from soil samples. Each sample was diluted in sterilized water and an aliquot was placed in Petri dishes containing PDA (potato dextrose agar) medium. After seven days a monosporic isolation of one *Trichoderma* sample for each farm spot was selected.

Production and quantification of Trichoderma spp. CFU g^{-1} in rice grains

Trichoderma inoculum was produced utilizing 200 g of rice grains to each strain. The rice grains were moistened with distilled water, then placed in Erlenmeyer flasks and autoclaved for 40 min twice. Five flasks were utilized for each *Trichoderma* spp. strain and to each flask we added five disks (10 mm diameter) containing fresh mycelium and spores of *Trichoderma* spp. These remained in an acclimatized chamber at 22°C with a photoperiod of 12 h for 15 days, until complete colonization of the rice grains took place. For storing, the already colonized grains were dried for 48 h in a closed chamber at a constant temperature of 37ºC. Then they were ground in a blender to a powder and stored in refrigerated at 4ºC until to be used in the biocontrol assay.

To quantify the *Trichoderma* in the ground rice grains, a sample of 1 g was taken from each powdery previously produced. The colony forming unit (CFU) g^{-1} was estimated by serial dilution using a Neubauer Chamber. Each *Trichoderma* strain was diluted four times and was counted at the Neubauer Chamber.

Production of S. sclerotiorum in rice grains

The *S. sclerotiorum* inoculum was obtained from sclerotia presents in lettuce plants containing white mold. The propagules were disinfected and placed in Petri dishes with PDA medium for seven days. The colonies containing with only *S. sclerotiorum*, without other fungi contaminants was selected.

S. sclerotiorum inoculum was produced only two weeks before application, utilizing moistened rice grains previously autoclaved for 40 min twice. To each flask containing rice grains, five disks (10 mm diameter) containing fresh mycelium and sclerotia of *S. sclerotiorum* were added. These remained at acclimatized chamber at 22°C with a photoperiod of 12 h for 15 days, until complete colonization of the rice grains took place. It was only used on the assays samples of rice grains containing only *S. sclerotiorum.*

Biocontrol activity of Trichoderma spp. strains

In this assay, polypropylenes pots containing commercial substrate specific to lettuce growth were used. Around two days before the assay began, each pot received rice grains infested with *S. sclerotiorum* and powder colonized with *Trichoderma* spp. at a ratio of 5 g per kg of substrate at opposite ends of the vessel.

After the applications of *S. sclerotiorum* and *Trichoderma* spp., healthy selected seedlings were transplanted to the central area of each pot. A week after the transplantation, the evaluations of disease severity was carried out once a week following the scale from Cotxarrera et al. (2002), which is based on external symptoms, in which: zero means plants without symptoms; 1 means less than 50% of yellow leaves (chlorosis) or wilted; 2 means more than 50% of leaves yellow (chlorotic) or wilted (live plants) and 3 means fallen or dead plants.

It was also calculated the area under the disease progress curve (AUDPC) from the disease observations using following formula.

$AUDPC = \sum[(X_i+X_{i+1})/2]/t_i$

AUDPC was calculated for individual plants from the severity values. Where X_i and X_{i+1} and are severity value on date i and date i + 1, respectively, and t_i is the number of days between date i and date i + 1.

At 35 days after transplantation, the blank control plants were at the point of harvest and a final evaluation was carried out. The following variables were analyzed: percentage of surviving plants; number of leaves, obtained by the total count of the number of fully expanded leaves; stem diameter, obtained with the aid of a digital caliper; length of root system (LRS), obtained with a graduated ruler, measuring from the neck of the plant to root end; fresh weight of shoot and root (FWS and FWR), done at harvest; dry matter of shoot and root (DMS and DMR), done by separating the material to dry in a circulation stove with forced air at 65 °C until constant weight was obtained; and total dry matter (TDM), obtained by the sum of the dry mass of shoots and roots. All the above weightings were determined with a precision balance.

The experiment was fully conducted in a protected greenhouse and it was irrigated every day or according to the plants necessity. The experiment was completely randomized in a factorial 12x2 design (*Trichoderma* spp. X substrate inoculated with or not with *S. sclerotiorum*) and control plants without any of the fungi. It contained eight plants per treatment and each one corresponded to an experimental unit.

Statistical analysis

Data were subjected to analysis of variance, and when a significant effect was observed, regression analysis or comparison of means was performed by the Scott-Knott test at 5% probability. The analyses were performed by the computer program SISVAR (Ferreira, 2014).

Conclusion

Isolates of *Trichoderma* spp. obtained from different sources showed different ability to grow. The isolated *Trichoderma* spp. were able to reduce the severity of damage to lettuce plants caused by *S. sclerotiorum*. Isolates UFSMT15.1, ETSR20, WM-13 and WM+21 positively helped growth of lettuce plants, while TC1.15, WM+22 and WM-13 were good drivers pathogen.

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