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SUBSTRATE INCUBATION TIME AFTER FUNGI INOCULATION IN THE CONTROL TOMATO SEEDLING DAMPING-OFF

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ABSTRACT - The use of fungi of the genus *Trichoderma* spp. for the control of plant diseases it has proved to be an important and promising tool, mainly for the tomato crop production system, however, there are difficulties in establishing the bioagent. This work aimed to evaluate the effect of substrate incubation time after inoculation with *Trichoderma harzianum* to control the damping-off of tomato seedlings. The experimental design was completely randomized, consisting of six treatments and four replications. The treatments are two incubation times in two doses of *T. harzianum* (0 and 10 days of substrate incubation [DIST] after inoculation with *T. harzianum* in 1.0 or 5.0 g of *T. harzianum*) and two controls (control inoculated and not inoculated with *Rhizoctonia solani*). The variables analyzed were incidence of damping-off, area under the disease progress curve, percentage of emergence, emergency speed index, average emergency time, germination speed coefficient, total fresh mass, root length (cm) and height of the area part (cm). The treatments containing *Trichoderma harzianum* have proven to be promising for the control of *R. solani* and for the growth of tomato seedlings.
Keywords: *Rhizoctonia solani, Trichoderma harzianum,* bioagent, damping-off.

TEMPO DE INCUBAÇÃO DO SUBSTRATO APÓS INOCULAÇÃO COM FUNGOS NO CONTROLE DO TOMBAMENTO DE MUDAS DE TOMATEIRO

RESUMO - A utilização de fungos do gênero *Trichoderma* para o controle de doenças de plantas tem-se mostrado uma ferramenta importante e promissora, principalmente para o sistema de produção do tomateiro, porém, há dificuldades no estabelecimento do bioagente. Este trabalho teve como objetivo avaliar o efeito do tempo de incubação do substrato após inoculação com *Trichoderma harzianum* para o controle do tombamento de mudas de tomateiro. O delineamento experimental utilizado foi inteiramente casualizado, constituído de seis tratamentos e quatro repetições. Os tratamentos são dois tempos de incubação em duas doses de *T. harzianum* (0 e 10 dias de incubação do substrato [DIST] após inoculação com *T. harzianum* em 1,0 ou 5,0 g de *T. harzianum*) e duas testemunhas (testemunha inoculada e não inoculada com *Rhizoctonia solani*). As variáveis analisadas foram incidência de tombamento, área abaixo da curva de progresso da doença, porcentagem de emergência, índice de velocidade de emergência, tempo médio de emergência, coeficiente de velocidade de germinação, biomassa fresca total (g), comprimento radicular (cm) e altura da parte área (cm). Pode-se concluir que os tratamentos contendo *Trichoderma harzianum* se mostraram promissores no controle de *R. solani* e na promoção do crescimento de mudas de tomateiro.
Palavras-chave: *Rhizoctonia solani, Trichoderma harzianum,* bioagente, damping-off.

INTRODUCTION

Diseases are among the main factors affecting tomato crop productivity, including damping-off, caused mainly by the fungus *Rhizoctonia solani* (RAJENDRAPRASAD et al., 2017). For its control, the use of antagonistic microorganisms via seeds and/or soil or substrate treatment is recommended, aiming to reduce costs and damage caused. Among these microorganisms, stand out species of the genus *Trichoderma* spp. (ANDRADE et al., 2014), considered potential biocontrol agents and growth promoters for many species of plants (SAVAZZINI et al., 2009).

Species of the genus *Trichoderma* are used successfully in the control of various plant diseases, especially those related to the soil, due to the different mechanisms of action (parasitism, antibiosis, competition, and resistance induction), and for efficiently colonizing the substrate and the root system of several plant species. In addition, many of their strains are prolific producers of spores and powerful antibiotics (HARMAN et al., 2004; WOO et al., 2006).

However, when performing the inoculation of *Trichoderma* spp. in field or green house conditions, the establishment of the fungus can be hindered due to adverse environmental factors, such as high temperature, low humidity and the presence of substances toxic to the microorganism (CARRERAS-VILLASENÕR; SÁNCHEZ-ARREGUÍN; HERRERA-ESTRELLA, 2012),

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making it essential to manage or use techniques that favor the initial development of the bioagent (GHAZANFAR et al., 2018).

In the literature there are few works that study the incubation period of microorganisms in the substrate in the production of seedlings, especially *Trichoderma* spp. Soares et al. (2010) observed a positive effect of the longer incubation period of streptomycetes isolates in tomato seedlings, and suggest that this positive effect is due to the longer time for the establishment of the microorganism and the production of extracellular enzymes.

Several studies on the growth of *Trichoderma* spp. in petri dish, they evaluate after the incubation period 3 to 10 days (MAMO; ALEMU, 2002; RINI; SULOCHANA, 2008; MUSTAFA; POULSEN; SHENG, 2016; LÓPEZ et al., 2019), with Rini and Sulochana (2008) obtained the highest biomass of *T. harzianum* after 10 days of incubation. In field conditions, there is a lack of studies as to which incubation period is necessary for the efficient establishment of the microorganism, thus, preliminary studies are essential in order to define such techniques.

In this sense, the present work was conducted with the objective of evaluating the effect of the incubation time of the substrate after inoculation with *T. harzianum* to control the damping-off of tomato seedlings.

MATERIAL AND METHODS

The test was conducted in a greenhouse, in plastic trays with 128 cells, containing commercial substrate Tropstrato HT hortaliças®. The tomato cultivar sown was Sakata's® 'Débora Max' and the inoculated *Trichoderma* spp. was the commercial product Ecotrich®, based on *T. harzianum* (10^{10} UFC g^{-1}). The isolate of R. solani was obtained from the Phytopathology Laboratory of the State University of Londrina and multiplied in BDA culture medium (Agar Potato Dextrose).

The experimental design was completely randomized, with of six treatments and four repetitions, in which each repetition was represented by seven cells, with three seedlings each. The treatments are two incubation times in two doses of *T. harzianum* [0 and 10 DIST - days of incubation of the substrate after inoculation with *T. harzianum*] and in two doses of *T. harzianum*, 1.0 or 5.0 g (D1 and D5); two witnesses (witness inoculated and not inoculated with *R. solani*). All treatments with *T. harzianum* were inoculated with *R. solani*.

The inoculation of *T. harzianum* was performed in two situations: 10 days before sowing and keeping the substrate incubated for that period, at a temperature of 25°C (10 DIST) and inoculation on the day of sowing (0 DIST), adding 1.0 and 5.0 g of the product formulated in 1.5 kg of substrate. For the assembly of the trays, 15 g of substrate, three seeds and one *R. solani* disc were added per cell. In one witness, the inoculation of *R. solani* and *T. harzianum* was not performed and in the other, only the inoculation of *R. solani* was performed.

The incidence evaluations were performed weekly, verifying the percentage of seedlings damping-off through the counting of symptomatic seedlings. From the incidence results, the area under the disease progress curve (AUDPC) was calculated, as proposed by Campbell & Madden (1990). The emergency percentage was evaluated in parallel to the incidence evaluation, and the following were performed: emergency speed index (ESI) calculations, according to the methodology described by Maguire (1962); emergency mean time (MET) in days, according to the Labouriau methodology (1983); and emergency speed coefficient (ESC), described by Furbeck et al. (1993). After 30 days of installation of the experiment, the length of the aerial and root part was measured in centimeters with the aid of a graduated ruler, and the total fresh mass (TFM) in milligrams, using a precision scale.

The data were submitted to variance analysis and the means were compared by Tukey's test ($p \leq 0.05$). The assumptions of normality and homogeneity of variances were tested by Shapiro-Wilk and Bartlett at 5% significance level. Quantitative statistical analyses were processed using the software R (R CORE TEAM, 2020).

RESULTS AND DISCUSSION

Several studies indicate the negative effect of climate adversities on *Trichoderma* spp. and the lack of correlation between *in vitro* and field tests (ELSHAHAWY et al., 2017). However, these problems can be minimized when using management or techniques that favor the initial development and the establishment of the bioagent, facilitating its mechanisms of action, as well as its ability to promote the development of the culture of interest.

According to Table 1, a significant difference was observed in the height variable of plants, in which treatments with a dose of 1.0 g of *T. harzianum* + 10 DIST, 5.0 g of *T. harzianum* + 0 DIST, 5.0 g of *T. harzianum* + 10 DIST and the non-inoculated witness showed greater height. The same behavior was observed for root length. However, in the variable total fresh mass, the dose of 5.0 g + 0 DIST and 1.0 g + 10 DIST were higher than the inoculated witness (Table 1).

The growth stimulation of tomato seedlings, especially in the D5 + 0 DIST and D1 + 10 DIST treatments in the presence of the phytopathogen *R. solani* were statistically equivalent to the control not inoculated with the disease. This confirms many works in the literature that observed the positive effect of the fungus *Trichoderma* spp. on plant development. In this sense, one of the possible explanations is that many strains of *Trichoderma* spp. are capable of producing compounds that act as growth regulators, resulting in changes in plant metabolism, such as the production of auxins and their derivatives, besides improving the absorption of nutrients (BATTAGLIA et al., 2013; ZHAO et al., 2014; LORITO; WOO, 2015; RUOCCO et al., 2015). However, the relationship between the use of species of the genus *Trichoderma* and the synthesis of auxin and the promotion of growth in soil-based systems is still unclear (NIETO-JACOBO et al., 2017).

TABLE 1 - Height of seedlings; root length; total fresh mass (TFM) in tomato seedling submitted to two incubation times (0 and 10 DIST) in two doses of *Trichoderma* (1.0 and 5.0 g - D1 and D5).

Tratamentos	Height (cm)	Root length (cm)	TFM (mg)
D5 + 10 DIST	15.09 a	9.81 a	23.00 ab
D1 + 10 DIST	15.56 a	10.61 a	23.64 a
D5 + 0 DIST	14.98 a	10.16 a	25.15 a
D1 + 0 DIST	13.56 b	8.63 b	21.59 ab
Witness without *R. solani*	14.90 a	9.68 a	21.31 ab
Witness with *R. solani*	13.30 b	8.78 b	19.41 b
CV (%)	7.75	10.53	12.48

*Means followed by the same letter in the column do not differ statistically through Tukey's test ($p \leq 0.05$). CV = coefficient of variation. D1 = 1.0 g, D5 = 5.0 g e DIST = days of incubation of the substrate.

There was no significant difference for the EMT and ESC variables. The results of emergency percentage and ESI presented similar behavior (Table 2), and for the non-inoculated witness, the treatments with a dose of 1.0 g + 10 DIST and of 5.0 g + 0 DIST presented the highest means. However, the dose of 5.0 g was lower when submitted to 10 DIST. Thus, the treatment with a lower dose proved to be superior to the one with a higher dose, indicating that the treatment with *T. harzianum* in the lower dose needs a longer time of contact with the substrate to obtain a better effect, while the higher dose acts immediately after the inoculation, not needing time for multiplication, observing the opposite effect and thus hampering the emergence.

TABLE 2 - Emergency percentage; emergency speed index (ESI); emergency mean time (EMT); emergency speed coefficient (ESP) in tomato seedling submitted to two incubation times (0 and 10 DIST) in two doses of *Trichoderma* (1.0 and 5.0 g – D1 and D5).

Treatment	Emergence (%)	ESI	EMT	ESP
D5 + 10 DIST	83.33ab	8.05 ab	10.76 a	9.32 a
D1 + 10 DIST	97.61 a	9.06 a	11.25 a	8.95 a
D5 + 0 DIST	94.04 a	9.42 a	11.15 a	8.98 a
D1 + 0 DIST	73.80 b	6.70 b	11.44 a	8.76 a
Witness without *R. solani*	94.04 a	9.04 a	11.05 a	9.08 a
Witness with *R. solani*	78.56 b	7.72 ab	12.08 a	8.34 a
CV (%)	8.64	11.13	7.75	7.36

*Means followed by the same letter in the column do not differ statistically through Tukey's test ($p \leq 0.05$). CV = coefficient of variation. D1 = 1.0 g, D5 = 5.0 g and DIST = days of incubation of the substrate.

According to You et al. (2016), it is well known that some phytohormones, such as gibberellins and brassinosteroids, can promote seed germination and that these compounds may be produced by species of the genus *Trichoderma*, as reported by Chowdappa et al. (2013). These two phytohormones synthesized by *Trichoderma* spp. may be responsible for promoting the germination of tomato seeds, as well as the subsequent growth of seedlings (YOU et al., 2016), as observed in the present study. Finally, for the results of seedling damping-off and area under the disease progress curve (AUDPC), the treatments with a dose of 1.0 g + 10 DIST and that of 5.0 g + 10 DIST proved to be superior to the ones witnessed with *R. solani*, there being no significant difference between them, indicating that the control of the disease was efficiently performed by the biological agent (Figure 1). These results showed that even the smallest dose (1.0 g) of *T. harzianum*, when inoculated to the substrate and submitted to 10 days of incubation, presents a good response, thus enabling the establishment of the fungus and greater control efficiency.

The application of high concentrations of *Trichoderma* spp. propagules is necessary for the satisfactory control of phytopathogens, although considerable inhibition of the germination of the conidia themselves can occur when this fungus is applied in very high doses (HJELJORD; TRONSMO, 2003), inhibitory effect on the culture of interest (ETHUR et al., 2008; HASSAN et al., 2013; WIETHAN et al., 2018), possibly due to competition for substrate, nutrients and water. Thus, the application of low doses to the substrate in advance of sowing, avoids this inhibitory effect due to the low microbial load, allowing the better establishment of the bioagent, in addition to obtaining satisfactory concentrations for disease control, as observed by Soares et al. (2010).

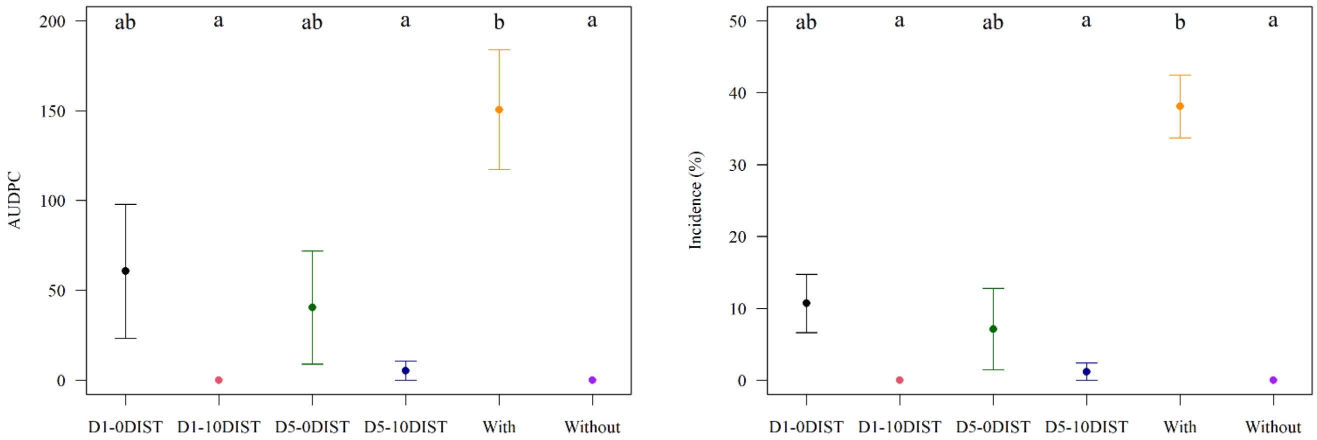

FIGURE 1 - Incidence of *Rhizoctonia solani* and AUDPC of *Rhizoctonia solani* in tomato seedling submitted to two incubation times (0 and 10 DIST) in two doses of *Trichoderma* (1.0 and 5.0 g), witness with *R. solani* (with), witness without *R. solani* (without). *Equal letters do not differ according to Tukey's test ($p \geq 0.05$). Bars indicate confidence interval.

Thus, the incubation of the bioagent in substrate in advance of sowing is an interesting strategy for the producer of tomato seedlings, since it allows reducing the cost of production due to the lower dose needed, thus contributing to the control of *R. solani* and the improvement of quality attributes of the seedlings, allowing similar results to the application of higher doses without incubation.

CONCLUSION

The treatments containing *Trichoderma harzianum* have proven to be promising in the control of *R. solani* and in the growth of tomato seedlings.

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