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Use of *Trichoderma* for disease control, development and growth stimulus in *Lactuca sativa* L.

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Abstract: The current pressure for healthy food implies in use of alternative methods to the conventional use of agrochemicals. In this scenario, biological control is fundamental for food safety. The use of *Trichoderma* to control diseases in lettuce appears as a viable alternative. The objective of this work was to verify the efficiency of *Trichoderma* to control diseases (white mold) in lettuce and to evaluate its influence in the stimulus of development and growth of this crop. Six treatments were used: T1 = control; T2 = application of *Trichoderma* in liquid formulation at intervals of 7 days; T3 = application in liquid formulation at 14 days intervals; T4 = application in granulated + liquid formulation at intervals of 7 days; T5 = application of granulated + liquid formulation at 14 days intervals, and T6 = application of *Trichoderma* in granulated formulation only in planting. The final number of leaves (NFF), phyllochron (°Cday.leaf^{-1}), fresh mass and diameter were evaluated and was observed the occurrence of diseases in the different treatments. The results indicate the introduction of *Trichoderma* is recommended for the control of white mold on lettuce but does not promote significant increases in the development and growth of plants.

Keywords: horticulture, biological control, white mold.

Uso de *Trichoderma* para controle de doença e estímulo de desenvolvimento e crescimento em *Lactuca sativa* L.

Resumo: A atual pressão por alimentos saudáveis implica na utilização de métodos alternativos ao convencional uso de agrotóxicos. Neste cenário, o uso do controle biológico é de fundamental importância para a segurança alimentar e o emprego de *Trichoderma* no controle de doenças em culturas comercialmente exploradas, como a *Lactuca sativa* L., surge como uma alternativa viável. O objetivo do trabalho foi verificar a eficiência do uso do *Trichoderma* para controle de doenças (mofo branco) em alface e avaliar a sua influência no estímulo de desenvolvimento e crescimento da cultura. Foram utilizados seis tratamentos: T1 = testemunha (sem aplicação); T2 = aplicação do *Trichoderma* em formulação líquida em intervalos de 7 dias; T3 = aplicação em formulação líquida em intervalos de 14 dias; T4 = aplicação em formulação granulada + líquida em intervalos 7 dias; T5 = aplicação de formulação granulada + líquida em intervalos 14 dias, e T6 = aplicação de *Trichoderma* em formulação granulada apenas na cova de plantio. Foram avaliados o número final de folhas (NFF), filocrono (°Cdia.folha^{-}

[1]), massa fresca (g) e diâmetro (g) e observada a ocorrência de doenças nos diferentes tratamentos. Os resultados obtidos indicam que a aplicação de *Trichoderma* é recomendada para o controle de mofo-branco em alface, porém não promove incrementos significativos no desenvolvimento e crescimento das plantas.

Palavras-chave: horticultura, controle biológico, mofo branco.

Introduction

Lettuce *(Lactuca sativa* L.) has a big importance in the Brazilian scenario, as much for the economic factor, since it represents a culture widely produced and commercialized in all the states of the country; as well as by the food factors, being the most consumed vegetable, tending to increase its consumption, since the profile of the consumer changed, tending to eat in increasingly healthy way (SALA and COSTA, 2012).

Being part of the Asteraceae family, it is an herbaceous plant with an annual cycle, with the emission of leaves in rosette, from a stem with non-elongated internodes. The leaves make up the edible part, which has a commercial interest, being smooth or curly, with the formation or not of a "head", presenting green or purple coloration (TRANI et al., 2014). It is a crop widely affected by fungal diseases, of which the white mold (*Sclerotinia sclerotium*) stands out, especially in conditions of high soil moisture and warm air temperature (CARNEIRO et al., 2011).

The use of biological control in agriculture, especially in the production of vegetables, has been highlighted, since, at the same time that there is an environmental pressure due to the wide use of pesticides, in order to provide safer and healthier food production, free of residues, the market for organic products, in continuous expansion, is one of the sectors of agriculture with very high demand for products of biological origin or zero residual (BETTIOL and MORANDI, 2009; SEDIYAMA et al., 2014). In this scenario, an introduction of fungi of the genus *Trichoderma* to control diseases in cultivated plants, appears as a good alternative, with an advantage of the absence of residues.

The genus *Trichoderma* combats saprophytic fungi, which compete with pathogens present in the soil, that is, they are microorganisms that feed on dead organic matter, promoting the decomposition of plant and animal residues that, after being decomposed, still return to the soil, serving as source of nutrients for plants (LUCON et al., 2009). Therefore, in addition to reducing the severity of plant diseases by inhibiting the pathogens present in the soil through antagonistic or mycoparasitic activity (HERMOSA et al., 2012) they can also interact directly with the roots of plants, increasing their growth potential, increasing also the tolerance to abiotic stresses (HERMOSA et al., 2012).

It has a positive effect on the development of tree seedlings (MACHADO et al., 2015; AZEVEDO et al., 2017), on the yield of grain crops (CHAGAS et al., 2017), and specifically in lettuce plants (SILVA et al., 2015). The objective of this work was to verify the efficiency of the use of *Trichoderma harzianum* in the control of white mold (*Sclerotinia sclerotium*) in lettuce and to evaluate its influence in the stimulus of development and growth of the crop.

Material and Methods

The experiment was carried out from lettuce seedlings, cultivar type Veneranda, purchased in the local trade establishments and transplanted to beds, inserted in the experimental area of the Horticulture Sector of the Department of Plant Science, Federal University of Santa Maria, Santa Maria, RS. The municipality of Santa Maria is in the physiographic region of the Central Depression of Rio Grande do Sul (latitude 29°43'S, longitude 53°43'W and altitude of 95m) and the climate, by classification of Köppen, is Cfa (Subtropical).

The beds were previously prepared and fertilized according to the recommendations of the Manual of Fertilization and Liming for the States of Rio Grande do Sul and Santa Catarina (SBCS, 2004). Each bed was 22 meters long and 1 meter wide, totaling 22 m^2. The seedlings were transplanted to the beds on July 13, 2017, when they presented approximately 4 to 5 true leaves, spaced 0.25 m between rows by 0.25 m between plants in the rows, totalizing 4 plant lines per bed. The total area of the plot was divided into 6 plots of 3 m^2 each, where treatments were applied in randomized blocks with three replicates for each treatment. In each repetition (3m^2), 10 central plants were evaluated, totaling 30 plants for each treatment.

The treatments applied were: T1 = control; T2 = application of *Trichoderma* in liquid formulation at intervals of 7 days; T3 = application in liquid formulation at 14 days intervals; T4 = application in granulated + liquid formulation at intervals of 7 days; T5 = application of granulated + liquid formulation at 14 days intervals, and T6 = application of *Trichoderma* in granulated formulation only in planting. The liquid formulation, at the concentration of 5x108 spores/ml, was applied in each repetition (3.6 ml of liquid formulation diluted in 2 liters of water). A granulated formulation, at a concentration of 3 x 108 spores/g, was applied to each repetition (0.8 g of the granulated formulation, totaling 38.4 g per each repetition).

In all demarcated plants, the number of leaves was evaluated at the frequency of twice a week. The last evaluation was performed on July 31, 2017, in which the final number of leaves (NFF) of each plant was counted. In order to evaluate the influence of the treatments on the increase in lettuce development, the phyllochron was calculated by the inverse of the coefficient of a linear regression between the number of leaves of the plant (NF) and the accumulated thermal sum (*STa*), being expressed ºCday.$leaf^{-1}$.

The accumulated thermal sum (*STa*, °Cday) is obtained from the sum of the daily thermal sum (*STd*), that is:

$$STa = \Sigma STd$$

The *STd* is obtained from the arithmetic mean of the minimum and maximum daily air temperatures, subtracting the base temperature (MCMASTER and WILHELM, 1997), which for the lettuce is 10°C (BRUNINI et al., 1976). Therefore, the daily thermal sum is calculated from the equation:

$$STd = \left(\frac{T_{max} + T_{min}}{2} - T_b\right) * 1\ day$$

where *STd* is the daily thermal sum, T_{max} is the daily maximum air temperature (°C), T_{mim} is the minimum daily air temperature (°C) and T_b is the base temperature (°C) (GILMORE and ROGERS, 1958).

For thermal sum determination, the data available from the conventional station of Santa Maria on the website of the National Institute of (INMET) was used for this research.

The harvest of the demarcated plants was performed on August 01, 2017, when the fresh mass (g) and diameter (cm) of each plant were determined. The parameters of fresh mass and diameter were measured in order to evaluate the application of the treatments on the development in lettuce growth.

It was observed the occurrence of diseases in the demarcated plants in every single treatment, being made annotations. The NFF, phyllochron, fresh mass, and plant diameter data were submitted to analysis of variance and the means were compared by the Tukey test, at a 5% probability of error, in the software SISvar 5.6.

Results and Discussion

Figure 1 shows the maximum, minimum and mean air temperatures for the experimental period in the municipality of Santa Maria, RS. The mean air temperature throughout the experiment was 18°C, the maximum absolute air temperature was 29.4°C and occurred on 17/06/17 (dd/mm/yy) while the absolute minimum air temperature was -1.2°C and occurred on 19/07/17 (dd/mm/yy). The temperatures observed in the experimental period are within the range favoring the development of fungi, such as *Sclerotinia sclerotium,* which according to Carneiro et al. (2011) are more capable of damage when temperatures are between 20 and 25°C.

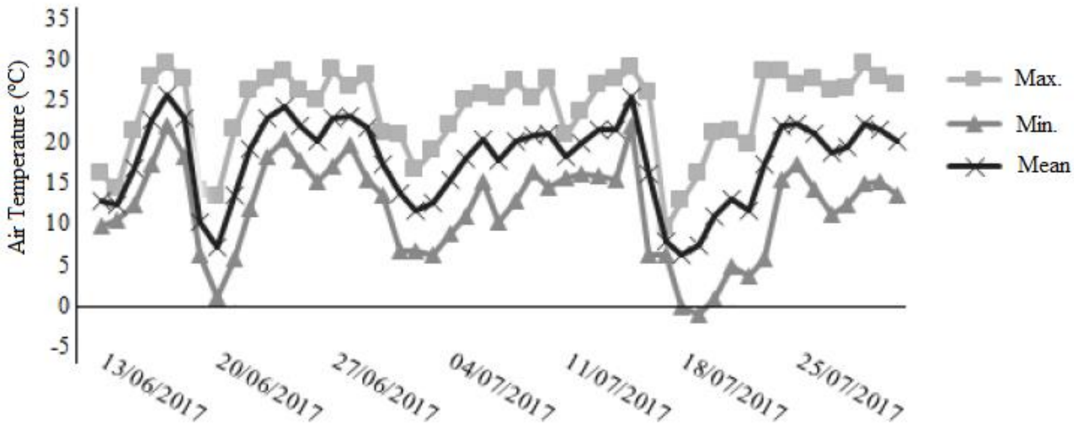

Figure 1. Maximum, minimum and mean air temperatures (°C) for the experimental period with lettuce culture in Santa Maria, Rio Grande do Sul (dd/mm/yyyy).

Figure 2 shows linear regressions between the number of leaves (NF) and the accumulated thermal sum (*STa*), in all different treatments. The phyllochron averages obtained in each treatment were submitted to statistical analysis and are presented in Table 1. For the T1, the lowest phyllochron value is equal to 24.51 °Cday.leaf^{-1}; while the highest phyllochron value was found for T6, which was 30.67 °Cday.leaf^{-1}, which did not differ statistically from T2 (phyllochron = 30.39 °Cday.leaf^{-1}), that is, the emission rate of leaves was slightly higher for plants where no application of *Trichoderma* was observed, demonstrating that *Trichoderma*, in this case, did not act as a plant growth promoter. Probably the beneficial effects provided by the application of *Trichoderma* in cultivated plants were not verified in this work due to the very short cycle between planting and NFF evaluation in lettuce, which was 20 days. This observation corroborates data obtained in a study by Chagas et al. (2017) with maize, rice, soybean and cowpea crops. These authors observed that inoculation with *Trichoderma* promoted plant growth only from 25 days after plant emergence, and treatments with *Trichoderma* were superior for the characteristics evaluated in relation to the control (CHAGAS et al., 2017).

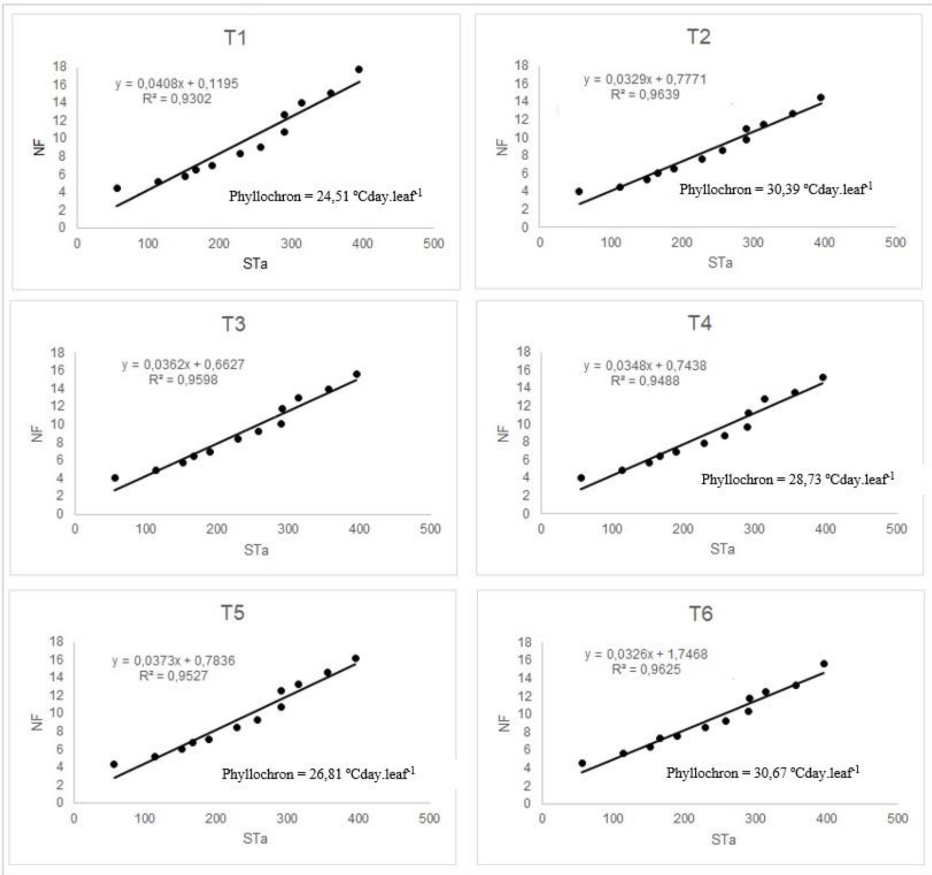

Figure 2. Linear regression between accumulated thermal sum (*STa*) and number of leaves (NF) accumulated in lettuce grown under different treatments in Santa Maria, RS, Brazil: T1 = control; T2 = application of *Trichoderma* in liquid formulation at intervals of 7 days; T3 = application in liquid formulation at 14 days intervals; T4 = application in granulated + liquid formulation at intervals of 7 days; T5 = application of granulated + liquid formulation at 14 days intervals, and T6 = application of *Trichoderma* in granulated formulation only in planting.

Table 1 shows that T1 and T5 obtained the highest mean final number of leaves (NFF) and did not differ statistically from the other treatments, again indicating that the use or not of *Trichoderma* did not influence plant development parameters, not being recommended for this purpose.

When we related the phyllochron, which is the time interval between the appearance of two successive leaves (WILHELM and MCMASTER, 1995) and the final number of leaves (NFF), it was possible to visualize that the plants with the highest NFF in the harvest (17.65 and 16.08, corresponding to T1 and T5, respectively) were those that had the lowest phyllochron value (24.51 and 26.81 °Cday.leaf^{-1}, for T1 and T5, respectively), that is, the lower phyllochron levels allowed an accelerated leaf emission rate, resulting in plants with higher NFF. The growth parameters (fresh mass and diameter) of the plants with higher NFF were therefore expected to be higher than the other treatments, which was not verified, as presented in Table 1.

Table 1. Phyllochron (°Cday.leaf-1), final number of leaves (NFF), fresh mass (g) e diameter (cm) of lettuce grown under different treatments in Santa Maria, RS, Brazil

Treatment	Phyllochron (ºCday.leaf-1)	NFF	Fresh mass (g)	Diameter (cm)
T1	24,51 a*	17,65 ab	128,75 a	67,30 a
T2	30,39 b	14,53 b	100,00 a	59,82 a
T3	27,62 ab	15,67 b	115,18 a	63,85 a
T4	28,73 ab	15,26 b	101,74 a	62,78 a
T5	26,81 ab	16,08 ab	120,00 a	66,64 a
T6	30,67 b	15,69 b	116,54 a	68,61 a
CV (%)	19,58	12,57	36,40	16,95

* Averaged followed by the same letter do not differ statistically from each other by the Tukey test at 5%. T1 = control; T2 = application of *Trichoderma* in liquid formulation at intervals of 7 days; T3 = application in liquid formulation at 14 days intervals; T4 = application in granulated + liquid formulation at intervals of 7 days; T5 = application of granulated + liquid formulation at 14 days intervals, and T6 = application of *Trichoderma* in granulated formulation only in planting.

As for the observed growth parameters (fresh mass and diameter), there was no statistical difference between the applied treatments (Table 1), a result also observed by Cassiolato et al. (1996), which tested the application of different mutants of *T. harzianum* (TW5 and WT-T95) and found no statistical difference between treatments with respect to lettuce diameter.

It should be noted that in T6 (application of *T. harzianum* in granulated formulation only in the planting, next to the pit) was observed higher mortality of plants in relation to the other plots, which may indicate that this form of application is not the most appropriate for the cultivation of lettuce. Possibly the contact between the seedling and the granulate may have negatively interfered with the soil-to-ground contact, making it difficult or less rooting and, consequently, the seedling take on growth. Another hypothesis is that the granulated formulation may have the same interference that the fertilizer causes in seed germination when in direct contact, as observed by Fois et al. (2017) in maize plants, which verified that the more distant the fertilizer is placed from the seed, the less the damage to them and the development of the seedlings.

Regarding the incidence and control of diseases, the results obtained with the application of *T. harzianum* were satisfactory (Figure 3). In the plots where the plants that did not receive application of *T. harzianum* (T1 = control), the incidence of white mold (*Sclerotinia sclerotium*) was observed in 23.3% of the plants. In the plots where *T. harzianum* was applied (T2, T3, T4, T5, and T6), disease control was equal or greater than 90%, reaching 100% control for T5 and T6. This demonstrates the ability of fungi of the genus *Trichoderma* to control *S. sclerotium*, as demonstrated by Ethur et al. (2001) in vitro and in lettuce plants by Silva et al. (2015).

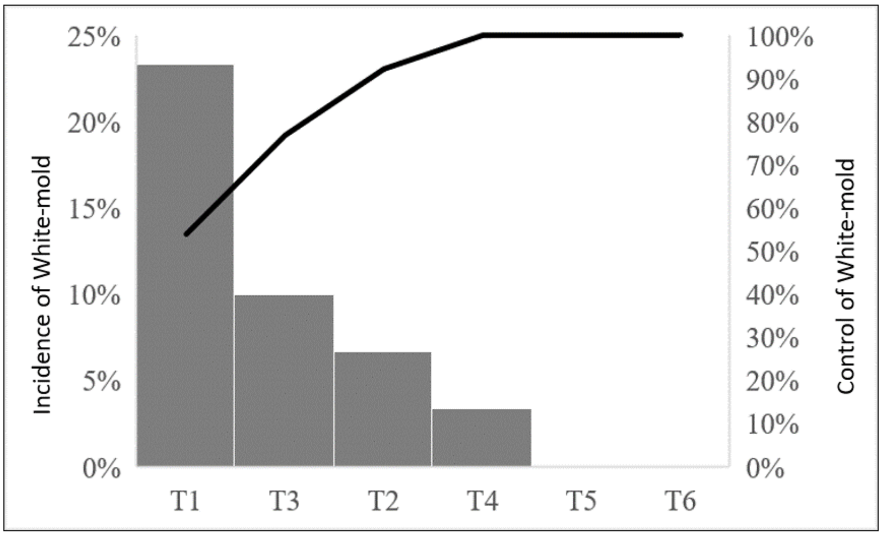

Figure 3. Incidence and control of white mold (*Sclerotinia sclerotium*) observed for treatments T1 = control; T2 = application of *Trichoderma* in liquid formulation at intervals of 7 days; T3 = application in liquid formulation at 14 days intervals; T4 = application in granulated + liquid formulation at intervals of 7 days; T5 = application of granulated + liquid formulation at 14 days intervals, and T6 = application of *Trichoderma* in granulated formulation only in planting. Santa Maria, RS, Brazil.

Conclusion

The introduction of *Trichoderma* is recommended for the control of white mold on lettuce but does not promote significant increases in the development and growth of plants.

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