

## Effect of *Trichoderma* spp. on the Propagation of *Maytenus ilicifolia* Mart. ex Reissek

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### Abstract

The interest in using biotechnology tools that contribute to reducing the need for chemical inputs in agroforestry production has increased in recent years, aiming at higher quality for the environment and for society. This interest is also applied to medicine species consumed *in natura*. The objective of this study was to evaluate the effect of *Trichoderma* spp. on germination and initial growth of *Maytenus ilicifolia* seedlings. Laboratory and greenhouse tests were carried out using *Trichoderma* spp. isolates obtained from three different strains identified as *Trichoderma asperelloides* (T1 & T2 strains) and *Trichoderma virens* (T10 strain). In laboratory, *M. ilicifolia* seeds without aryl were inoculated in solutions containing fungal spores and distributed in substrate paper in four replicates of 25 seeds/treatment. The first germination count, accumulated germination, percentages of accumulated dead seeds and firm seeds were evaluated at 7, 14, 21 and 28 days of incubation. The same isolates were used to evaluate the effect on the initial growth of seedling in a greenhouse through a completely randomized design with 40 replicates, considering a seedling as each replicate. *Trichoderma* spp. isolates were inoculated on the substrate used as the basis for seeding. Seeds of *M. ilicifolia* were used from the same batch of the laboratory test, but without removing the aryl. The variables of total height, diameter at root collar and number of leaves were evaluated at 90, 120, 150 and 180 days after seeding. A positive effect of *Trichoderma* inoculation on seed germination and vigor was observed in the laboratory, with emphasis on the T2 isolate. Growth promoting effects on the *M. ilicifolia* seedlings were not observed in greenhouse. We suggest to better investigate the interaction between the tested *Trichoderma* isolates and *M. ilicifolia* seeds in the presence of aryl.

**Keywords:** *Maytenus ilicifolia*, medicinal species, growth promoters, beneficial fungi, forest seeds

### 1. Introduction

The search for treatments from medicinal and phytotherapeutic plants in Brazil increased 161% between 2013 and 2015. This increase is largely due to the National Policy on Medicinal Plants and Phytotherapeutics promoted by the Health Ministry, which has been able to promote efforts to disseminate and aggregate species of medicinal value in the Unified Health System-SUS (Brasil, 2016). *Maytenus ilicifolia* Mart. ex Reiss. (Celastraceae) is popularly known as espinheira-santa, is one of the most exploited native forest species in Brazil. Due to its applicability in traditional medicine over the years, and mainly after the pharmacological confirmation of its antiulcerogenic and anti-gastric properties (Di Stasi, 2004; Brasil, 2018) there was an expressive increase of predatory extractive activities among its population in naturally.

It is a sub-shrub or small tree, of perennial habit, branched from the base and height between 2 and 5 meters (Carvalho-Okano, 1992). The main form of propagation is through seeds that should be harvested when the fruits are brownish-red color and open spontaneously, exposing the aryl (Montanari Junior, Scheffer, & Radomski, 2004; Negrelle, Doni, Ohlson, & Herr, 1999). In Rio Grande do Sul, fruit maturation occurs between November and December in the southern region and in January in the northeast region of the state (Mariot, 2005). The seeds are orthodox but need to be stored in a cold chamber, since they gradually lose viability (Eira, Dias & Mello, 1995; Lima, 2010).

Seed germination is quite uneven being extend up to six months after seeding (Lima, 2010) and growth is considered slow, a factor that hinders the process of obtaining seedlings in the short term (Kowalski, Signor, Machado, Biasi, & Lima, 2008; Nicoloso, Fotunato, Zanchetti, Cassol, & Eisinger, 2000).

Because it was considered priority in conservation strategies in the Southern region of Brazil (Steenbock & Reis, 2011), it is believed that the insertion of *M. ilicifolia* into the family farming of small properties can diversify production, strengthen the productive chain of medicinal species and contribute to the *in situ* conservation of species, leading to sustainable extractive practices (Mariot & Barbieri, 2007, Carvalho & Rosa, 2014, Rovedder et al., 2016).

However, inserting it in agriculture requires compatible agricultural production methods with the use of raw vegetable materials, since the product supply is often insufficient due to production scarcity and/or low phytosanitary quality (Gahukar, 2017). Moreover, information on the behavior of medicinal plants when submitted to agricultural production techniques is still very scarce (Pravuschi, Marques, Rigolin, & Santos, 2010). When it comes to native forest species, this lack is even greater, even when presented with enormous potential for biological diversity in the Brazilian territory.

In this context, the use of *Trichoderma* spp. as biological input represents an opportunity of use in ecological farming for producing medicinal species, acting in promoting growth and plant development and as a biocontrol agent of pathogens (Harman, Howell, Viterbo, Chet, & Lorito, 2004). These characteristics provide greater food safety for the consumer, considering that the application of agrochemicals is reduced (Akhtar & Siddiqui, 2008). Moreover, there are reports that their use may present increases of up to 200% in total biomass (Stewart & Hill, 2014), an aspect of extreme importance in the commercialization of medicinal species.

However, the application of *Trichoderma* spp. in producing native forest species and its effects on the growth process are concentrated in a limited number of species, such as *Theobroma cacao* (Bae et al., 2009; Tchameni et al., 2011), *Gochnatia polymorpha* (Machado, Tavares, Lopes, & Silva, 2015), *Prunus* sp. (Sofu, Milella & Tataranni, 2010), *Myrcianthes pungens*, *Eugenia pyriformis* (Soldan, 2014) and *Jacaranda micrantha* (Amaral, Steffen, Maldaner, Missio, & Saldanha, 2017). Given this gap, the objective of this study was to evaluate the effect of *Trichoderma* on the germination and initial growth of *M. ilicifolia* seedlings, aiming to develop production technologies compatible with the final use of the raw material.

## 2. Material and Method

This study was conducted in the *Laboratório de Análise de Sementes Florestais (LASF)* and in a greenhouse of the *Centro de Pesquisa em Florestas do Departamento de Diagnóstico e Pesquisa Agropecuária (DDPA)* of the *Secretaria Estadual da Agricultura, Pecuária e Irrigação do RS (SEAPI/RS)*, located in the municipality of Santa Maria, Rio Grande do Sul, Brazil, in the period of November 2015 to May 2016.

In the laboratory, inoculation effects of three different *Trichoderma* spp. isolates on the germination and seed vigor of *M. ilicifolia* were evaluated. The seeds used were collected from matrices located in the municipality of Santa Maria, RS, in November 2016. Four treatments were evaluated corresponding to the inoculation of three fungal isolates of the *Trichoderma* genus (T1, T2 and T10 isolates) belonging to the collection of the *Centro de Pesquisas em Florestas (DDPA)* and control treatment without inoculation.

*Trichoderma* spp. isolates were identified at the species level by the *Laboratório de Bioquímica Fitopatológica do Instituto Biológico da Universidade de São Paulo (USP)* as: *Trichoderma asperelloides* (T1 & T2) and *Trichoderma virens* (T10). Molecular identification was performed through the translation elongation factor gene sequencing. *Trichoderma asperelloides* (T1 & T2) were isolated of soil with native forest vegetation their origin, obtained through samples collected at different points of the site while *Trichoderma virens* (T10) was isolated from the superficial seed layer of the *Delonix regia* Raf. that were in germination and vigor analysis at the LASF. From the observation of fungal mycelium growth with morphology similar to the *Trichoderma* genus, mycelial fragments were transferred to Petri dishes containing BDA culture medium species.

The solutions containing the inoculum of each fungal isolate were previously prepared in the laboratory. To that end, 100 grams of parboiled rice colonized with each *Trichoderma* isolate were placed in an Erlenmeyer glass flask in which 1000 mL of distilled water was added. Double gauze filtration was carried out after five minutes of stirring the solutions to release spores contained in the surface of the rice grains. The spore concentration was determined under an optical microscope with the aid of Neubauer chamber. For this, dilution was performed of 1 mL of each solution of *Trichoderma* sp. in 99 mL sterile distilled water. From this suspension, 10 microliters were analysed in Neubauer's chamber, the final concentration adjusted to  $2 \times 10^7$  spores per mL. The solutions containing the fungal inoculum remained refrigerated until the time of its use.

Seeds without the aryl were separated into four batches of 100 units for each of the four treatments and arranged in gerbox plastic boxes disinfested with 70% alcohol solution. Then, we added a volume of solution containing spores for each treatment (seeds inoculated with *T. asperelloides* (T1), seeds inoculated with *T. asperelloides* (T2), seeds inoculated with *T. virens* (T10) and seeds without *Trichoderma* spp. inoculation/control). The seeds remained submerged for five minutes in the solution. The same procedure was performed in the control treatment, however we used distilled water in place of solutions with spores. Removal of aryl from the seeds occurred manually using scissor.

At the end of the five-minute period, the liquids contained in the gerbox boxes were discarded and the seeds were immediately distributed onto substrate paper suitable for germination in four replicates of 25 seeds, adapted from Brasil (2013).

The treatments were placed in a Biomatic<sup>®</sup> Mangelsdorf seed germinator at a temperature of 25 °C ( $\pm 2$ ) following the methodology described by Brasil (2013). For standardization, germinated seeds that showed radicle emission greater than or equal to 2 mm were considered. The first germination count, accumulated germination, percentages of accumulated dead seeds and firm seeds at 7, 14, 21 and 28 days of incubation were evaluated, adapted from the methodology described by Brasil (2013).

The data were transformed by the Arcsen formula, being the statistical methodology used when working with percentage data (Gomes, 1985). The following formula was used:  $\sqrt{x}/100$ . The data were subsequently submitted to the Tukey test at 5% probability of error, using the Sisvar v.5.6 statistical program (Ferreira, 2011).

The greenhouse trial was installed in a completely randomized design, with three treatments consisting of *Trichoderma* spp. inoculation in the substrate and one control treatment without fungal isolate for comparison. A total of 40 replicates per treatment were used, considering one plant per tube as repeat.

The substrate composition consisted of a mixture of non-sterile soil from the Horizon A of arsenic dystrophic red argisol (Santos, Jacomini, & Anjos, 2013) and Carolina Soil<sup>®</sup> commercial substrate in a 1:1 (v/v) ratio. *Trichoderma* spp. inoculum were prepared according to the methodology proposed by Steffen & Maldaner (2017).

*M. ilicifolia* fruits containing the seeds remained in shade until maturity was reached, as verified by the epicarp rupture and aryl exposure. The seeding was done 7 days after the epicarp opening in tubes with a volume of 180 cm<sup>3</sup>, filled with the respective substrates of each test. Two *M. ilicifolia* fruits (seeds + aril) were sown in each tube. After seeding, the plastic grids containing the tubes remained inside the greenhouse to protect the seedlings from excessive heat until 180 days of evaluation. Irrigation was performed daily throughout the experiment with tap water, without addition of nutrients. Seedling thinning occurred 60 days after seeding, leaving one plant per tube.

The following variables were evaluated at 90, 120, 150 and 180 days after seeding: total height (cm) using a graduated ruler, diameter at root collar (mm) with a digital pachymeter and the number of completely expanded leaves.

The data were processed and analyzed using Microsoft Excel and Assistat 7.7 pt software (Santos & Silva, 2016). As the data did not show a normal distribution as verified by the Lilliefors and Shapiro-Wilk tests, and homogeneity of the variances by the Bartlett test, the means for the total height, diameter at root collar and number of leaves variables were analyzed by the Kruskal-Wallis test to 5% probability of error (Filho, Lúcio, Lopes, & Storck, 2012).

### 3. Results and Discussion

A positive effect of *Trichoderma* spp. inoculation was observed on germination of *M. ilicifolia* seeds under laboratory conditions, with differences in relation to the evaluated isolates. All *Trichoderma* spp. isolates expressed significantly higher percentages at the first germination count in relation to the control treatment, without inoculation (Table 1). These results with higher initial seed germination percentages are very interesting

for application in forest nurseries, because the seeds are totally exposed at this stage and sensitive to biotic and abiotic factors, which can prevent their germination and the successive development of the seedlings.

Therefore, the adoption of strategies that contribute to the anticipation of the germination of forest seeds under nursery conditions presents innumerable advantages for the production of seedlings because it reduces the time when the seed remains below the ground, where it is totally vulnerable to numerous microorganisms phytopathogenic which cause rotting and seed unfeasibility. Reductions in seed germination potential represent economic damages for forest producers, which, in addition to direct loss in relation to seedling production, may lead to the production of seedlings less vigorous or ununiform without commercial standards. Thus, technologies which have a direct positive effect on this crucial seed germination period certainly favor plant production.

The evaluated isolates also influenced the percentages of accumulated germination, dead and firm seeds, especially the T2 isolate. This isolate showed a 21% increase in the accumulated germination compared to the control treatment, and reduced the percentage of firm seeds by seven times, meaning that they remained in good condition, but did not germinate at the end of the 28 days of evaluation. Another important consideration in relation to this isolate is that it seems to have protected the seeds from phytopathogen attack which causes seed death, preventing their germination. The treatment corresponding to the T2 isolate inoculation was the only one where no seed death was observed for the total of 100 evaluated seeds (Table 1). Based on the results obtained under laboratory conditions, it is possible to state that the tested *Trichoderma* spp. isolates had positive effects on the germination and vigor of the *M. ilicifolia* seeds, proving to be potential microorganisms for forest use.

Table 1. First germination count (FGC), accumulated germination (AG), accumulated dead (AD) and firm (F) *Maytenus ilicifolia* seeds treated with different *Trichoderma* spp.

Treatment	FGC (%)	AG (%)	AD (%)	F (%)
Control	2 b	76 b	3 ab	21 a
<i>T. asperelloides</i> T1	18 a*	86 ab	6 a	8 ab
<i>T. asperelloides</i> T2	23 a	97 a	0 b	3 b
<i>T. virens</i> T10	19 a	90 ab	3 ab	7 ab
CV (%)	42.8	8.1	83.1	65.7

Note. \*Values followed by the same letter in the column did not differ significantly by the Tukey test ( $\alpha = 0.05$ ); CV: Coefficient of variation.

According to Garnica-Vergara et al. (2016), metabolites such as auxins and volatile organic compounds such as 6-PP synthesized by *T. atroviride* in the early interaction stages are perceived by roots and generate a series of alterations in the hormonal mechanisms that control the growth and development of plants. These changes are potentiated when *Trichoderma* spp. colonize the root system, creating a protection area against pathogenic microorganisms and also providing an increase in root volume, making it system more efficient for the absorption of nutrients and water (Contreras-Cornejo, Macías-Rodrigues, del-Val, & Larsen, 2016).

However, the action mechanisms of *Trichoderma* spp. involved in plant growth are complex and have not yet been fully explained. Effects of promotion, suppression and no growth effect on tomato plants were observed by Bharti, Sharma, Pandey, and Mall (2012) from different strains of *Trichoderma harzianum*. These variations may occur due to the ability of a strain to express higher levels of either action mechanism in relation to the others (Marzano, Gallo, & Altomare, 2013), because there are different degrees of adaptation to biotic and abiotic factors between different strains of a same species (Sariah, Choo, Zakaria, & Norihan, 2005) and also because the interactions may vary according to the plant species studied.

In relation to the initial growth of *M. ilicifolia* seedlings inoculated or not with *Trichoderma* spp. there were no statistical differences observed between the treatments for the variables of total height, diameter at root collar diameter and number of leaves in the four analyzed seasons (Table 2). These results may be related to physiological factors, such as the slow rate of growth of the species, or because the aryl was not removed from the seeds at the time of seeding.

Table 2. Total height, diameter at root collar diameter and number of leaves of *Maytenus ilicifolia* seedlings at 90, 120, 150 and 180 days after seeding cultivated with different *Trichoderma* spp. isolates inoculated on the substrate

Treatment	Days after seeding			
	90	120	150	180
<i>Total height (cm)</i>				
Control	5.2 a*	6.0 a	6.9 a	7.2 a
<i>T. asperelloides</i> T1	5.1 a	5.7 a	7.1 a	7.9 a
<i>T. asperelloides</i> T2	5.1 a	6.3 a	7.2 a	7.9 a
<i>T. virens</i> T10	4.6 a	5.3 a	6.7 a	7.1 a
CV (%)	27.7	26.8	28.1	26.8
<i>Diameter at root collar (mm)</i>				
Control	0.5 a	0.6 a	0.7 a	0.7 a
<i>T. asperelloides</i> T1	0.5 a	0.7 a	0.8 a	0.8 a
<i>T. asperelloides</i> T2	0.6 a	0.6 a	0.8 a	0.8 a
<i>T. virens</i> T10	0.4 a	0.6 a	0.8 a	0.8 a
CV (%)	30.3	24.6	27.4	26.8
<i>Number of leaves</i>				
Control	5 a	6 a	7 a	8 a
<i>T. asperelloides</i> T1	5 a	6 a	8 a	9 a
<i>T. asperelloides</i> T2	5 a	6 a	7 a	8 a
<i>T. virens</i> T10	5 a	7 a	8 a	8 a
CV (%)	26.5	16.6	11.4	19.5

Note. \*Values followed by the same letter in the column did not differ significantly by the Kruskal-Wallis test ( $\alpha = 0.05$ ); CV: Coefficient of variation.

Aryl is a fleshy white-colored excrescence which is exposed when the fruit capsule opens, indicating its maturity (Carvalho-Okano, 1992). Although there are no studies which characterize its chemical composition, it is believed to be a substance with nutritive content, since it is attractive to fauna (Martins, Vasconcellos, Rossetto, & Carvalho, 2010). Therefore, it is possible that the presence of aryl in the surrounding seed may have prevented or hindered the positive action of *Trichoderma* spp. on seedling emergence, as was expected based on the results obtained in the laboratory test when an anticipative effect was observed in germination of the seeds treated with the fungal isolates (Table 1).

Although the presence of aryl does not influence the seed germination process under normal conditions (Mariot, Barbieri, Sinigaglia, Bento, & Ribeiro, 2005), a possible negative interaction effect with fungi of the *Trichoderma* spp. genus should not be ruled out, which deserves to be investigated in greater detail.

It is also worth noting that the use of non-sterile soil in the substrate composition may have influenced this result with the possibility that other microorganisms may be present in the substrate system, allowing a non-beneficial interaction with *Trichoderma* spp. isolates, differed from the sterile conditions in the laboratory test.

Although *M. ilicifolia* has antifungal properties, it is unlikely that the viability of the isolates in the substrate was impaired through the release of root exudates. Brand et al. (2007) found that the aqueous extract from *M. ilicifolia* leaves had no fungitoxic effect on fungi of the *Trichoderma* spp. genus *in vitro*. However, based on the positive results that the three *Trichoderma* spp. isolates presented on the germination and vigor of *M. ilicifolia* seedlings under laboratory conditions, these interactions deserve to be better explored in future studies.

In general, the use of *Trichoderma* spp. in plant production confers immediate benefits against adverse effects of the environment, acting on the physiology of seeds and seedlings even before the radicle protrusion occurs, as it can grow endophytically within the plant tissue (Stewart & Hill, 2014). In a study by Zachow, Fatehi, Cardinale, Tilcher, and Berg (2010) the authors applied a *T. velutinum* GI/8 spore suspension to lettuce seedlings and two weeks after examined the roots. Was observed that there were nongerminated spores adhered to seeds, roots and root hairs, with extensive hyphal colonization growing of the surface and inside the root.

However, studies that explain the initial interactions involved in this process are still incipient (Mastouri, Bjorkman, & Harman, 2010). Therefore, the results presented in this study reinforce the need to broaden

discussions and research regarding the interactions established between *Trichoderma*/seeds/seedlings, especially for forest species that have an intrinsic socioeconomic value such as *M. ilicifolia*, and which lack technologies for sustainable agricultural production to support the demands of the phytotherapeutic market, in addition to generating a confidence margin in usage of the produced raw material at the same time.

#### 4. Conclusions

Inoculation of *M. ilicifolia* seeds with *Trichoderma* T1, T2 and T10 isolates had a positive effect on seed vigor and initial germination percentage under laboratory conditions, especially T2 that significantly increased the accumulated germination percentage in relation to uninoculated seeds. *Trichoderma* spp. inoculation on the substrate for seedling production did not present an initial growth promoting effect on *M. ilicifolia*. It is suggested to better investigate the interaction between the tested *Trichoderma* spp. strains and *M. ilicifolia* seeds with aryl.

#### References

- Akhtar, M. S., & Siddiqui, Z. A. (2008). Biocontrol of a root-rot disease complex of chickpea by *Glomus intraradices*, *Rhizobium* sp., and *Pseudomonas straita*. *Crop Protection*, 27, 3410-417. <https://doi.org/10.1016/j.cropro.2007.07.009>
- Amaral, P. P., Steffen, G. P. K., Maldaner, J., Missio, E. L., & Saldanha, C. W. (2017). Promotores de crescimento na propagação de caroba. *Pesquisa Florestal Brasileira*, 37(90), 149-157. <https://doi.org/10.4336/2017.pfb.37.90.1402>
- Bae, H. et al. (2009). The beneficial endophyte *Trichoderma hamatum* isolate DIS 219b promotes growth and delays the onset of the drought response in *Theobroma cacao*. *Journal of Experimental Botany*, 60(11), 3279-3295. <https://doi.org/10.1093/jxb/erp165>
- Bharti, M. K., Sharma, A. K., Pandey, A. K., & Mall, R. (2012). Physiological and biochemical basis of growth suppressive and growth promotory effect of *Trichoderma* strains on tomato plants. *The National Academy of Sciences*, 35(5), 355-359. <https://doi.org/10.1007/s40009-012-0058-2>
- Brand, S., Manzoni, C., Junges, E., Durigon, M., Milanesi, P., ... Muniz, M. (2007). Extrato de cancorosa (*Maytenus ilicifolia*) não inibe *Trichoderma* sp. *Revista Brasileira de Agroecologia*, 2(2), 1054-1057.
- Brasil. (2013). *Instruções para análise de sementes de espécies florestais*. Ministério da Agricultura, Pecuária e Abastecimento, Secretaria de Defesa Agropecuária. Retrieved from <http://www.agricultura.gov.br>
- Brasil. (2016). *Uso de fitoterápicos e plantas medicinais cresce no SUS*. Retrieved from [http://dab.saude.gov.br/portaldab/noticias.php?conteudo=\\_&cod=2162](http://dab.saude.gov.br/portaldab/noticias.php?conteudo=_&cod=2162)
- Brasil. (2018). *Primeiro Suplemento do Formulário de Fitoterápicos da Farmacopeia Brasileira* (1st ed., FFFB1S1), Comissão da Farmacopeia Brasileira. Retrieved from <http://portal.anvisa.gov.br/documents/33832/259456/Suplemento+FFFB.pdf/478d1f83-7a0d-48aa-9815-37dbc6b29f9a>
- Carvalho, C. A., & Rosa, M. (2014). Potencial de uso de espécies medicinais sob a perspectiva da preservação e recuperação ambiental. In A. C. Dörr et al. (Eds.), *Práticas e Saberes em meio ambiente* (pp. 333-352). Curitiba: Appris.
- Carvalho-Okano, R. M. (1992). *Estudos taxonômicos do gênero Maytenus Mol. emend. Mol. (Celastraceae) do Brasil extra-amazônico* (Doctoral dissertation, State University of the Campinas, São Paulo, SP). Retrieved from <https://www.bdpa.cnptia.embrapa.br>
- Contreras-Cornejo, H. A., Macías-Rodríguez, L., Del-Val, E., & Larsen, J. (2016). Ecological functions of *Trichoderma* spp. and their secondary metabolites in the rhizosphere: Interactions with plants. *FEMS Microbiology Ecology*, 92(4). <https://doi.org/10.1093/femsec/fiw036>
- Di Stasi, L. C. (2004). Aspectos químicos e farmacológicos da espinheira-santa: uma análise da utilidade dos dados. In M. S. Reis, & S. R. Silva (Eds.), *Conservação e uso sustentável de plantas medicinais e aromáticas: Maytenus spp., espinheira-santa* (pp. 67-92). Brasília: IBAMA.
- Eira, M. T. S., Dias, T. A. B., & Mello, C. M. C. (1995). Comportamento de espinheira-santa (*Maytenus ilicifolia*) no armazenamento. *Horticultura Brasileira*, 13(1), 32-34.
- Ferreira, D. F. (2011). Sisvar: A computer statistical analysis system. *Ciência e Agrotecnologia (UFPA)*, 35(6), 1039-1042. <https://doi.org/10.1590/S1413-70542011000600001>

- Filho, A. C., Lúcio, A. D. C., Lopes, S. J., & Storck, L. (2012). *Testes não-paramétricos para pesquisas agrícolas* (p. 97). Santa Maria: UFSM/ CCR/Departamento de Fitotecnia.
- Gahukar, R. T. (2017). Pest and disease management in important medicinal plants in India: A review. *Official Journal of the Society of Nutrition and Food Science*. <https://doi.org/10.1016/j.nfs.2017.02.001>
- Garnica-Vergara, A., Ortiz, S. B., Parra, E. M., Gonzáles, J. R., Bravo, A. M., Rodríguez, L. M., ... Bucio, J. L. (2016). The volatile 6-pentyl-2H-pyran-2-one from *Trichoderma atroviride* regulates *Arabidopsis thaliana* root morphogenesis via auxin signaling and ethylene insensitive 2 functioning. *New Phytologist*, 209(4), 1496-1512. <https://doi.org/10.1111/nph.13725>
- Gomes, F. P. (1985). *Curso de Estatística Experimental* (p. 466). Piracicaba: Nobel.
- Harman, G. E., Howell, C. R., Viterbo, U., Chet, I., & Lorito, M. (2004). *Trichoderma* species-opportunistic, avirulent plant symbionts. *Nature Reviews Microbiology*, 2, 43-56. <https://doi.org/10.1038/nrmicro797>
- Kowalski, A. P. J., Signor, D., Machado, E. M., Biasi, L. A., & Lima, D. M. (2008). Influência da qualidade da semente e do tipo de substrato na formação de mudas de espinheira-santa. *Scientia Agraria*, 9(1), 15-20. <https://doi.org/10.5380/rsa.v9i1.10127>
- Lima, D. M. (2010). Caracterização e produção de mudas de espinheira-santa (*Maytenus muelleri* Schwacke). In T. N. Martin et al. (Eds.), *Sistemas de Produção Agropecuária (Ciências Agrárias, Animais e Florestais)*. Ed. UTFPR, Dois Vizinhos, PR.
- Machado, D. F. M., Tavares, A. P., Lopes, S. J., & Silva, A. C. F. (2015). *Trichoderma* spp. na emergência e crescimento de mudas de cambará (*Gochnatia polymorpha* (Less.) Cabrera). *Revista Árvore*, 39(1), 167-176. <https://doi.org/10.1590/0100-67622015000100016>
- Mariot, M. P., Barbieri, R. L., Sinigaglia, C., Bento, L. H., & Ribeiro, M. V. (2005). Presença do arilo na produção de mudas de *Maytenus ilicifolia*. *Ciência Rural*, 35(2), 468-470. <https://doi.org/10.1590/S0103-84782005000200038>
- Martins, C. M., Vasconcellos, M. A. S., Rossetto, C. A. V., & Carvalho, M. G. (2010). Prospecção fitoquímica do arilo de sementes de maracujá amarelo e influencia em germinação de sementes. *Ciência Rural*, 40(9), 1934-1949. <https://doi.org/10.1590/S0103-84782010000900013>
- Marzano, M., Gallo, A., & Altomare, C. (2013). Improvement of biocontrol efficacy of *Trichoderma harzianum* vs. *Fusarium oxysporum* f. sp. lycopersici through UV-induced tolerance to fusaric acid. *Biological Control*, 67(3), 397-408. <https://doi.org/10.1016/j.biocontrol.2013.09.008>
- Mastouri, F., Björkman, T., & Harman, G. E. (2010). Seed treatment with *Trichoderma harzianum* alleviates biotic, abiotic, and physiological stresses in germinating seeds and seedlings. *Phytopathology*, 100(11), 1213-1221. <https://doi.org/10.1094/PHYTO-03-10-0091>
- Montanari Junior, I., Scheffer, M. C., & Radomski, M.I. (2004). Cultivo de espinheira-santa. In M. S. Reis, & S. R. Silva (Eds.), *Conservação e uso sustentável de plantas medicinais e aromáticas: Maytenus spp., Espinheira-Santa* (Vol. 1). Brasília, DF: Ibama.
- Negrelle, R. B., Doni, M. E., Ohlson, O. C., & Herr, S. (1999). Tecnologia de produção de sementes de Espinheira-Santa (*Maytenus ilicifolia* Mart. ex Reiss.-Celastraceae). *Revista Brasileira de Sementes*, 21(1), 76-81. <https://doi.org/10.17801/0101-3122/rbs.v21n1p76-81>
- Nicoloso, F. T., Fotunato, R. P., Zanchetti, F., Cassol, L. F., & Eisinger, S. M. (2000). Recipientes e substratos na produção de mudas de *Maytenus ilicifolia* e *Apuleia leiocarpa*. *Ciência Rural*, 30(6), 987-992. <https://doi.org/10.1590/S0103-84782000000600011>
- Pravuschi, P. R., Marques, P. A. A., Rigolin, B. H. M., & Santos, A. C. P. (2010). Efeito de diferentes lâminas de irrigação na produção de óleo essencial do manjeriço (*Ocimum basilicum* L.). *Acta Scientiarum Agronomy*, 32(4), 687-693. <https://doi.org/10.4025/actasciagron.v32i4.3160>
- Rovedder, A. P. M., Piazza, E. M., Thomas, P. A., Felker, R. M., Hummel, R. B., & Farias, J. A. (2016). Potential medicinal use of forest species of the Deciduous Seasonal Forest from Atlantic Forest Biome, South Brazil. *Brazilian Archives of Biology and Technology*, 59. <https://doi.org/10.1590/1678-4324-2016150329>
- Santos e Silva, F. de A. (2016). *Assistat: Assistência Estatística*. DEAG-CTRN-UFMG, Campina-Grande-PB.
- Santos, H. G., Jacomine, P. K. T., & Anjos, L. H. C. (2013). *Sistema brasileiro de classificação de solos* (3rd ed.). Rio de Janeiro: Embrapa Solos.

- Santos-Oliveira, R., Coulaud-Cunha, S., & Colaço, W. (2009). Revisão da *Maytenus ilicifolia* Mart. ex Reissek, Celastraceae. Contribuição ao estudo das propriedades farmacológicas. *Revista Brasileira de Farmacognosia*, 19(2B), 650-659. <https://doi.org/10.1590/S0102-695X2009000400025>
- Sariah, M., Choo, C. W., Zakaria, H., & Norihan, M. S. (2005). Quantification and characterisation of *Trichoderma* spp. from different ecosystems. *Mycopathologia*, 159(1), 113-117. <https://doi.org/10.1007/s11046-004-4432-6>
- Sofo, A., Milella, L., & Tataranni, G. (2010). Effects of *Trichoderma harzianum* strain T-22 on the growth of two *Prunus* rootstocks during the rooting phase. *Journal Horticultural Science Biotechnology*, 85(6), 497-703. <https://doi.org/10.1080/14620316.2010.11512704>
- Soldan, A. M. (2014). *Desenvolvimento e estado nutricional de mirtáceas sob o efeito de Trichoderma spp. e fosfato natural* (Master's thesis, State University of Central-West, Guarapuava, PR). Retrieved from <http://www.unicentroagronomia.com>
- Steenbock, W., & Reis, M. S. (2011). *Maytenus ilicifolia*: Espinheira-Santa. In L. Coradin, A. Siminski, & A. Reis (Eds.), *Espécies Nativas da Flora Brasileira de Valor Econômico Atual ou Potencial* (p. 936). Brasília: MMA. Retrieved from [http://www.creasp.org.br/biblioteca/wp-content/uploads/2012/11/Regiao\\_Sul.pdf](http://www.creasp.org.br/biblioteca/wp-content/uploads/2012/11/Regiao_Sul.pdf)
- Steffen, G. P. K., & Maldaner, J. (2017). Methodology for *Trichoderma* sp. multiplication in organic substrates. *International Journal of Current Research*, 9(01), 44564-44567.
- Stewart, A., & Hill, R. (2014). Applications of *Trichoderma* in Plant Growth Promotion. *Biotechnology and Biology of Trichoderma* (Chap. 1, pp. 415-428). <https://doi.org/10.1016/B978-0-444-59576-8.00031-X>
- Tchameni, S. N., Ngonkeu, M. E. L., Begoude, B. A. D., Nana, L. W., Fokom, R., Owona, A. D., ... Kuate, J. (2011). Effect of *Trichoderma asperellum* and arbuscular mycorrhizal fungi on cacao growth and resistance against black pod disease. *Crop Protection*, 30(10), 1321-1327. <https://doi.org/10.1016/j.cropro.2011.05.003>
- Zachow, C., Fatehi, J., Cardinale, M., Tilcher, R., & Berg, G. (2010). Strain-specific colonization pattern of *Rhizoctonia* antagonists in the root system of sugar beet. *FEMS Microbiol Ecol.*, 74, 124-135. <https://doi.org/10.1111/j.1574-6941.2010.00930.x>

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