Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Volatile organic compounds (VOCs) of essential oils for the control of *Fusarium oxysporum* in cherry tomato seeds

Compostos orgânicos voláteis (COV) de óleos essenciais no controle de *Fusarium oxysporum* em sementes de tomate cereja

M. C. Schuster-Russiano[a*], C. G. S. Russiano[b], P. V. D. Moraes[b], R. D. B. Ducatti[a] and S. M. Mazaro[a]

[a]Universidade Tecnológica Federal do Paraná – UTFPR, Programa de Pós-graduação em Agronomia, Pato Branco, PR, Brasil
[b]Universidade Tecnológica Federal do Paraná – UTFPR, Programa de Pós-graduação em Agroecossistemas, Dois Vizinhos, PR, Brasil

Abstract

Fusarium oxysporum is the causal agent of *Fusarium* wilt in tomato plants. The most common form of control of this disease is through seed chemical treatment. However, the present work presents an alternative method, through the fumigation technique with essential oils. The pathogen *F. oxysporum* was inoculated on organic cherry tomato seeds through contact with sporulated Petri® plates. Thereafter, seeds were placed in stainless steel crucibles containing a 1.0 x 1.0 cm filter paper adhered to the lid and kept for 24 hours. This paper received 20 µL of each essential oil: tea tree, chia, citronella, lavender, anise basil, clove basil, and deionized water as control. This process was called "seed fumigation by essential oil". After this process, a germination test was carried out in germ boxes with Germitest® paper to verify the variables Germination Speed Index (GSI), Germination (G%), and Mean time to germination (MGT). Mycelial growth was verified in Petri® plates containing PDA medium. The plates containing mycelial growth were observed through scanning electron microscopy to verify possible morphological damage in the hyphae of the pathogen. Tea tree essential oil was the one that allowed the greatest suppression of the phytopathogen. Therefore, new tests were carried out with this specific oil. In germ boxes, tests of germination (G%), Abnormal seedlings count (ASC), and percentage of seedlings with mycelial growth were carried out. In addition, plant elicitation tests were performed in tomato seedlings through the analysis of chitinase, glucanase, and total proteins. All tests were carried out in completely randomized designs with four replications. All data were submitted to the Lilliefors normality test, followed by the analysis of variance, and Tukey's HSD (5% significance) for mean comparison. It was found that tea tree essential oil inhibited the mycelial growth of *F. oxysporum* without affecting the germination of cherry tomato seeds. Subsequent tests with this oil also demonstrated that there is a reduction in mycelia present in the seeds and a reduction in abnormal seedlings compared to the control. There was no significant difference between the variables tested for plant elicitation.

Keywords: seed fumigation, plant elicitation, germination, phytopathogen, mycelial growth.

Resumo

Fusarium oxysporum é o agente causal da murcha de Fusarium em tomateiro, a forma mais comum de controle da doença é através do tratamento químico das sementes, porém o presente trabalho apresenta uma alternativa, através da técnica de expurgo com óleos essenciais. O patógeno, Fusarium oxysporum, foi inoculado em sementes de tomate cereja orgânicas, através do contato com placas de Petri® esporuladas. Em seguida, estas sementes dotadas do inóculo da doença, foram alocadas em cadinhos de inox contendo um papel filtro de 1 cm x 1cm, aderido à tampa contento 20 uL de cada óleo: óleo essencial de melaleuca, chia, citronela, lavanda, alfavaca anis, alfavaca cravo e água deionizada como testemunha. Esse processo em que as sementes foram alocadas em cadinhos contendo óleo essencial na tampa por um período de 24 horas, foi chamado de expurgo das sementes pelo óleo essencial. Após este processo, foi realizado o teste de germinação em caixas Gerbox com papel Germitest®, verificando as variáveis IVG, %G, TMG; e crescimento micelial em placas de Petri® contendo meio BDA. As placas de crescimento micelial foram observadas em microscópio eletrônico de varredura, a fim de verificar possíveis danos morfológicos nas hifas do patógeno. Após tais ensaios, constatou-se que o óleo essencial de melaleuca foi o que obteve maior supressão do fitopatógeno; e dessa forma novos ensaios laboratoriais foram realizados apenas com este óleo. Em caixas Gerbox realizou-se o teste de germinação (%), contagem de sementes anormais e porcentagem de sementes com micélio, além de testes de indução de resistência em plântulas de tomate, realizado por meio da análise de quitinase, glucanase e proteínas totais. O delineamento adotado em todos os ensaios foi o inteiramente ao acaso, com quatro repetições. Os dados obtidos foram submetidos ao teste de normalidade de Lilliefors, e à análise de variância, com as médias comparadas pelo teste de Tukey (significância de 5%). Constatou-se que o óleo de melaleuca inibiu o crescimento micelial de F. oxysporum, sem prejudicar a germinação das sementes de tomate cereja. Os testes subsequentes com este óleo, também demonstram que há a redução de micélio nas sementes e redução de plântulas anormais em comparação com a testemunha. Não ocorreu diferença significativa entre as variáveis testadas para indução de resistência.

Palavras-chave: expurgo, indução de resistência, germinação, fitopatógeno, crescimento micelial.

*e-mail: maira.schuster@hotmail.com
Received: April 29, 2023 – Accepted: August 8, 2023

1. Introduction

Tomato (*Solanum lycopersicum*) belongs to the Solanaceae family. Besides tomatoes, this family also includes superior plants with world prominence, such as potatoes, eggplant, pepper, and tobacco. All these crops have great importance in the economy and for human nutrition (Ghatak et al., 2017; Seguí-Simarro, 2016). Tomatoes can be consumed "*in natura*" as salads and juices or industrialized for the production of sauces and extracts. Their high concentration of lycopene stands out as a natural antioxidant that eliminates free radicals (Li et al., 2015).

Because of climatic conditions and soil types, tomato farming in Brazil is concentrated in the states of São Paulo, Goiás, and Minas Gerais. Combined, these states have more than 50% of the Brazilian farmed area for this crop (CONAB, 2019). In 2021, the Brazilian production accounted for 3.68 million tons in a farmed area of 51.9 thousand hectares, with an average yield of 70.8 tons ha^{-1} (IBGE, 2021).

Several challenges affect tomato farming, including pests, diseases, and weeds. *Fusarium* wilt (*Fusarium oxysporum*) is one of the diseases that most affect the crop and whose dissemination occurs via seeds. Symptoms of the disease can be seen at any stage. The main symptom is the wilting of the upper leaves during the hottest hours of the day (Silva and Bettiol, 2006). *F. oxysporum* inocula can remain for more than five years in the soil or in crop residues in the form of chlamydospores (Zambolim et al., 2001).

Given the problems involving synthetic pesticides and treatments, alternative forms of control have been sought to reduce the negative impacts on human health and the environment. Essential oils have become a viable form of antimicrobial control, as demonstrated in studies where essential oils from *Allium sativum*, *Copaifera langsdorffii*, *Eugenia caryophyllata,* and *Cinnamomum zeylanicum* were used to reduce anthracnose in banana fruits (Cruz et al., 2013). Essential oils from *Aloysia citriodora*, *Cymbopogon winterianus*, *Lippia alba,* and *Ocimum americanum* were also shown to be effective in the post-harvest control of *Botrytis cinerea* in strawberries (Fontana et al., 2021). Volatiles from peppermint essential oil were able to control *F. sambucinum*, the causal agent of soft rot in pepper plants (Pérez-Vázquez et al., 2022).

Fumigation is a seed disinfection technique based on the use of gas. It is commonly used for grains stored in bags or bulk. Phosphine is the most used product for this purpose (Krzyzanowski et al., 2013). Thus, this work aimed to evaluate the effects of seed fumigation by Volatile Organic Compounds (VOCs) coming from the essential oils of tea tree, chia, citronella, lavender, anise basil, and clove basil in organic cherry tomato seeds contaminated with *F. oxysporum* inocula.

2. Material and Methods

2.1. Seed fumigation

Essential oils coming from tea tree (*Melaleuca alternifolia*), chia (*Salvia hispanica*), citronella (*Cymbopogon winterianus*), lavender (*Lavandula stoechas*), anise basil (*Ocimum selloi*), and clove basil (*Ocimum gratissimum*) were commercially obtained for the tests. *F. oxysporum*, which causes the *Fusarium* wilt in tomato, was used as a challenging phytopathogen.

Cherry tomato seeds were transferred and portioned into Petri® plates containing PDA medium and sporulated culture of *F. oxysporum* with an average growth of 10 days. The plates were closed and sealed with plastic PVC film and kept in a Bio-Oxygen Demand Chamber (BOD) for a period of 48 hours so that *F. oxysporum* spores would adhere to the walls of the seeds.

In aluminum crucibles (6.0 cm in diameter and 6.0 cm in height) a 1.0 x 1.0 cm filter paper was stuck to the inside of the crucible lid. This filter paper received 20 µL of each essential oil: tea tree, chia, citronella, lavender, anise basil, clove basil, and deionized water for the Control (-). One-hundred ten *F. oxysporum*-infected cherry tomato seeds were transferred to each crucible. An additional treatment was included using the crucible with 20 µL of deionized water in the lid, and seeds without pathogenic infection. This additional treatment composed the Control (+). The crucibles were closed and sealed with PVC film and left in fumigation for a period of 24 hours at room temperature.

2.2. Germination test

For the germination test, a completely randomized experimental design was used with four replications. Fumigated seeds were used for the test. A total of eight treatments were used: Control (+) deionized water and seeds without pathogenic infection, Control (-) deionized water and seeds with pathogenic infection, and fumigated seeds with VOCs coming from the essential oils of tea tree, chia, citronella, lavender, anise basil, and clove basil.

Each experimental unit consisted of a germ box with 25 cherry tomato seeds distributed on Germitest® paper. The paper was moistened (deionized water) with 2.5 times the weight of the paper (Brasil, 2009). The germ boxes were placed in a germination chamber at 25 ± 2°C with no light for a period of eight days.

Daily assessments were carried out over eight days. Seeds were considered "germinated" when root protrusion was above 2 mm. The analyzed variables were Germination (%) (Brasil, 2009), Germination speed index (GSI) (Maguire, 1962), and Mean time to germination (MTG) (Silva and Nakagawa, 1995). The formulas used to calculate the GSI (1) and the MTG (2) were:

$$GSI = \Sigma\left(\frac{n_i}{t_i}\right) \quad (1)$$

$$MTG = \frac{\left(\Sigma n_i \times t_i\right)}{\Sigma n_i} \quad (2)$$

Where: n_i represents the number of seeds that were considered germinated in each day, and t_i represents the day in which the seeds were considered germinated. Seeds with the presence of pathogenic mycelia were counted.

All data were submitted to the Lilliefors normality test. With no need for transformation, data were submitted to the analysis of variance, and their means, when significant ($p \leq 0.05$), were compared by Tukey's SHD test ($p \leq 0.05$), using the Genes software (Cruz, 2016).

2.3. Mycelial growth

A completely randomized test with four replications was used to account for the mycelial growth. The treatments used were Control (+), Control (-), and fumigated seeds with VOCs coming from the essential oils of tea tree, chia, citronella, lavender, anise basil, and clove basil.

One fumigated cherry tomato seed from each treatment was transferred to the center of a Petri® plate containing commercial PDA medium. The plates were closed and sealed with plastic PVC film and placed at a BOD at 25 ± 2°C, and a photoperiod of 12 hours. With the aid of a graduated ruler, the mycelial growth of each plate was measured daily for six days (144 hours).

At the end of the experiment, scanning electron microscopy was performed to verify the effect that each essential oil had on the morphology of *F. oxysporum* hyphae. All data were submitted to the Lilliefors normality test followed by the analysis of variance and regression analysis using the Genes software (Cruz, 2016).

2.4. Germination test with tea tree essential oil

The tea tree essential oil was the most effective in controlling *F. oxysporum* without causing damage to the germinative aspects of the seeds. Therefore, other laboratory tests were carried out exploring only this essential oil. The fumigation of cherry tomato seeds was performed according to the procedure previously described in the "seed fumigation" section.

A bifactorial scheme arranged as a completely randomized design with four replications was carried out for this experiment. Factor A (quantitative factor) consisted of the germination time (3, 6, 9, and 12 days after the start of the experiment), and Factor B (qualitative factor) consisted of three treatments: 1) seeds inoculated with *F. oxysporum* and fumigated with tea tree oil at a dose of 20 µL; 2) Control (+) deionized water and seeds without pathogenic infection, and 3) Control (-) deionized water and seeds with pathogenic infection.

Evaluations were performed every three days (3, 6, 9, and 12 days) and consisted of checking the presence of abnormal seedlings, and the presence or absence of mycelial growth on the seedlings. Then, all the seedlings contained in the germ boxes were transferred to envelopes and frozen, for later weighing and analysis of β-1,3-glucanase, chitinase, and proteins.

All data were submitted to the Lilliefors normality test followed by the analysis of variance and regression analysis using the Genes software (Cruz, 2016).

2.5. Plant elicitation

To quantify the activity of chitinase and β-1,3-glucanase, the methodology developed by Wirth and Wolf (1992) were used. Total protein quantification was performed according to Bradford (1976).

3. Results and Discussion

Seeds fumigated with Lavender essential oil were the ones that presented the longest mean time to germination (MTG in days), followed by Citronella, Chia, and the Control (-). The shorter the MTG, the greater the advantage the plant will have under competition when sown in the field. Therefore, the use of treatments with a longer MTG is disadvantageous due to the slow establishment of the plant, which will benefit weeds to establish and complete for natural resources.

Hirata et al. (2018) observed that *Cymbopogon nardus* essential oils and *Annona muricata* extracts delayed the germination of *Bidens pilosa* and *Megathyrsus maximus*. *A. muricata* extracts also corroborated for a higher germination speed index (GSI) of *M. maximus*.

As for the Germination speed index (GSI), the treatment with Clove basil essential oil presented the highest GSI, followed by the Control (-), and Chia. The Tea tree essential oil did not differ from the Control (+). There was no significant difference in the germination percentage (G%) of seeds treated with the essential oils studied in this experiment (Table 1).

Table 1. Mean time to germination (MTG), Germination speed index (GSI), and Germination (G%) of tomato seeds submitted to fumigation with volatile organic compounds (VOCs) of different essential oils, and the remaining number of seeds containing *F. oxysporum* inocula after fumigation.

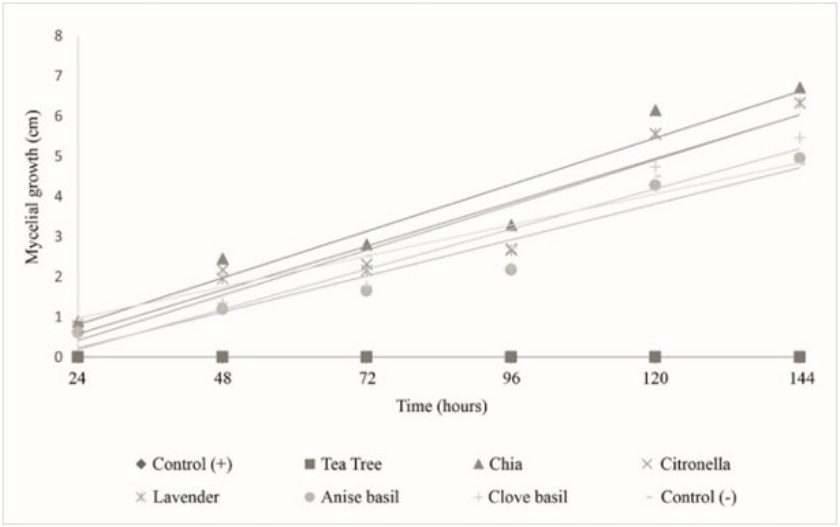
Essential oils	MTG (days)	GSI	G%	Number of seeds with remaining inocula
Control (+)	1.73 b	28.82 c	87 a	2.91 ab
Tea tree	1.96 b	23.76 c	81 a	0.00 c
Chia	2.17 ab	52.88 ab	90 a	3.72 a
Citronella	2.18 ab	43.88 b	78 a	3.31 ab
Lavender	2.48 a	45.58 b	81 a	3.23 ab
Anise basil	1.81 b	45.78 b	77 a	2.48 b
Clove basil	1.91 b	58.37 a	93 a	2.69 ab
Control (-)	2.01 ab	53.79 ab	84 a	0.00 c
CV%	10.61	9.86	8.96	21.95

Means followed by the same letter in each column are not significantly different according to Tukey's HSD test (5%). CV%: coefficient of variance. Control (+): seeds without pathogenic infection and without fumigation. Control (-): seeds with pathogenic infection and without fumigation.

Garbin et al. (2015) noticed that the use of *Eucalyptus* essential oil negatively interfered with the G%, MTG, and the GSI of lettuce and radish seeds. Lobato et al. (2007) verified in an experiment involving different concentrations of *Piper aduncum* essential oil, on seeds of *Vigna unguiculata*, that as the concentration of the oil increased there was a reduction in the germination. However, when compared to the control no significant differences were observed. It means that there is no phytotoxicity associated with the oil on the initial growth of the embryo or any tissue that compromises the development of the seedling. The same was observed for the Tea tree essential oil at 100% concentration (20 µL dose), in which the GSI did not differ from the Control (+).

The Control (+), and the treatment with the Tea tree essential oil (Figure 1), did not show mycelial growth of *F. oxysporum* for 144 hours (six days). The other treatments were not effective in controlling the mycelial growth of the phytopathogen.

Chia essential oil corresponded to the treatment that provided the greatest mycelial growth of *F. oxysporum*, followed by Citronella, Lavender, Anise basil, Clove basil, and the Control (-) (Figure 1 and Figure 2).



Treatments	Equation	R^2 adjusted
Control (+)	$\bar{y} = 0$	-
Tea tree	$\bar{y} = 0$	-
Chia	y = -0.3537+0.0485x	90.97
Citronella	y = -0.5121+0.0455x	87.68
Lavender	y = -0.697+0.0468x	88.21
Anise basil	y = -0.667+0.0374x	90.87
Clove basil	y = -0.8117+0.0417x	89.21
Control (-)	y = 0.2071+0.0322x	93.37

Figure 1. Mycelial growth (cm) of *F. oxysporum* during six days (daily assessments).

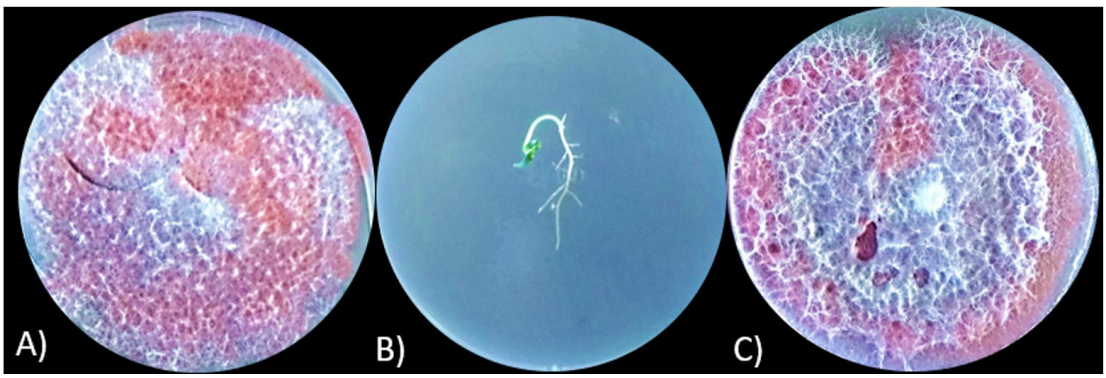


Figure 2. A) Petri® plate containing the Control (-) treatment at 144 hours; B) Petri® plate containing the treatment with seed fumigation performed with Tea tree at 144 hours; C) Petri® plate containing the treatment with seed fumigation performed with Chia at 144 hours.

Using the minimum inhibitory concentration method, the Tea tree essential oil was also effective in controlling *Paenibacillus* (anaerobic bacteria) in *Apis mellifera* colonies. The authors attribute the result to the antifungal composition of the oil, containing Terpinen-4-ol (29.09%), alpha-pinene (21.63%), and limonene (17.4%) (Fuselli et al., 2010).

Abdel-Kawy et al. (2021) also verified that the essential oil of *Citrus trifoliata* is effective in controlling the mycelial growth of *F. solani* and *F. oxysporum*.

As for the damage to the fungus hyphae, no mycelial growth of *F. oxysporum* was observed after seed fumigation with Tea tree essential oil. Therefore, this treatment was not evaluated by microscopy. As shown in Figure 3, all treatments allowed the sporulation of the phytopathogen (circled in red). Compared to the Control, seed fumigation with Chia, Lavender, Anise basil, and Clove basil essential oils damaged the *F. oxysporum* hyphae (narrowed hyphae (arrows in blue) and curled hyphae (arrows in yellow)).

Citronella essential oil treatment allowed higher sporulation of *F. oxysporum* when compared to the Control (-) (Figure 3). This overproduction of spores may be associated with the stress suffered by the pathogen, as described by Mello et al. (2018) when submitting *Corynespora cassiicola* to different light and stress regimes.

As determined by the germination and the mycelial growth tests, the treatment that allowed the best reduction of the pathogen inocula and that did not interfere directly with the tested germinative variables (Table 1) was the Tea tree essential oil. Therefore, other laboratory tests were carried out to better explore this essential oil. Three treatments were considered: 1) seeds inoculated with *F. oxysporum* and fumigated with tea tree oil at a dose of 20 µL; 2) Control (+) deionized water and seeds without pathogenic infection, and 3) Control (-) deionized water and seeds with pathogenic infection with evaluations performed at 3, 6, 9, and 12 days after the begin of the experiment.

For the variable G%, there was no significant difference between treatments, reaching an average of 90% of germination on day 3, which was maintained until day 12. Inoculation of *F. oxysporum* (control (-)) did not interfere with seed germination. However, it interfered with the percentage of abnormal seedlings, as seen in Figure 4. For the percentage of abnormal seedlings, no significant difference between the control (+) and the seeds fumigated with Tea tree essential oil was observed. Silva (2018) also observed that tomato seeds inoculated with *Pseudomonas syringae* had their height affected, but not their germination.

The Control (-) showed a linear growth, increasing the percentage of abnormal seedlings over time, and reaching 40% at the end of 96 h (3 days). The Tea tree essential oil, and The control (+) at the end of the period showed only 10% of abnormal seedlings.

The Control (+) did not present seedlings with mycelial growth, as there was no inoculation of the pathogen (Figure 5). On day 12, the treatment containing only the inoculation with *F. oxysporum* (Control (-)) presented 35% of the seedlings containing mycelial growth, whereas the treatment with the Tea tree essential oil presented 5%. It represents a reduction of 80% in the mycelial growth of *F. oxysporum* and shows the efficiency of fumigation when performed with Tea tree essential oil.

This happens because the essential oil acts on the cell wall of the fungus, causing lipid peroxidation and the leakage of the cell content, hence, leading to the death of the fungus. This interaction with the cell wall of the fungus might be linked to the components present in the essential oil (Amaral and Bara, 2005).

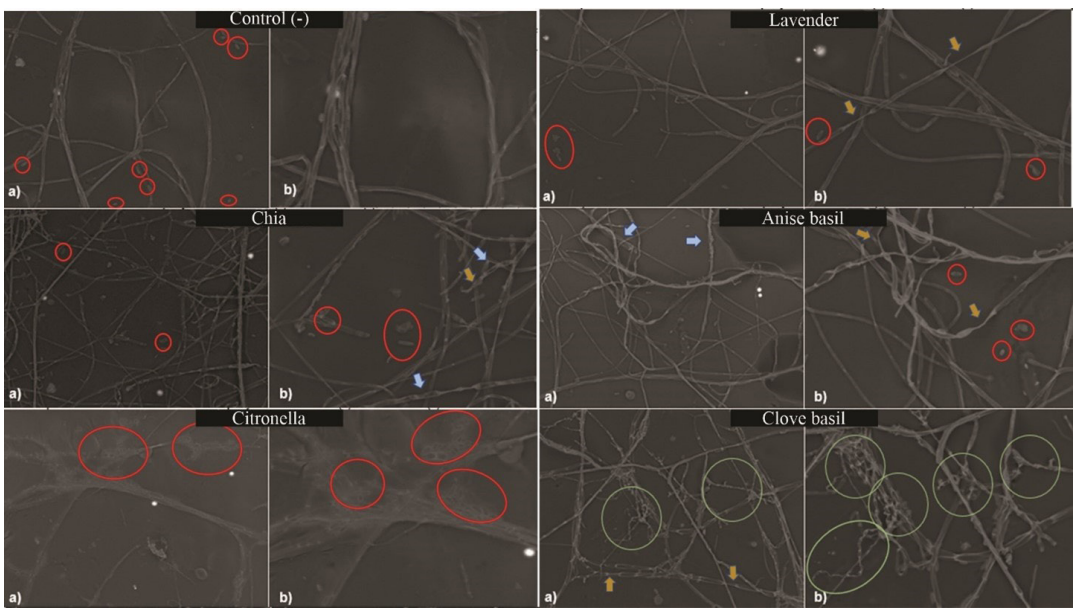


Figure 3. *F. oxysporum* hyphae after seven days of cherry tomato seed fumigation with Lavender, Chia, Anise basil, Citronella, Clove basil, and the Control (-) seen in scanning electron microscopy with a magnification of 500 (a) and 1000 times (b). Red circles: sporulation of the phytopathogen. Arrows: damages to the hyphae.

Terpinen-4-ol (37.4%) is the major component of the Tea tree essential oil, followed by γ-terpinene (19.4%) and α-terpinene (9%) (Bishop and Thornton, 1997). These components' antifungal and antibacterial activities have already been tested and shown by different authors (Gioppo et al., 2019; Souza et al., 2015; Martins et al., 2010).

A decrease in the protein content was observed over time, reaching the highest levels on the first day of evaluation (day three), and decreasing on the sixth, ninth, and twelfth days.

On the first day of assessment (day three), the protein content in the Control (-) was lower in comparison to the Control (+) and seeds fumigated with Tea tree essential oil (Figure 6). This can be explained due to the damage caused by the pathogen in the seed, reducing its viability.

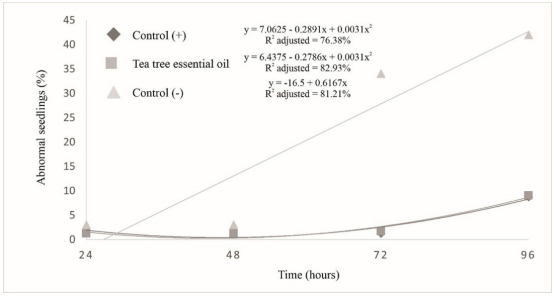


Figure 4. Percentage of abnormal cherry tomato seedlings as a function of three treatments [Control (+), Control (-), and seeds fumigated with Tea tree essential oil] and four evaluation times.

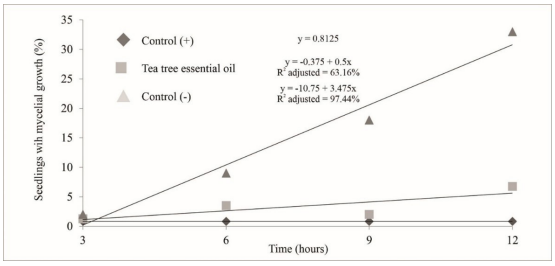


Figure 5. Percentage of cherry tomato seedlings containing mycelial growth as a function of three treatments [Control (+), Control (-), and seeds fumigated with Tea tree essential oil] and four evaluation times.

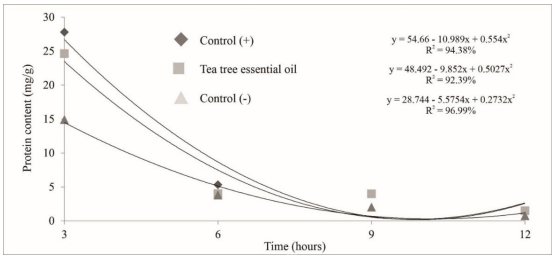


Figure 6. Protein content in cherry tomato seedlings as a function of three treatments [Control (+), Control (-), and seeds fumigated with Tea tree essential oil] and four evaluation times.

It is also noted that there is a constant reduction in the protein content in all treatments over time, this decrease can be justified by the germination process, which depends on the use of the seed reserves until the seedling becomes fully autotrophic. This was observed in an experiment with *Melanoxylon brauna*, where there was a reduction of soluble carbohydrates, lipids, and proteins during the germination process (Ataíde et al., 2017). Moreover, this tendency was also reported in *Caesalpinia peltophoroides*, where there was a gradual reduction of carbohydrates and soluble proteins during germination (Corte et al., 2006).

No significant differences were observed for the activities of β-glucanase and chitinase over time. During germination, hydrolytic enzymes such as β-glucanase degrade reserve substances to generate substrates for the respiratory process, hydrolyzing starch into smaller sugars. Therefore, there is an overall increase in the enzymatic activity of glucanase during the germination process (Popinigis, 1985).

Therefore, no plant elicitation relationships were observed for the treatments performed with the Tea tree essential oil, Control (-), and Control (+). For plant elicitation to be considered "on" there must be a significant increase in the activities of β-glucanases, and chitinases (Bettiol and Morandi, 2009). Borsatti et al. (2015) observed induction of resistance in blackberries by increased activity of β-1,3-glucanase after treatments with salicylic acid in the post-harvest of this crop.

References

ABDEL-KAWY, M.A., MICHEL, C.G., KIROLLOS, F.N., HUSSIEN, R.A.A., AL-MAHALLAWI, A.M. and SEDEEK, M.S., 2021. Chemical composition, and potentiation of insecticidal and fungicidal activities of Citrus trifoliata L. fruits essential oil against *Spodoptera littoralis, Fusarium oxysporum,* and *Fusarium solani* via nano-cubosomes. *Natural Product Research*, vol. 35, no. 14, pp. 2438-2443. http://dx.doi.org/10.1080/14786419.2019.1675063. PMid:31596140.

AMARAL, M.F.Z. and BARA, M.T.F., 2005. Avaliação da atividade antifúngica de extratos de plantas sobre o crescimento de fitopatógenos. *Revista Eletrônica de Farmácia*, vol. 2, pp. 5-8.

ATAÍDE, G.M., BORGES, E.E.L., PICOLI, E.A.T., LEITE-FILHO, A.T. and FLORES, A.V., 2017. Alterações nas reservas de sementes de *Melanoxylon brauna* Schott. (*Fabaceae Caesalpinoideae*) durante a germinação em diferentes temperaturas. *Agrarian*, vol. 12, no. 3, pp. 372-379. http://dx.doi.org/10.5039/agraria.v12i3a5454.

BETTIOL, W. and MORANDI, M.A.B., 2009. Biocontrole de doenças de plantas: uso e perspectiva. In R.S. ROMEIRO and F.A.O. GARCIA. *Indução de resistência em plantas a patógenos por eliciadores de natureza bacteriana*. Jaguariúna: Embrapa, cap. 6, pp. 85, 140-141.

BISHOP, C. and THORNTON, I., 1997. Evaluation of the antifungal activity on the essential oils of *Monarda citriodora* var. citriodora and *Melaleuca alternifolia* on post harvest pathogens. *The Journal of Essential Oil Research*, vol. 9, no. 1, pp. 77-82. http://dx.doi.org/10.1080/10412905.1997.9700718.

BORSATTI, F.C., MAZARO, S.M., DANNER, M.A., NAVA, G.A. and DALACOSTA, N.L., 2015. Indução de resistência e qualidade pós-colheita de amora-preta tratada com ácido salicílico. *Revista Brasileira de Fruticultura*, vol. 37, no. 2, pp. 318-326. http://dx.doi.org/10.1590/0100-2945-087/14.

BRADFORD, M.M., 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Analitycal Biochemistry*, vol. 72, no. 1-2, pp. 248-254. http://dx.doi.org/10.1016/0003-2697(76)90527-3. PMid:942051.

BRASIL. MINISTÉRIO DA AGRICULTURA, PECUÁRIA E ABASTECIMENTO. SECRETARIA DE DEFESA AGROPECUÁRIA, 2009. *Regras para análise de sementes*. Brasília: Ministério da Agricultura, Pecuária e Abastecimento. 399 p.

COMPANHIA NACIONAL DE ABASTECIMENTO – CONAB, 2019. Tomate: análise dos indicadores da produção e comercialização no mercado mundial, brasileiro e catarinense. *Compêndio de Estudos CONAB*, vol. 21, pp. 6-21.

CORTE, V.B., BORGES, E.E.L., PONTES, C.A., LEITE, I.T.A., VENTRELLA, M.C. and MATHIAS, A.A., 2006. Mobilização de reservas durante a germinação das sementes e crescimento das plântulas de *Caesalpinia peltophoroides* Benth. *Revista Árvore*, vol. 30, no. 6, pp. 941-949. http://dx.doi.org/10.1590/S0100-67622006000600009.

CRUZ, C.D., 2016. Genes Software - extended and integrated with the R, Matlab, and Selegen. *Acta Scientiarum. Agronomy*, vol. 38, no. 4, pp. 547-552. http://dx.doi.org/10.4025/actasciagron.v38i3.32629.

CRUZ, M.E.S., SCHWAN-ESTRADA, K.R.F., CLEMENTE, E., ITAKO, A.T., STANGARLIN, J.R. and CRUZ, M.J.S., 2013. Plant extracts for controlling the post-harvest anthracnose of banana fruit. *Revista Brasileira de Plantas Medicinais*, vol. 15, no. 4, suppl. 1, pp. 727-733. http://dx.doi.org/10.1590/S1516-05722013000500013.

FONTANA, D.C., DOURADO NETO, D., PRETTO, M.M., MARIOTTO, A.B., CARON, B.O., KULCZYNSKI, S.M. and SCHMIDT, D., 2021. Using essential oils to control diseases in strawberries and peaches. *International Journal of Food Microbiology*, vol. 338, p. 108980. http://dx.doi.org/10.1016/j.ijfoodmicro.2020.108980. PMid:33243629.

FUSELLI, S.R., GARCÍA DE LA ROSA, B., EGUARAS, M.J. and FRITZ, R., 2010. In vitro antibacterial effect of exotic plants essential oils on the honeybee pathogen *Paenibacillus* larvae, the causal agent of American foulbrood. *Spanish Journal of Agricultural Research*, vol. 8, no. 3, pp. 651-657. http://dx.doi.org/10.5424/sjar/2010083-1261.

GARBIN, T.H.S., ROMANO, E.D.B., CARNEIRO, S.M.T.P.G. and SOUZA, M.L.V., 2015. Efeito do óleo essencial de eucalipto sobre a germinação e o crescimento de rabanete e alface. *SaBios-Revista de Saúde e Biologia*, vol. 10, no. 1, pp. 52-58.

GHATAK, A., CHATURVEDI, P., PAUL, P., AGRAWAL, G.K., RAKWAL, R., KIM, S.T., WECKWERTH, W. and GUPTA, R., 2017. Proteomics survey of Solanaceae family: current status and challenges ahead. *Journal of Proteomics*, vol. 169, pp. 41-57. http://dx.doi.org/10.1016/j.jprot.2017.05.016. PMid:28528990.

GIOPPO, A., ZANCANARO, V. and BELLAVER, E.H., 2019. Atividade antibacteriana do óleo essencial de Melaleuca alternifolia frente a isolados multirresistentes produtores de ESBL e KPC causadores de infecções hospitalares. *Revista Biotemas*, vol. 32, no. 3, pp. 35-42. http://dx.doi.org/10.5007/2175-7925.2019v32n3p35.

HIRATA, D.B., LUZ, A.C.C., ZANETTI, L.V., WERNER, E.T., MILANEZ, C.R.D. and LEITE, I.T.A., 2018. Allelopathic effect of the essential oil of *Cymbopogon nardus* and *Annona muricata* extract on the germination of *Bidens pilosa* and *Megathyrsus maximus*. *Revista de Ciências Agrárias*, vol. 41, no. 3, pp. 712-728.

INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA – IBGE, 2021 [viewed 20 August 2022]. *Produção de tomate* [online]. IBGE. Available from: https://www.ibge.gov.br/explica/producao-agropecuaria/tomate/br

KRZYZANOWSKI, F.C., LORINI, I., HENNING, A.A. and FRANÇA-NETO, J.B., 2013. *Expurgo de sementes de soja e seu efeito na qualidade fisiológica durante o armazenamento*. Londrina: Empresa Brasileira de Pesquisa Agropecuária/Centro Nacional de Pesquisa de Soja.

LI, T., HEUVELINK, E. and MARCELLIS, L.F.M., 2015. Quantifying the source sink balance and carbohydrate contente in three tomato cultivars. *Frontiers in Plant Science*, v. 6, n. 416, p. 1-10. https://dx.doi.org/10.3389/fpls.2015.00416.

LOBATO, A.K.S., SANTOS, D.G.C., OLIVEIRA, F.C., GOUVEA, D.D.S., TORRES, G.I.O.S., LIMA-JÚNIOR, J.A., OLIVEIRA-NETO, C.F. and SILVA, M.H.L., 2007. Ação do óleo essencial de *Piper aduncum* L. utilizado como fungicida natural no tratamento de sementes de *Vigna unguiculata* (L.) Walp. *Revista Brasileira de Biociências*, vol. 5, suppl. 2, pp. 915-917.

MAGUIRE, J.D., 1962. Speeds of germination-aid selection and evaluation for seedling emergence and vigor. *Crop Science*, vol. 2, no. 2, pp. 176-177. http://dx.doi.org/10.2135/cropsci1962.0011183X000200020033x.

MARTINS, J.A.S., SAGATA, E., SANTOS, V.A. and JULIATTI, F.C., 2010. Avaliação do efeito do óleo de *Melaleuca alternifolia* sobre o crescimento micelial in vitro de fungos fitopatogênicos. *Bioscience Journal*, vol. 27, no. 1, pp. 49-51.

MELLO, F.E., SILVA, H.P., CELESTINO, G.G., LOPES, I.O.N., BALBI-PEÑA, M.I. and GODOY, C.V., 2018. Crescimento micelial radial e esporulação de isolados de *Corynespora cassiicola*. *Summa Phytopathologica*, vol. 44, no. 4, pp. 374-379. http://dx.doi.org/10.1590/0100-5405/177612.

PÉREZ-VÁZQUEZ, M.A.K., PACHECO-HERNÁNDEZ, Y., LOZOYA-GLORIA, E., MOSSO-GONZÁLEZ, C., RAMÍREZ-GARCÍA, A.S., ROMERO-ARENAS, O. and VILLA-RUANO, N., 2022. Peppermint essential oil and its major volatiles as protective agents against soft rot caused by *Fusarium sambucinum* in cera pepper (*Capsicum pubescens*). *Chemistry & Biodiversity*, vol. 19, no. 1, p. e202100835. http://dx.doi.org/10.1002/cbdv.202100835. PMid:34812593.

POPINIGIS, F., 1985. *Fisiologia da semente*. Brasília: AGIPLAN. 289 p.

SEGUÍ-SIMARRO, J.M., 2016. Androgenesis in Solanaceae. *Methods in Molecular Biology*, vol. 1359, pp. 209-244. http://dx.doi.org/10.1007/978-1-4939-3061-6_9. PMid:26619864.

SILVA, E.O., 2018. *Termoterapia e óleos essenciais no controle de Pseudomonas syringae pv. tomato em sementes de tomate*. Botucatu: Universidade Estadual Paulista "Júlio de Mesquita Filho". Tese.

SILVA, J.B.C. and NAKAGAWA, J., 1995. Estudo de fórmulas para o cálculo da velocidade de germinação. *Informativo ABRATES*, vol. 5, no. 1, pp. 62-73.

SILVA, J.C. and BETTIOL, W., 2006. *Possibilidade de controle da murcha de Fusarium do tomateiro com isolados de Fusarium oxysporum não patogênico*. Jaguariúna: Embrapa.

SOUZA, A.D., ROGGERIO, T.U., FURLAN, M.R. and AOYAMA, E.M., 2015. Óleo de melaleuca (*Melaleuca alternifolia* Maiden & Betche, Cheel) no controle de cercosporiose em beterraba. *Revista Brasileira de Plantas Medicinais*, vol. 17, no. 4, suppl. 3, pp. 1078-1082. http://dx.doi.org/10.1590/1983-084x/14_042.

WIRTH, S.J. and WOLF, G.A., 1992. Micro-plate colourimetric assay for endo-acting cellulose, xylanase, chitinase, 1,3-β-glucanase and amylase extracted from forest soil horizons. *Soil Biology & Biochemistry*, vol. 24, no. 6, pp. 511-519. http://dx.doi.org/10.1016/0038-0717(92)90074-8.

ZAMBOLIM, L., COSTA, H. & VALE, F.X.R. Efeito da nutrição mineral sobre doenças de plantas causadas por patógenos do solo. In: L. ZAMBOLIM, ed. *Manejo integrado, fitossanidade, cultivo protegido, pivô central e plantio direto*. Viçosa: Suprema Gráfica e Editora Ltda, 2001, pp. 347-408.