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Essential oil of *Mentha spicata* reduces the initial growth of *Corynespora cassiicola in vitro*

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ABSTRACT

Synthetic fungicides are one of the control strategies for the most common diseases in agriculture. However, more sustainable strategies are required. Thus, this study evaluates the potential of the essential oils of *Cedrela fissilis* and *Mentha spicata* in the *in vitro* control of the mycelial growth of the pathogen *Corynespora cassiicola*. The experiment was conducted at the Phytosanitary Laboratory of the Universidade Estadual do Mato Grosso do Sul (UEMS), University Unit of Cassilândia-MS. The essential oils of Argentine Cedar (*Cedrela fissilis*) and Spearmint (*Mentha spicata*) were acquired from commerce. A completely randomized design was used with a factorial scheme of two categories of essential oils (Spearmint and Cedar) x 5 concentrations (0, 25, 50, 100, and 200 μL L^{-1}). The data were submitted to the analysis of variance. The means were compared by the Tukey test at 5% probability. Quantitative data were submitted to regression analysis. The fungicide effect was determined by measuring the diameter of colonies (mean of two diametrically opposed measurements, using a caliper, every 72 hours until reaching 216 hours). Spearmint essential oil (*M. Spicata*) presented higher efficiency than Argentine Cedar essential oil (*Cedrela fissilis*) on reducing of *Corynespora cassiicola* growth in the periods between 72 and 144 hours *in vitro*.

Keywords: Phytopathology, fungicide resistance, alternative control.

Óleo essencial de *Mentha spicata* reduz o crescimento inicial de *Corynespora cassiicola* in vitro

RESUMO

O uso de fungicidas sintéticos é uma das estratégias de manejo de doenças mais utilizadas na agricultura, porém, busca-se manejos que sejam mais sustentáveis. Dessa forma, objetivou-se avaliar o potencial de óleos essenciais de *Cedrela fissilis* e *Mentha spicata* no controle, *in vitro*, do crescimento micelial do patógeno *Corynespora cassiicola.* O trabalho foi desenvolvido no Laboratório de Fitossanidade da Universidade Estadual do Mato Grosso do Sul (UEMS), Unidade Universitária de Cassilândia-MS. Os óleos essenciais, Cedro (*Cedrela fissilis*) e Hortelã (*Mentha spicata*) foram adquiridos comercialmente. O delineamento experimental utilizado foi inteiramente casualizado, em esquema fatorial 2 categorias de óleos essenciais (hortelã e cedro) x 5 concentrações (0, 25, 50, 100 e 200 μL L^{-1}). Os dados foram submetidos à análise de variância e as médias foram comparadas pelo teste de Tukey a 5% de probabilidade. Os dados quantitativos foram submetidos à análise de regressão. O efeito fungicida foi determinado através de medições do diâmetro das colônias (média de duas medidas diametralmente opostas), com auxílio de um paquímetro, a cada 72 horas até 216 horas. O óleo essencial de hortelã (*M. Spicata*) apresentou maior eficácia do que o óleo essencial de Cedro (*Cedrela fissilis*) na redução do crescimento de *Corynespora cassiicola* nos períodos de 72 e 144 horas *in vitro*.

Palavras-chave: Fitopatologia, resistência a fungicidas, controle alternativo.

1. Introduction

The species *Corynespora cassiicola* (Berk. and Curt.) C.T. Wei is a cosmopolitan Ascomycota fungus that uses a wide variety of crop plants as hosts (Dixon et al., 2009), causing leaf pathologies, such as target leaf spot in soybeans (*Glycine max* L. Merrill) (Soares and Arias, 2020), cotton (*Gossypium* spp.) (Rondon and Lawrence, 2020), tomato (*Lycopersicon esculentum* Mill.), and coffee (*Coffea* spp.) (Pereira and May-De-Mio, 2019). Depending on the interaction with the host, it can be found as a parasite, causing diseases on other vegetable organs (Far and Rossman, 2020).

Synthetic fungicides are a control strategy for the most common crop diseases, and, among these, the single-site fungicide presents excellent results for disease control. However, samples of *C. cassiicola* in Brazilian soybean crops showed isolation in different degrees of insensibility to the groups of fungicides carboxamides (SDHI), triazoles (DMI), and strobilurins (QoI), demonstrating that a selection for resistance is undergoing (Teramoto et al., 2017). These authors report isolated resistance to the fungicide Cyproconazole (DMI) so that a dose is 100 times higher than the one used in isolates sensible to this fungicide. The pressure to select molecules of single-site fungicides promotes insensibility to the alteration in a single target protein, a characteristic that leads to a high mutation risk (Lucas et al., 2015).

As the approval of new synthetic fungicides is costly and subjected to restrictive laws, the efficiency of available fungicides has been reduced by inadequate use and evolutive factors of the populations of phytopathogens (Lucas et al., 2015). Besides, there is a growing global concern about the consequences of synthetic molecules, both for the environment and human beings. The study of economically viable and ecologically correct control strategies is necessary and urgent. In this context, the use of natural bioactive products of botanic origin has shown a considerable alternative, fulfilling such demands (Santos et al., 2015; Gwinn, 2018).

Currently, biofungicides formulated with essential oil or vegetal extract are available in the market (Reuveni et al., 2020). Essential oils generally are of easy obtention and use, besides being more ecologically correct and safer than the synthetic molecules currently used in agriculture (Marques et al., 2004). A vast literature supports their fungicide and fungistatic effect (Sing et al., 1980; Gwinn, 2018). *Cedrela fissilis* Vell species, from the family Meliaceae, commonly known as Argentine Cedar, has an essential oil rich in limonoid compounds widely used in studies by its insecticide effect (Nogueira et al., 2020), also presenting antimicrobial effects (Tan et al., 2011).

Mentha spicata L, known as Spearmint, of the family Lamiaceae, is rich in carvone and limonene, secondary compounds that can present bactericide effect on *Xanthomonas* spp. and fungicide on *Rhizoctonia solani* Kuhn (Bayan and Küsek, 2018). Thus, associated with the fact that no reports were found about the use of essential oils of *Cedrela fissilis* or *Mentha spicata* for the control of *C. Cassiicola*, our study evaluates the potential of *Cedrela fissilis* and *Mentha spicata* essential oils on the *in vitro* control of the mycelial growth of the pathogen *Corynespora cassiicola*.

2. Material and Methods

The experiment was conducted at the Phytosanitary Laboratory of the Universidade Estadual do Mato Grosso do Sul (UEMS), University Unit of Cassilândia-MS. Both essential oils, Argentine Cedar (*Cedrela fissilis*) and Spearmint (*Mentha spicata*) were acquired from a specialized company registered in Anvisa under the n. CEVS 354340218-477-000401-1-8. *Corynespora cassiicola* strains were obtained from a fungi collection of Instituto Biológico de São Paulo and also pertained to the collection of the Phytosanitary Laboratory of the UEMS. The anti-fungi activity of the essential oils was determined through the mycelial development of the fungus in a PDA medium, added by the treatments mentioned above. Then, colony disks of 5 mm in diameter were introduced in the PDA medium (potato-dextrose-agar) with the addition of the essential oils according to the treatment.

The addition of the treatments to the PDA was conducted when the medium reached a considerable temperature (45-50 ºC). The treatments were conditioned in Petri dishes (90 mm x 10 mm). The fungicide effect was determined by measuring the diameter of colonies (mean of the two diametrically opposed measurements), using a caliper, every 72 hours until the witness ultimately reached the dish.

The completely randomized design arranged in a 2x5 factorial scheme was used. Two essential oil categories (Spearmint and Cedar) x five concentrations (0, 25, 50, 100, and 200 $\mu L\ L^{-1}$) were evaluated. The data were submitted to the analysis of variance. The means were compared by the Tukey test at 5% probability. Quantitative data were submitted to regression analysis. Statistical analyses and graphics production were performed with the software R version 3.6.1 (R Development Core Team, 2019).

3. Results and Discussion

The effect of essential oil concentrations (C) on the mortality of the fungus *Corynespora cassiicola* was significant ($p < 0.01$). It was possible to organize treatments in a factorial scheme O*C ($p < 0.05$) to evaluate the mortality of *C. cassiicola* in periods of 72 hours and 144 hours of exposition to the essential oils. Nevertheless, the interaction (O*C) presented no significant effect ($p > 0.05$) in 216 hours (Table 1).

Table 1. Summary of the Analysis of Variance, the significance of the F-test for the mortality periods of the fungus *C. cassiicola*, and the coefficient of variation (CV) in different exposition periods (72, 144, and 216 hours) to the essential oils concentrations of Spearmint and Argentine Cedar.

		------------- Exposition period -------------		
S.V.	D.F.	72h	144h	216h
O	1	0.00**	0.03*	0.28ns
C	4	0.00**	0.00**	0.00**
O*C	4	0.04*	0.03*	0.77ns
CV (%)		19.23	7.76	5.58

ns: not significant, *: significant at 5%, and **: significant at 1% by the F-test.

The effect of the categories of essential oils (EO) was significant for the exposition periods of 72 hours ($p < 0.01$) and 144 hours ($p < 0.05$). Nevertheless, this factor had no significant effect on the 216 hours (Table 1). This fact means that the 216 hours exposition presented the same result for every essential oils category.

There were differences in the means of the concentrations of 100 and 200 μL L^{-1} between the categories of essential oils in the period of 72 hours of exposition (Figure 1A). Nevertheless, regarding the exposition period of 144 hours, the dose of 200 μL L^{-1} presented a difference between the essential oils, and the highest mean was obtained by EO of Argentine Cedar (Figure 1B). This fact indicates that the spearmint essential oil was more efficient in controlling the mycelial growth in these two observation periods, especially in the concentration of 200 μL L^{-1}.

It was possible to perform a regression analysis for the periods of 72 and 144 hours of exposition. For 72 hours of exposition, the data regarding the essential oil of Argentine Cedar was not significant for the regression. For the 144-h period, the regression equation was possible for both essential oils. In both exposition periods, a descending linear relation was observed. Thus, the higher the concentrations of spearmint essential oil, the lower the mycelial growth area of the pathogen *C. cassiicola* (Figure 2). There was possibly an interference on the mycelial growth of this fungus when treated with the essential oil of *M. spicata*. Essential oils can affect the protective spores or act before fungus formation, resulting in abnormal development of the germ tubes (Dantigny and Nanguy, 2009).

The analyses show that the essential oil of Spearmint was more effective in inhibiting the pathogen than the Cedar essential oil. According to Shahbazi and Shavisi (2016), the main chemical components of the essential oil of *M. spicata* are carvone, limonene, and β-bourbonene. Carvone is an unsaturated ketone monoterpenoid of the class of terpenes. This compound is considered a microbial agent, acting on fungi and bacteria (Carvalho and Fonseca, 2006).

Diniz et al. (2008), while conducting an *in vitro* experiment, observed an inhibition close to 80% of the mycelial growth of *C. cassiicola* with the dose of 100 μL of the *Mentha arvensis* L essential oil. Thus, it is believed that the essential oil of *Mentha arvensis* inhibits more of this pathogen than the essential oil of *M. spicata*. According to Teramoto et al. (2017), in Brazil, the populations of *C. cassiicola* already presented organisms resistant to strobilurins, which are widely used for the chemical control of crops. An alternative to control this use is employing natural bioactive products.

According to Gwinn (2018), their action mode generally involves more than one physiological mechanism, showing a low mutation risk for resistance in the target population. Thus, the control of pathogens with the essential oil of *M. spicata* is not toxic to humans (Kedia et al., 2014), which makes it safer and ecologically cleaner than synthetic molecules. Regarding the concentrations (Table 2), it is noticed that besides the first periods of 72 h presented differences between the essential oils, in the 216-h period, both essential oils had the same result. Such results suggest that the doses used can be low to inhibit the growth of the pathogen for extended periods because during the time the fungus adapts to such compounds or the effects of the oil are lost, especially with volatile bioactive compounds (Gwinn, 2018).

Monoterpenoids, such as carvone and limonene, have limitations such as low solubility in water, high volatility, and low chemical stability, which can minimize their effects (Maróstica Junior and Patores, 2007). Regarding such data, new hypotheses emerge, such as: would the same results occur under field conditions? Maybe the permanent effects depend on the reapplication of essential oil solutions. Nevertheless, new studies should be conducted using microscopy to observe the interaction between the essential oils and the pathogen *C. cassiicola* to check permanent effects or new concentration intervals. Moreover, *in vitro* experiments are required to clarify this phenomenon.

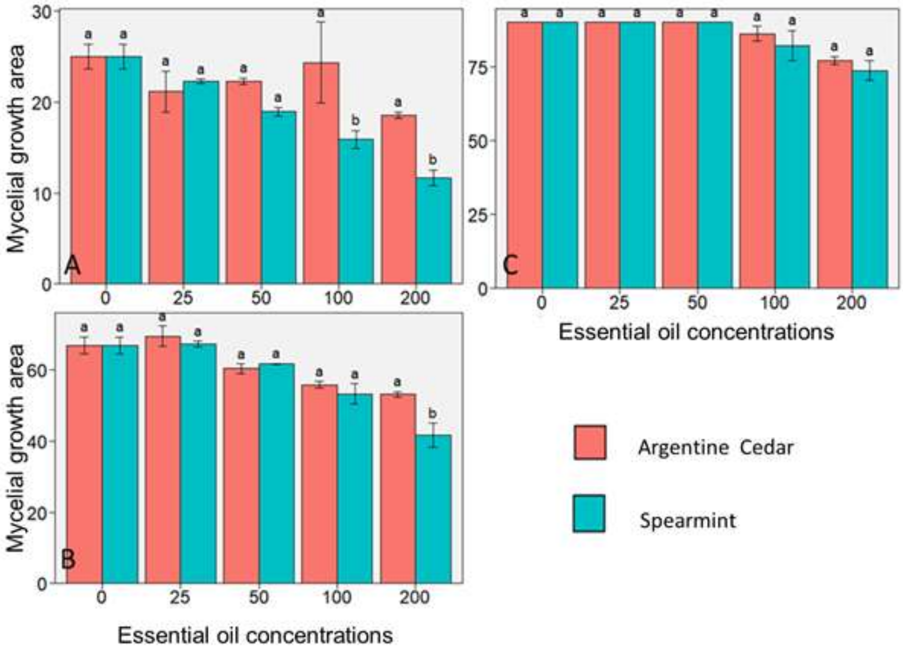

Figure 1. Area of mycelial growth of *C. cassiicola* with the application of doses of essential oils from Argentine Cedar and Spearmint at 72 hours exposition period (A), 144 hours exposition period (B), and 216 hours exposition period (C).

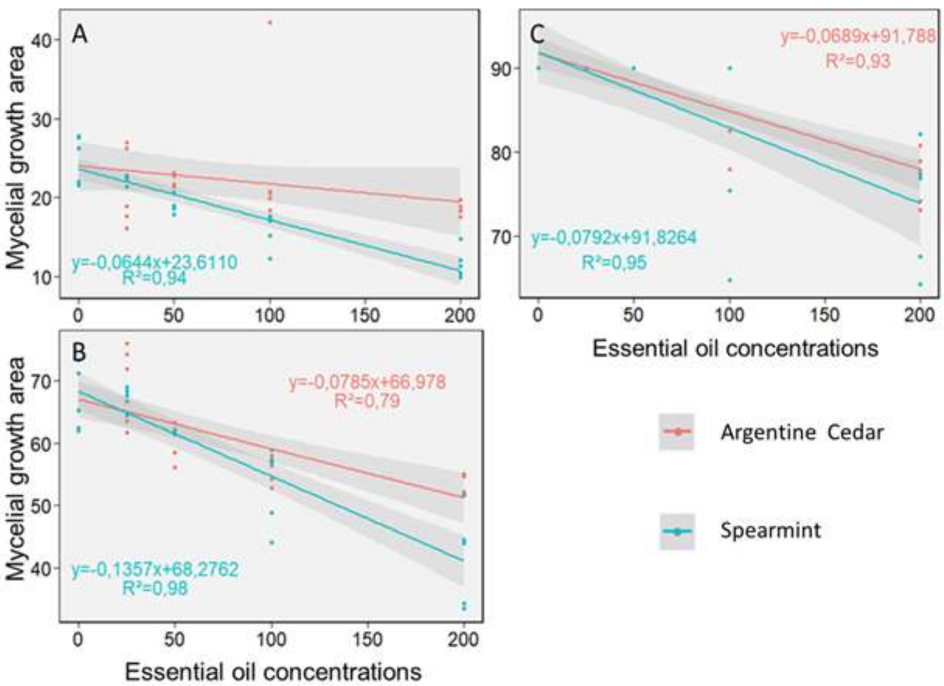

Figure 2. Regression equations for the relation between the essential oils of Argentine Cedar and Spearmint and their concentrations. A: 72 hours exposition period; B: 144 hours exposition period, and C: 216 hours exposition period.

Table 2. Comparison in each essential oil category regarding the mortality periods of the fungus *Corynespora cassiicola* submitted to different exposition periods (72, 144, and 216 hours) to the essential oils of Spearmint, Argentine Cedar, and their concentrations.

	- - - - - 72h - - - - -		- - - - - 144h - - - - -		216h
Concentration	Cedar	Spearmint	Cedar	Spearmint	Essential Oil
0	25.04 a	25.04 a	69.44 a	67.26 a	90.00 a
25	21.17 a	22.30 ab	66.84 ab	66.84 a	90.00 a
50	22.34 a	18.99 ab	60.32 bc	61.65 a	90.00 a
100	24.37 a	15.90 bc	55.77 c	53.18 b	84.08 a
200	18.55 a	11.69 c	53.10 c	41.56 c	75.34 a

Means followed by the same letter in the column do not differ statistically from each other by the Tukey test ($p < 0.05$).

4. Conclusions

The essential oil of Spearmint (*Mentha spicata*) presented higher efficiency than the essential oil of Argentine Cedar (*Cedrela fissilis*) on the control of the *Corynespora cassiicola* mycelial growth in the exposition periods of 72 and 144 hours.

Authors' Contribution

Abimael Gomes Silva contributed to writing the manuscript and statistical analysis. Ana Caroline de Souza contributed to setting up the experiment, collecting data, and writing the manuscript. Beatriz Garcia Lopes contributed to statistical analysis, creating the figures, and writing the manuscript. Eliamara Marques da Silva contributed to writing the manuscript. Gustavo Haralampidou da Costa Vieira contributed to guide the experiment assembly and writing the article.

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