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Fungitoxity of *Mentha spicata* oil on the growth of fungi of the genera *Colletotrichum* and *Fusarium*

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ABSTRACT

Increasingly, the population has been concerned that the production of these foods is free of pesticide residues, so it is necessary to search for viable and effective alternatives to control pests and diseases. Thus, the use of essential oil from plants with insecticidal and fungicidal potential has been successfully studied. The study aimed to demonstrate the effect of *Mentha spicata* essential oil on the control of *Fusarium* and *Colletotrichum* fungi *in vitro*. The essential oil of *M. spicata* used was purchased from the chemical industry Ferquima, being diluted in a 2% Tween 20 solution (being 98 mL of distilled water + 2 mL of Tween 20), which was added in 60 mL of a PDA culture medium, resulting in final essential oil concentrations: 0 ppm; 0.83 ppm; 1.67 ppm; 8.33 ppm, and 16.67 ppm and poured into 90 mm diameter Petri dishes. The evaluations took place periodically every 48 hours for eight days, performing measurements of the diameters of the colonies. The results evidenced that the analyzed fungi, when submitted to treatments of different doses, suffered fungistatic action according to the gradual increase of the doses. Thus, it is possible to conclude that *M. spicata* oil provided the *in vitro* growth control of *Fusarium* sp. and *Colletorichum* sp.

Keywords: Alternative control, Fungi, Rots.

Fungitoxidade do óleo de *Mentha spicata* sobre crescimento dos fungos dos gêneros *Colletotrichum* e *Fusarium*

RESUMO

Cada vez mais, a população tem se preocupado que a produção desses alimentos seja livre de resíduos de agrotóxicos, portanto se faz necessário a busca por alternativas viáveis e eficazes para controle de pragas e doenças. Desta forma, a utilização de óleo essencial de plantas com potencial inseticida e fungicida vem sendo estudada com sucesso. O objetivo deste trabalho foi demonstrar o efeito do óleo essencial de *Mentha spicata* no controle dos fungos dos gêneros *Fusarium* e *Colletotrichum in vitro*. O óleo de essencial de *M. spicata* utilizado, foi adquirido da indústria química Ferquima, sendo diluído em uma solução Tween 20 a 2% (sendo 98 mL de água destilada + 2 mL de Tween 20), que foram adicionados em 60 mL de meio de cultura BDA, resultando nas concentrações finais de óleo essencial: 0 ppm; 0,83 ppm; 1,67 ppm; 8,33 ppm e 16,67 ppm e vertidos em placas de Petri de 90 mm de diâmetro. As avaliações ocorreram periodicamente a cada 48 horas, por 8 dias, realizando medições dos diâmetros das colônias. Os resultados apresentados mostraram que os fungos analisados quando submetidos a tratamentos de diferentes doses, sofreram ação fungistática, de acordo com o aumento gradativo das doses. Assim, é possível concluir que o óleo de *M. spicata* proporcionou o controle do crescimento *in vitro* dos fungos *Fusarium* sp. e *Colletorichum* sp.

Palavras-chave: Controle alternativo, Fungos, Podridões.

Modern agriculture has been based on two fundamental points: production quantity and quality. Increasingly, the population has been concerned about the consumption of foods that offer a guarantee of the absence of chemical residues, as a result, there has been an increasing search for disease management in crops, employing the reduction of economic damage, aligned with a smaller loss to the ecosystem (Dal Soglio and Kubo, 2016).

Currently, numerous active chemical principles on the market can be used to control diseases in different cultures. However, many of these compounds are highly toxic to humans and the biota in which the culture of interest is inserted (Anvisa, 2018). The considerable increase in the use of pesticides has brought a series of disorders and changes to the environment, destroying numerous microorganisms that do not harm the production process but help in the metabolization of compounds, being beneficial to the environment (Pereira et al., 2019).

Post-harvest-related pathogens cause major negative impacts on producers, especially product losses due to fungal attacks. One of the limiting factors for papaya cultivation is anthracnose, the most common disease in the culture, caused by the fungus *Colletotrichum gloeosporioides*, which can establish itself in the immature fruit and remain in a quiescent state throughout its development, without the appearance of symptoms, until there are conditions for the infection process to occur (Ventura and Rezende, 2016). Another predominant fungus in the papaya crop is *Fusarium solani*. Fungi of the genus *Fusarium* can survive for long periods in the soil by forming survival structures called chlamydospores, and the symptoms observed are small (± 15 mm), dry and depressed lesions (Ventura and Rezende, 2016).

Silva et al. (2009) studied the effect of extracts and essential oils of some medicinal and/or native plants on the germination of spores and mycelial growth of the fungus *C. gloeosporioides*, concluding that the compounds showed control over the fungus, which demonstrates the effectiveness of compounds from plants. As a result, more and more viable alternatives have been sought that effectively control these diseases in the papaya crop, using natural compounds, which can be extracted from leaves, stems, fruits, or roots of plants. Several authors prove the effectiveness of several essential oils in controlling fungal growth, however, there is no standard methodology, which makes it difficult to compare and discuss results.

The present study aimed to demonstrate the effect of the essential oil of *M. spicata* in the *in vitro* control of the fungi *Fusarium* sp. and *Colletorichum* sp and it was developed at the Federal Institute of Mato Grosso do Sul, Campus Nova Andradina, in the Microbiology and Phytopathology laboratory. *Fusarium* sp. and *Colletorichum* sp. used in the experiments were obtained from direct isolation of papaya fruits showing disease symptoms and signs of pathogens, such as those obtained in commercial establishments in Nova Andradina (Alfenas et al., 2016). Isolation and multiplication were performed using a PDA culture medium (Potato, Dextrose, and Agar) from Kasvi, and the plates were incubated in BOD at 26±10C with a photoperiod of 24h.

The essential oil of *M. spicata* used was purchased from the chemical industry Ferquima, being diluted in a 2% Tween 20 solution (being 98 mL of distilled water + 2 mL of Tween 20), which was added in 60 mL of culture medium Potato-Dextrose-Agar (PDA), resulting in final concentrations of (0 ppm, 0.83 ppm, 1.67 ppm, 8.33 ppm, and 16.67 ppm) and poured into 90 mm Petri dishes with 15 ml each. In the center of the Petri dishes, a 5 mm disc of PDA containing fungal growth (*Fusarium* sp. or *Colletotrichum* sp.) with five days of growth was added. After inoculation, the plates were incubated in BOD (26±10C in a 24h light photoperiod).

The evaluations took place periodically every 48 hours for eight days, through the measurements of the diameters of the colonies, using a digital caliper, obtaining two opposed averages. The experimental design was completely randomized, with five treatments (concentrations) and four replications each, and each fungal species was considered an independent experiment and installed at different times. Colony growth data were used to calculate the growth inhibition percentage (PIC) obtained by the formula:

$$PIC = [(\text{control diameter} - \text{treatment diameter}) / \text{control diameter})] * 100$$

Subsequently, the data were tested. When they met the assumptions, they were submitted to analysis of variance (ANOVA), and the F test evaluated the effect at 5% significance. When there was a significant effect, average tests were conducted. The test used in this study was the Tukey test at 5% significance. A regression analysis was also performed to model the relationship between the products and the effectiveness of controlling the pathogens. The effective concentration leading to a 50% reduction in mycelial growth and conidial germination (EC_{50}) and the respective standard error (SE) was estimated using the 'ec50estimator' and 'drc' packages (Ritz et al. 2015; Alves 2020).

All analyses and graphs were produced using the R software (R Core Team, 2022). Tables 1 and 2 and Figure 1 have showed that the fungi analyzed, when submitted to treatments with different doses of *Mentha spicata* oil, suffered a fungistatic action according to the gradual increase in doses, as can be seen in the doses of 8.33 ppm and 16.67 ppm which showed growth inhibition greater than 80% for both fungi.

Table 1. Data on the percentage inhibition of mycelial growth of *Fusarium* sp.

Fungus	Concentration (ppm)	% inhibition
Fusarium sp.	0	0.00 C
	0.83	25.2 B
	1.67	28.4 B
	8.33	90.5 A
	16.67	92.7 A

Table 2. Data on the percentage inhibition of mycelial growth of *Colletotrichum* sp.

Fungus	Concentration (ppm)	% inhibition
Colletotrichum sp.	0	0.00 B
	0.83	15.6 B
	1.67	27.0 B
	8.33	83.8 A
	16.67	100 A

As presented in the tables, it is possible to observe a tendency where both isolates present a certain similarity when submitted to treatment with the oil, in which the doses of 0.83 ppm and 1.67 ppm presented lower percentages of control, being 25.2 and 28.4% of inhibition for *Fusarium* sp., while *Colletotrichum* sp., presented 15.6 and 27% of inhibition, respectively. Carnelossi et al. (2009) evaluated *in vitro* and *in vivo* control of *C. gloeosporioides* in post-harvest papaya using essential oils of *Cymbopogon citratus*, *Eucalyptus citriodora*, *Mentha arvensis*, and *Artemisia dracunculus* at different doses. They concluded that for the oils of *M. arvensis* and *E. citriodora*, the inhibition was dose dependent, that is, as the dose applied to the fungus increases, consequently, we have an increase in the percentage of inhibition. Thus, there is a similarity with the present work, which showed a gradual increase in the inhibition of fungal mycelial growth according to the increase in doses. Chemically, most essential oils are composed of phenylpropanoid or terpenoid derivatives, which may explain the fungitoxic capacity of oils (Robbers et al., 1997).

Regarding the fungistatic capacity of *M. spicata* oil, Silva et al. (2021) evaluated the potential of essential oils from *Cedrela fissilis* and *M. spicata* in controlling, *in vitro*, the mycelial growth of the pathogen *Corynespora cassiicola*, and concluded that the essential oil of Spearmint (*M. spicata*) was more efficient than the essential oil of Argentine Cedar (*Cedrela fissilis*) in controlling the growth of *Corynespora cassiicola*, strengthening the effectiveness of *M. spicata* oil in controlling phytopathogenic fungi.

Nosrati et al. (2011) also evaluated the antifungal activity of *M. spicata* oil in controlling *Fusarium oxysporum* f. sp. *radicis-cucumerinum*, obtaining a growth inhibition percentage that ranged from 27.14% in seven days related to a 3 µL concentration to 65.59% related to a 5 µL concentration in four days, which indicated a different concentration establishment rate on different days, which is possible to observe an inhibitory potential in the use of *M. spicata* oil.

Paiva et al. (2021) assessed the antifungal effect of three essential oils, including *M. spicata* oil, on the mycelial growth of *Pythium* sp. This study made it possible to conclude the fungistatic capacity of the tested oils, showing the presence of compounds capable of inhibiting the mycelial growth of an oomycete. Based on the results of this work, we also calculated the EC_{50}, which represents the concentration of *M. spicata* oil necessary for inhibiting 50% of the fungal mycelial growth (Figure 2). Observing the data above, it is once again possible to observe the effectiveness of the oil in controlling fungi, which was able to inhibit 50% of growth at concentrations of 2.66 ppm (± 0.43 SE) and 2.96 ppm (± 0.75 SE) for *C. gloeosporioides* and *F. solani*, respectively.

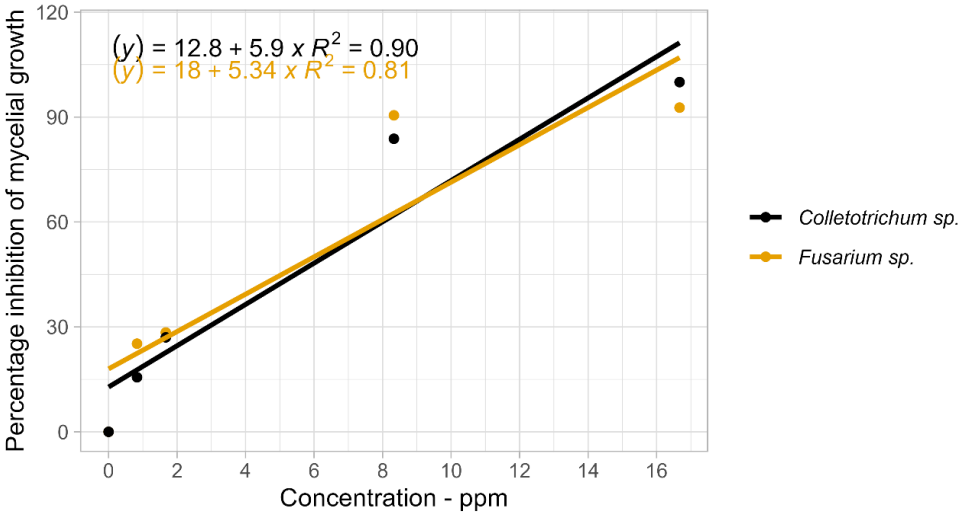

Figure 1. Representation of the percentage of mycelial growth inhibition

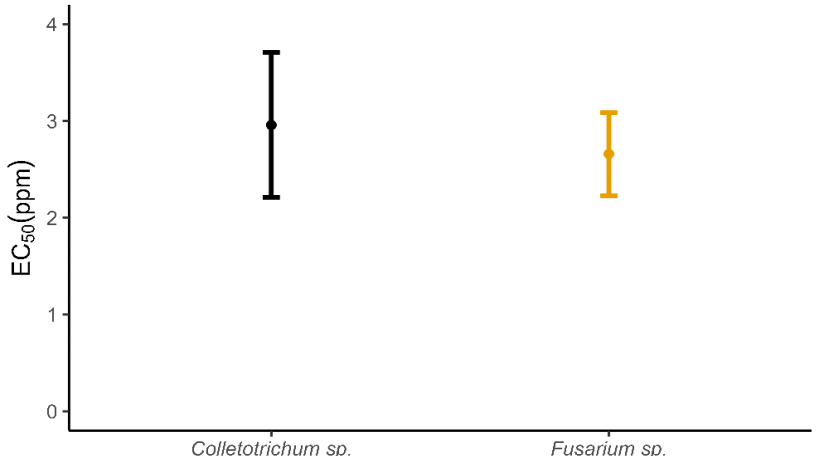

Figure 2. Representation of the concentration in ppm to inhibit 50% of fungal mycelial growth.

Based on these results, it is possible to define ways of approaching future research, such as the use of these data for research with *in vivo* tests, being conducted in a greenhouse and the field, and with that, propitiating the development of commercial products, which can be used on a commercial scale in crops and post-harvest fruits. *M. spicata* oil, at concentrations between 8.33 and 16.67 ppm, has fungistatic activity against *Fusarium* sp. and *Colletotrichum* sp., requiring further studies on application modes and their use on a commercial scale.

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