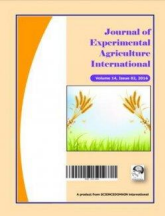

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In vitro Effect of Essential Oil of Peppermint (*Mentha x piperita* L.) on the Mycelial Growth of *Alternaria alternata*

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The research aimed to evaluate the *in vitro* antifungal effect of the essential oil of peppermint (*Mentha x piperita* L.) in the control of *Alternaria alternata*.
Study Design: The experiment was conducted in a completely randomised experimental design with five treatments in four replicates each.
Place and Duration: The work was conducted at the Center of Science and Technology Agri-food of the Federal University of Campina Grande, Pombal-PB, Brazil, between February and March of 2018.
Methodology: The essential oil was added to the PDA culture medium (Potato-Dextrose-Agar) autoclaved and subsequently poured into Petri plates. The treatments comprised five concentrations of the oil (0.0, 0.4, 0.6, 0.8, and 1.0%). After the inoculation with fungi, the plates

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were incubated for 14 days in a B.O.D incubator at 27±2°C. With the data of mycelial diameters, the percentage of mycelial growth inhibition (PGI) and index of mycelial growth speed (IMGS) were calculated.
Results: All concentrations of peppermint oil reduced the mycelial growth of *A. alternata*. The minimum and maximum inhibitions occurred in the concentrations of 0.4 and 0.8%, which reached -13.27 and 72.45%, respectively. Although the maximum inhibition was 72.45%, the average percentages were 41.67 and 37.18% at the highest concentrations, showing an intermediate power of inhibition. A dose-dependent behaviour, was observed which suggests that further increases in concentration may enhance the inhibition effect of the oil.
Conclusion: The peppermint oil can be used as a viable and sustainable antifungal product in the control of this pathogen. Due to the possible dose-dependent effect, the development of studies is recommended with concentrations around 2.25% to test if the oil reaches the total inhibition. The *in vivo* experiments are required to verify the oil feasibility on the control of *A. alternata* on plants and fruits.

Keywords: Antifungal; disease control; fungitoxic effect; phytopathogens; postharvest diseases.

1. INTRODUCTION

Agroecological systems link agricultural production to ecological perspectives, aiming a food production with low environmental impact. The preservation of soil and biodiversity comprises some benefits of using agroecological management techniques.

However, the control of diseases has hampered the progress of many agroecosystems. Approximately 70% of tree diseases are caused by pathogens that reduce productivity and damage fruits intended for export [1,2]. The rotting stands out among the diseases that cause post-harvest losses. This disease appears early and causes severe injuries under tropical climate, because of the high temperatures and humidity which increases the propagation of microorganisms leading to depreciation of the fruit during the marketing phase [3].

Rotting usually occurs through latent infections, when the phytopathogen infects healthy fruits, but the symptoms arise later in the physiological maturation of fruits [4], during the storage and transport before marketing. The fungi of the genus *Alternaria* are among the main pathogens causing post-harvest diseases. These fungi comprise saprophytic species and plant parasites [5]. The species *Alternaria alternata* affects various economically important crops, causing rot in melon (*Cucumis melo* L.) [2], grapes (*Vitis vinifera* L.) [6], and papaya (*Carica papaya* L) [7].

Conventional farmers fight this problem with highly toxic agrochemicals, which pollute the environment [8], affect human health, increase production costs, and hinder the export of products [9]. The inadequate management of pesticides causes several environmental damages, such as the accumulation of harmful substances in the soil, water, and organisms along the food webs. Also, it may lead to the emergence of resistant strains of pathogens, making the use of ever stronger pesticides necessary, worsening even more the environmental impacts [10].

The use of less harmful products in the control of plant pathogens became urgent to attain sustainability in agroecosystems. Such products must be efficient, cheap, and safe for human health and the environment. Among the products widely tested for this purpose, one finds the essential oils extracted from the aromatic plants whose raw material is easy to acquire, the production has low cost, and many present antifungal activities [7,11,12,13,14,15].

The essential oil of peppermint (*Mentha* x *piperita* L.) has been tested as an antimicrobial agent for several years. Studies proved its *in vitro* fungitoxic effect, obtaining satisfactory results on the control of *Macrophomina phaseolina* [16], *Colletotrichum gloeosporioides* [14], *Fusarium solani*, *Rhizoctonia solani*, *Sclerotium rolfsii* [17], *Aspergillus niger* and *A. flavus* [9]. Aiming to provide a healthy and environmentally safe product to fight the diseases caused by *A. alternata*, this study assesses the *in vitro* fungitoxic effect of peppermint essential oil on the control of this fungus.

2. MATERIALS AND METHODS

2.1 Conduction of the Experiments

The study was carried out in the Laboratory of Phytopathology, Federal University of Campina Grande, Campus of Pombal-PB, in October 2017. The experiment comprised a completely randomised design with five treatments and four replicates each.

The 0878 strain of *A. alternata* was used, provided by the collection of pathogenic fungi of Prof. Maria Menezes of the Federal Rural University of Pernambuco. The pure essential oil of peppermint was purchased at a local store specialising in natural products.

The oil was added to autoclaved PDA culture medium (Potato Dextrose Agar), on the following concentrations (treatments): 0.0; 0.4; 0.6; 0.8 and 1.0%. The 0.0% treatment comprised a negative control. After cooling, the medium was poured into Petri plates of 9 cm in diameter under aseptic conditions. Discs of culture medium with 1 cm in diameter containing propagules of the fungus were transferred to the centre of each plate containing the treatments. Then, the plates were wrapped in plastic film and incubated for 14 days in a B.O.D incubator (Biochemical Oxygen Demand) at a temperature of 27±2°C.

The mycelial growth was assessed through daily measurements of colony diameters with the aid of a graduated ruler. For each colony, a daily data comprised the average of two perpendicular measurements. With the result of the measures, the percentage of mycelial growth inhibition (PGI; [18]) and the index of mycelial growth speed (IMGS; [19]) were calculated, according to the formulas (1) and (2):

$$PGI = \frac{[(negative\ control\ growth - treatment\ growth)]\times 100}{negative\ control\ growth} \quad (1)$$

$$IMGS = \sum \frac{current\ mycelial\ growth - previous\ mycelial\ growth}{number\ of\ days\ of\ incubation} \quad (2)$$

2.2 Statistical Analysis of Data

The data were submitted linear regression using statistical software R 3.5.1 [20] and Past 3.12 [21]. Differences were considered significant at a 95% level of confidence.

3. RESULTS

All concentrations of peppermint oil reduced the mycelial growth of *A. alternata* (Fig. 1). The increase in oil concentration improved significantly the percentage of mycelial inhibition ($F = 21.9$; $p = 0.0001$) (Fig. 2). The minimum and maximum inhibitions occurred in the concentrations of 0.4 and 0.8%, which reached -13.27 and 72.45%, respectively. Applying the regression equation, the total inhibition was calculated, which may occur with the use of oil at a concentration of 2.26%.

The rate of mycelial growth decreased significantly with the addition of oil ($F = 22.08$; $p = 0.0001$; Fig. 3). The minimum and maximum rates occurred in the concentrations of 0.8 and 0.0%, ranging from 0.10 to 0.42 cm day^{-1}, respectively. The equation suggests that the oil concentration of 2.25% may preclude the mycelial growth.

4. DISCUSSION

The peppermint oil inhibits the growth of *A. alternata* under *in vitro* condition. The presence of terpenes in the composition of peppermint oil gives its antimicrobial properties. The menthone, menthol, and menthyl acetate comprise the main constituents of this oil [22,23], they turn the fungal cell membrane permeable, causing the loss of cytoplasmic content [24].

The results corroborate the findings of several studies. A previous study evaluating the seasonal variation of chemical composition and antimicrobial and cytotoxic activity of essential oils from four species of the genus Mentha (*M. arvensis, M. piperita, M. longifolia, M. spicata*), found that *Alternaria alternata* was one of the most sensitive strains to the oils [22]. In the control of *Macrophomina phaseolina*, the peppermint essential oil obtained growth inhibition percentage between 37.38 and 60.04% [16], which are close to the present result, while on control of *Colletotrichum gloeosporioides* the use of this oil reached the best inhibition effect (100% of inhibition in the concentration of 0.4%) [14].

Although the best results had occurred with the concentration of 0.8%, a dose-dependent behaviour was observed, which suggests that further increases in concentration may enhance the inhibition effect of the oil. In a study using the peppermint oil in higher concentrations

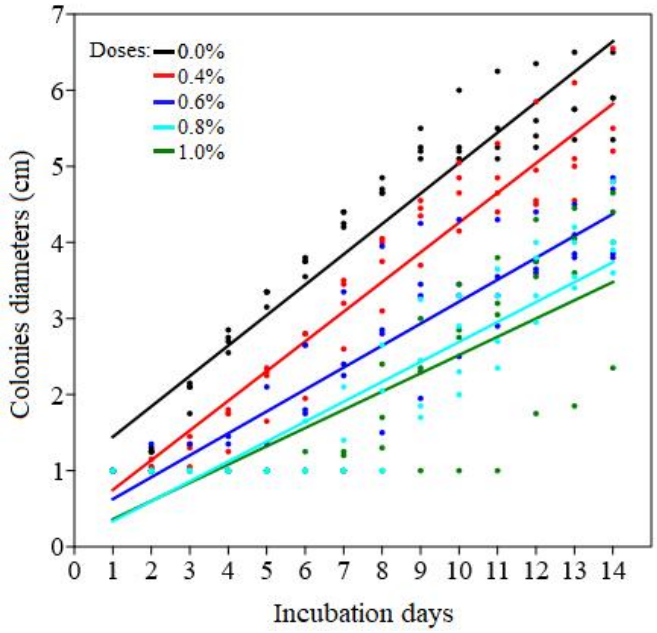

Fig. 1. Effect of different doses of peppermint essential oil on the growth of *Alternaria alternata* fungal colonies over time

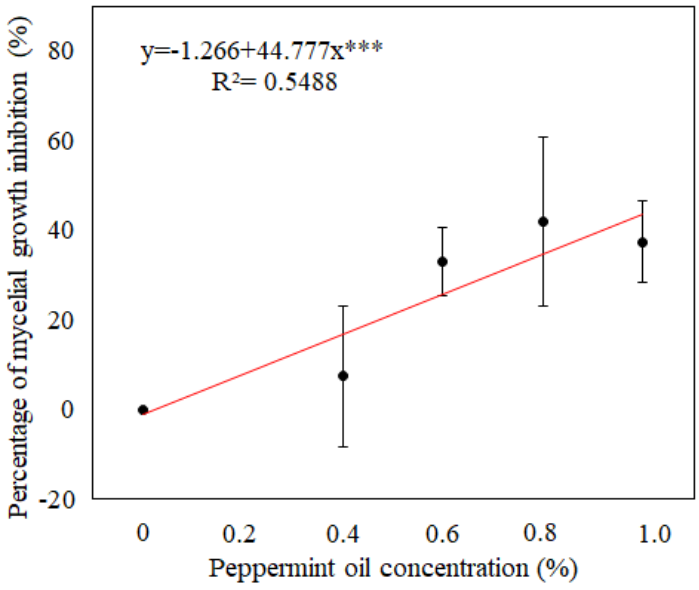

Fig. 2. Effect of peppermint oil concentration on the percentage of mycelial growth inhibition of *Alternaria alternata* on cultural media. The red line shows the direction of effect estimated by linear regression

(1.0, 2.0, and 4.0%) on the control of *Fusarium solani*, *Rhizoctonia solani*, *Sclerotium rolfsii*, and *M. phaseolina*, a dose-dependent inhibitions was observed, that is, with the higher concentration of the oil, the inhibition effect gets stronger [17]. In another study using peppermint oil reported reduced growth of *Fusarium sp*. only at low concentrations of the oil, while the high

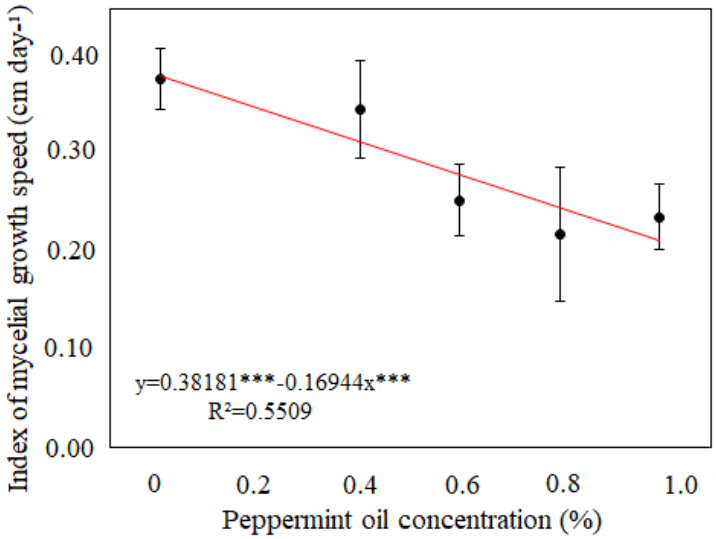

Fig. 3. Effect of peppermint oil concentration on the index of mycelial growth speed (IMGS) of *Alternaria alternata* on cultural media. The red line shows the direction of effect estimated by linear regression

concentrations did not affect the mycelial growth [11]. Thus, the antimicrobial effect of peppermint oil as well, as its dose-dependent effect, will vary among species of pathogen evaluated [11,17,25].

In the present study, although the maximum inhibition has been 72.45%, the average percentages were 41.67 and 37.18% at the highest concentrations, showing an intermediate power of inhibition, for not having reached 50% of inhibition. Superior inhibitions were found with the use of other vegetable oils. The essential oil of *Lippia gracilis* reached the inhibition of 100% on the control of *Alternaria* sp, with the concentration of 750 µl L^{-1} [7]. With the essential oil of mandarin orange (*Citrus reticulate*) against *Alternaria alternata* was observed inhibition of 84% at a concentration of 0.1 mL.100 mL^{-1}, and 100% with the dose 0.2 mL.100 mL^{-1} [26]. The essential oil of Peruvian pepper (*Schinus molle*) has shown a fungicidal effect against *Alternaria* spp. and other pathogenic fungi [27]. The total inhibition of *Alternaria alternata* was obtained using the essential oil of the *Piper hispidinervum* pepper at a concentration of 1000 mg L^{-1} [28].

The results obtained in this study show the existence of biologically active compounds in the essential oil of peppermint, which guaranteed *in vitro* antifungal activity against *A. alternata*. The use of the essential oil of peppermint in the control of *A. alternata* presented several advantages over the agrochemical traditional use, such as the low toxicity, fast degradation in the environment [29], low cost of production, and lack of health risks to the producer and final consumer.

In this perspective, the results of this study can be applied to the formulation of natural plant products that may be implemented in agroecological crops aiming to replace or reduce the application of conventional fungicides. The study suggests the development of further studies addressing the *in vivo* control of *A. alternata* to assess the activity of the oil on different plant species of commercial value and to determine the secure inhibitory concentrations of the product.

5. CONCLUSION

Despite having moderate fungitoxic activity, the essential oil of peppermint can be used as a viable and sustainable alternative for the control of *A. alternata*. Due to the possible dose-dependent effect, the study recommends the development of future studies with concentrations around 2.25% to test if the oil reaches the total inhibition.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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