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Original Research Article

In vitro effect of essential oil of peppermint (*Mentha x piperita* L.) on the mycelial growth of *Alternaria alternata*

ABSTRACT

Aims: Aiming to provide a healthy and environmentally safe product to fight these diseases, this study evaluates the in vitro antifungal effect of the essential oil of peppermint (*Mentha x piperita* L.) in the control of *Alternaria alternata*.
Study desing: The experiment was conducted in a completely randomized 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 PDA culture medium (Potato-Dextrose-Agar) autoclaved and subsequently poured into Petri plates. The treatments comprised five concentrations of the oil (0.0, 0.2, 0.6, 0.8, and 1.0%). After the inoculation with fungi, the plates were incubated for 14 days in a B.O.D incubator the temperature of 27±2 °C. With the data of mycelial diameters, we calculated the percentage of mycelial growth inhibition (PGI) and index of mycelial growth speed (IMGS).
Results: All concentrations of peppermint oil reduced the mycelial growth of *A. alternata*. The percentage of inhibition varied between 7.31 and 41.67%, showing a moderate power of inhibition. The concentration of 0,8% reached the highest inhibition effect.
Conclusion: We conclude that the peppermint oil can be used as a viable and sustainable antifungal product in the control of this pathogen. The in vivo experiments are still needed to verify the oil feasibility on the control of *A. alternata* on plants and fruits.

Keywords Antifungal; Disease control; Fungitoxic effect; Postharvest diseases; Phytopathogens;

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 [2], grapes [6], and papaya [7].

UNDER PEER REVIEW

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 necessary the use of ever stronger pesticides, 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 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.) have 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. MATERIAL 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 randomized design with five treatments and four replicates each.

We used the 0878 strain of *A. alternata* provided by the collection of pathogenic fungi Prof. Maria Menezes of the Federal Rural University of Pernambuco. The essential oil of peppermint was purchased in local trade.

The oil was added to autoclaved PDA culture medium (Potato Dextrose Agar), on the following concentrations (treatments): 0.0%; 0.2%; 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 center 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, we calculated the percentage of mycelial growth inhibition (PGI; [18]) and the index of mycelial growth speed (IMGS; [19]), 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

UNDER PEER REVIEW

The data were submitted to analysis of variance (ANOVA) followed by Tukey test to check significant differences among treatments using statistical software Past 3.12 [20]. Differences were considered significant at 95% level of confidence.

3. RESULTS

All concentrations of peppermint oil reduced the mycelial growth of *A. alternata* (Figure 1). However, the percentage of mycelial inhibition differ significantly among treatments (g.l. = 3; F = 3.824; p = 0.039) (Figure 2). The strongest inhibitions were obtained at the concentrations of 0.6, 0.8, and 1.0% with an average inhibition of 32.8, 41.6, and 37.1%, respectively. The concentration of 0.4% showed the lowest percentage of inhibition of growth (6.1%) but diffed significantly only from the 0.8% dose.

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

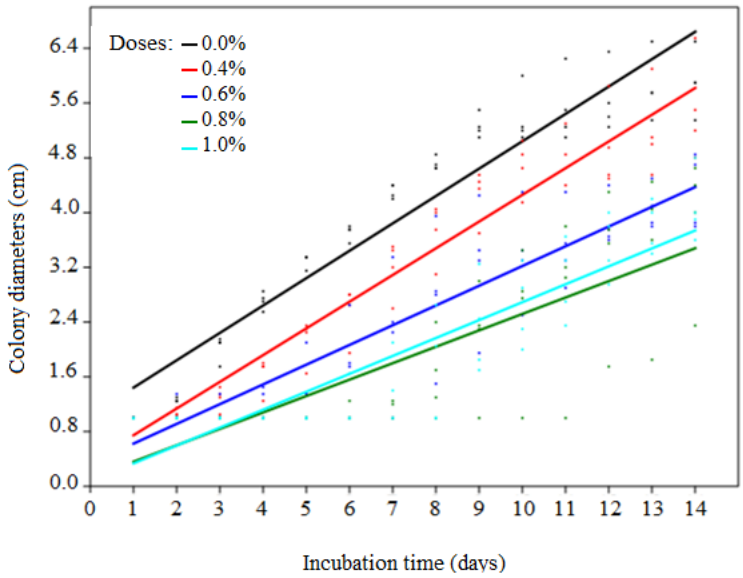
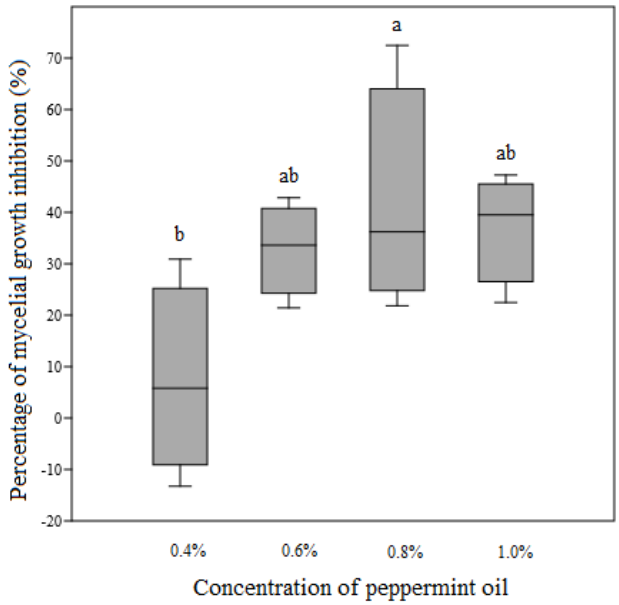


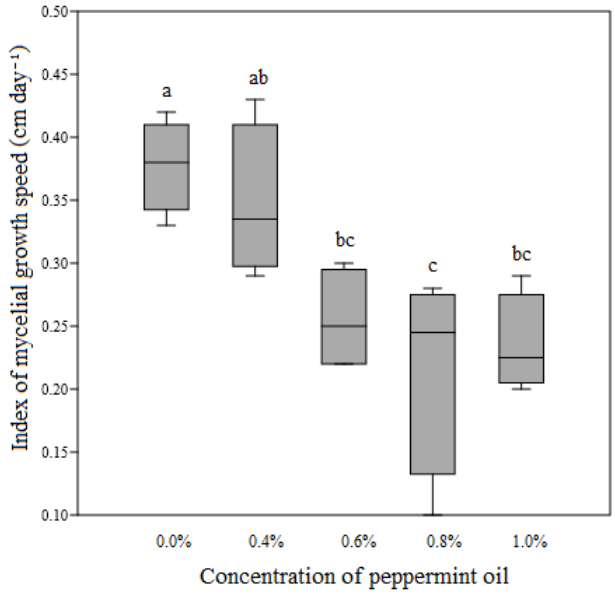
Figure 2. Percentage of mycelial growth inhibition (*PGI*) of *Alternaria alternata* on cultural media with different concentrations of peppermint essential oil. Boxes of concentrations with the same letter did not differ significantly between themselves according to the Tukey test at 95% of confidence.

UNDER PEER REVIEW



The rate of mycelial growth differed significantly between treatments (g.l. = 4; F = 6.922; p = 0.002). The treatments containing 0.6, 0.8, and 1.0% of peppermint oil reduced more effectively the growth speed *A. alternata*, with averages of 0.25, 0.22, and 0.24 cm day-1, respectively, differing from the negative control treatment that showed the highest growth rate (0.38 cm day-1; Figure 3). The concentration of 0.4% did not differ significantly from the control treatment.

Figure 3. Index of mycelial growth speed (*IMGS*) of *Alternaria alternata* in media containing different concentrations of the peppermint oil and in the negative control treatment. The boxes of concentrations with the same letter did not differ significantly between themselves according to the Tukey test at 95% of confidence.



UNDER PEER REVIEW

4. DISCUSSION

The peppermint oil inhibits the growth of the fungus *A. alternata* under *in vitro* condition, with the best results obtained with 0.8% concentration. The presence of terpenes in the composition of peppermint oil gives its antimicrobial activities. The menthone, menthol, and menthyl acetate comprise the main constituents of this oil [21,22]), they turn the fungal cell membrane permeable, causing the loss of cytoplasmic content [23].

Our results corroborate the findings of several studies. A previously 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 [21]. In the control of *Macrophomina phaseolina*, the peppermint essential oil obtained percentage growth inhibition between 37.38 and 60.04% [16], which are close to our results, 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].
In the present study, the growth of *A. alternata* was significantly affected between the doses 0.6 and 0.8 %, where as at the highest dose (1.0%) the inhibition was slightly reduced and rate of growth increased, showing no dose-dependent effect.

Our results are similar to those obtained by previous studies whose relate that the growth of *Fusarium* sp. was reduced only at low concentrations of the oil, while the high concentrations pose no effect on the mycelial growth [9]. A hypothesis for this behavior is that some innocuous components of the oil become nutritional resources for the fungus at high concentrations, supporting its development even in the presence of fungitoxic components.

Because the strength of oil antimicrobial effect varies among the species of studied microorganisms [24], the dose-dependent increase in the inhibitory power also vary among the species. In a study using the peppermint oil in concentrations higher than ours (1.0, 2.0, and 4.0%) on the control of *Fusarium solani*, *Rhizoctonia solani*, *Sclerotium rolfsii*, and *M. phaseolina*, was observed dose-dependent inhibitions, that is, the higher the concentration of oil the stronger the inhibition effect. Thus, the inhibitory effect of peppermint oil depends on the species of pathogen evaluated [17].

Several factors determine the chemical composition of essential oils, which directly influences its biological activity, for example, intraspecific genetic diversity [25], ecological context, and climatic conditions (climate, temperature, and soil type) [26]. Crop management techniques, such as the period of harvest and preparation of the material before the extraction, also affect the oil chemical composition [27].

In the present study, the percentage of inhibition of mycelial growth ranged from 7.31 to 41.67%, 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.100mL^{-1}, and 100% with the dose 0.2 mL.100 mL^{-1}. The essential oil of Peruvian pepper (*Schinus molle*) has showed a fungicidal effect against *Alternaria* spp. and other pathogenic fungi [29]. The full inhibition of *Alternaria alternata* was obtained using the essential oil of the *Piper hispidinervum* pepper at a concentration of 1000 mg L^{-1} [30].

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* present several advantages over the agrochemical traditionally used, such as the low toxicity, fast degradation in the environment [31], 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 reducing the application of conventional fungicides. We suggest 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.

UNDER PEER REVIEW

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*. Therefore, we recommend the use of oil at a concentration of 0.8%.

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UNDER PEER REVIEW

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UNDER PEER REVIEW

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