

## Antimicrobial activity of essential oils on pathogenic fungi

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### Abstract

Since the induction of chemical defensives in agriculture, human beings have suffered from our own actions, caused by the indiscriminate and abusive use of those substances; therefore, researches have been motivated to look for alternative ways, aiming to use plant inputs to control pathogenic agents in agriculture. As the essential oils from the species *Eucalyptus globulus* (eucalyptus), *Callistemon viminalis* (weeping bottlebrush), *Cymbopogon winterianus* (citronella grass) and *Tetradenia riparia* (misty plume bush) have proved effectiveness and an immense applicability, this research studied the use of those essential oils, aiming the effectiveness against plant pathogens. The plants were collected from Universidade do Vale do Sapucaí, Pouso Alegre (MG). The fungi's samples belong to the mycology collection from the institution and the tests were based on the mycelial development comparison of the control fungi on the dishes with essential oils. Notice that the inhibition caused by the oils over fungi's mycelial developing and the analysis of the data have been made through Turkey's statistic. From the data analyzed, it was possible to realize that the citronella's grass essential oil was efficient to control the mycelial development of fungi analyzed, followed by, in order of efficiency, the eucalyptus oil, the weeping bottlebrush oil and the misty plume bush oil.

**Keywords:** Citronella grass. Essential oil. Fungal plant pathogens.

### Introduction

In 2008 Brazil assumed the first place at the defensive world market (AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA - ANVISA, 2015). Since the induction of chemical defensives in agriculture, population suffers direct and indirect actions by abusive and indiscriminate use of these synthetic substances due to the fact that many of those have systemic elements and cumulative effects in organisms and go through all the food chains, contaminating soil and water sources (FERRARI, 1985; FLORES et al., 2004). Therefore, researches are induced to search for alternative ways such as plants inputs to control pathogens agents in agriculture.

The main pathogens in agriculture are fungi, responsible for 70 % of the illness that compromise the productivity of most crops (POZZA, et al., 1999). The fungus success is caused by their reproduction, which is usually based formation of spores, and can be transported by water, wind and plants, being resistant to temperature oscillation and capable of staying inert in the soil for many years (KIMATI et al., 1997).

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As the demands for food to fulfill the necessity of a rising population and the attempt to reduce the impact on the environment intensify, new technics have been discussed and developed to re-establish the food quality we produce. Innovating studies, which use inputs from microorganisms (SILVA; COELHO, 2006) and plant essential oils (MEDICE et al., 2007), have been standing out in science as possible substitutes for the defensives that are harmful to the environment.

Plant extracts and essential oil utilization have been known for a long time, since these compounds have medicinal and aromatic properties (NÓBREGA et al., 2003). The essential oils are found in different parts of a plant and they can be extracted from leaves, flowers, seeds, twigs or roots (KOKETZU; GONZALES, 1991). Studies showing essential oil activities over fungitoxic action (SILVA; BASTOS, 2007), antibacterial activities (PEREIRA et al., 2004), antioxidant (MORAIS et al., 2006), insecticide (MARANGONI et al., 2013) and herbicide (BARBOSA et al., 2008) prove plant essential oils efficiency.

Due to the evidences collected from tests with essential oil, the challenges to turn these technologies viable are many; however, it is necessary to intensify research and to turn the technology cheap and profitable. Since the essential oils from the species *Eucalyptus globulus*, *Callistemon viminalis*, *Cymbopogon winterianus* and *Tetradenia riparia* have proved effective against antimicrobial activity (PEREIRA, 2009; PIATTI et al., 2011), the present research aimed to study their application to control phytopathogens with tests on fungi for future use in agriculture.

## Material and methods

The present study was developed at the Microbiology Lab at the Universidade do Vale do Sapucaí, located in Pouso Alegre city, Minas Gerais, Brazil, from February to November of 2015. The plants utilized in the present study were the eucalyptus (*Eucalyptus globulus*), the weeping bottlebrush (*Callistemon viminalis*), the misty plume bush (*Tetradenia riparia*) and the citronella grass (*Cymbopogon winterianus*). The species studied are found on the institution campus.

### Extraction of the essential oils

The process used to obtain the essential oils from the studied plants was by hydrodistillation in a Clevenger-type apparatus, method developed by Koketsu and Gonçalves (1991). The part from the plants used for extractions of essential oils were the leaves; at first, they were left to dry at room temperature for two days aiming to eliminate the excess of water; after that, 1 kilogram of each plant was submitted to hydrodistillation for one hour. Then, the essential oils were collected with a Micro-pipette, stored in amber flasks and kept at 5 °C temperature to be used afterward.

### Preparation of culture medium

To reactivate the fungus and to do the antibiosis test in vitro, Potato Dextrose Agar (PDA) medium, made with 200 g of potato extract, 20 g of Dextrose and 15 g of Agar for each 1000 ml of water, was employed. Powdered PDA was diluted in distilled water at the concentration demanded at the product's labels; afterwards, it was autoclaved for 20 minutes at 121°C/1Atm temperature. Following, the medium was cooled at temperature around 45 °C, in a sterile environment, in the Laminar Flow Cabinet, and bedded in 9 cm Petri dishes.

## Antimicrobial test “in vitro”

The studied plant essential oils were diluted in dimethyl sulfoxide (DMSO) to obtain different concentrations of oils: 0,5 %, 1 %, 5 %, 10 %, 15 %, 25 %, 35 %, 45 %, 50 % and 100 %. Before it, the DMSO was tested to check if it could contribute to the inhibition of the fungus; as the results were negative, the experiment was carried out.

The fungi used in the experiment were previously cultivated at 28 °C for seven (7) days. After the growth, circular fungus fragments of 1 cm of diameter were transferred to the center of the Petri dish, with the PDA medium. Subsequently, four circular fragments of sterilized paper filters were added in accordance to Bauer et al. (1966) disk diffusion method. In every paper disk, 10 μL of each essential oil in concentrations from 0,5 % to 100 % were added, each treatment was placed at 3 cm distance from the fungus. The negative controls were made from Petri dishes with the fungus and sterilized paper filter. All the tests were carried out three times and the dishes were incubated at 28 °C temperature.

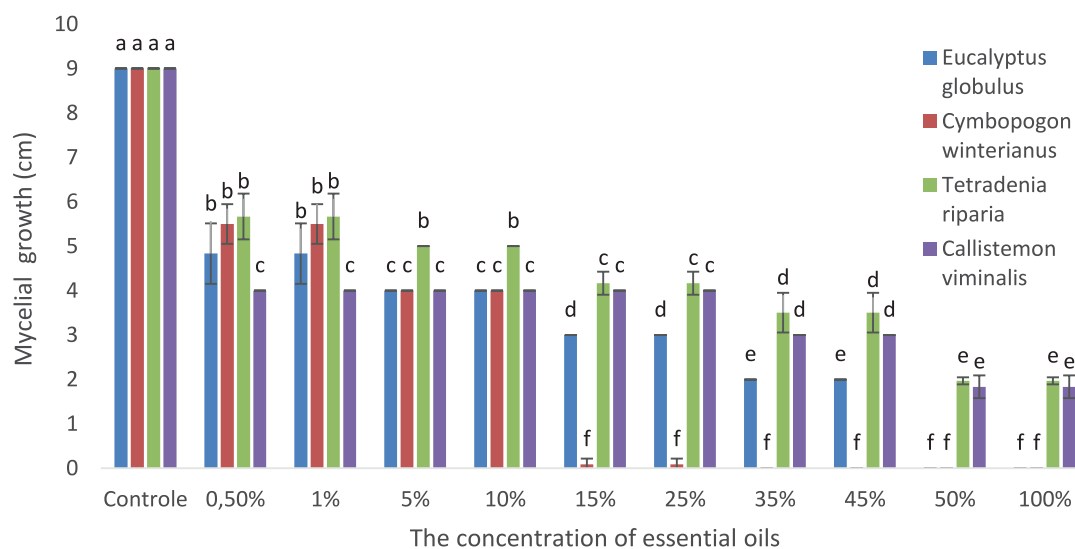
The determination for the mycelial growth of the plant pathogen fungus from the oil was made in six sets, through metrical evaluation of the mycelial growth size, with the help of a pachymeter; four oils to each fungus were compared by Tukey test at 5 %, using Assistat software (Statistical Assistance), version 7.7 beta.

## Results and discussion

### Essential oils activity towards *Pestalotiopsis sp.* fungus

The experiment carried out with the four essential oils on *Pestalotiopsis sp.* fungus showed this microorganism has the mycelial growth reduced when exposed to higher concentration of oils, as it can be observed in figure 1.

**Figure 1.** Comparative of Mycelial growth for *Pestalotiopsis sp.* towards the essential oil of *Eucalyptus globulus*, *Cymbopogon winterianus*, *Tetradenia riparia* and *Callistemon viminalis* with different concentration when compared with the fungus control, average with different letters are significantly different, according to Tukey test ( $P > 0,05$ )



Source: Elaborated by the authors (2015)

The essential oil of *Cymbopogon winterianus* showed greater inhibition for *Pestalotiopsis sp.* fungus, demonstrating a complete mycelial inhibition at 35 % concentration, followed by the *Eucalyptus globulus* oil, which had a complete mycelial inhibition at 50 % concentration.

Many authors have also proved the fungicide action for the essential oil from *Cymbopogon winterianus* against the fungus *Trichophyton rubrum*, *Trichophyton mentagrophytes* (PEREIRA, 2009) and towards the fungus *Alternaria sp.*, *Cladosporium sp.*, *Rhizopus spp.* (ARAUJO NETO; ARAÚJO, 2012) and *Fusarium solani* (CRUZ et al., 2015), which emphasizes that oil as a potential fungicide.

The *E. globulus* oil has also demonstrated a great inhibition for the *Penicillium* pathogenic fungus in the Vegetative state as well as in the reproductive stage and spore germinations with the concentration equal or higher than 0,25 %. According to Piatti et al. (2011), the eucalypt oil has demonstrated to be more efficient than the fungicide Azoxystrobin.

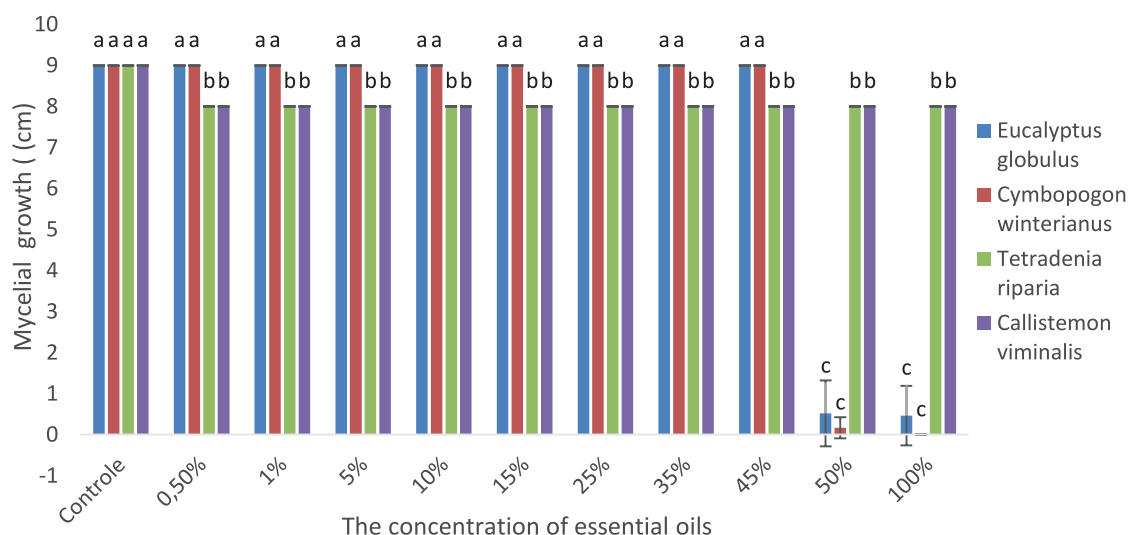
The chemical compounds from the eucalypt essential oil are many and very complex, for example, 1,8-cineol, citral, citronellal, geranylol ethyl, among many others that have antifungal action (NAGPAL et al., 2010). The results support the data for the antimicrobial potentiality of *Eucalyptus* oil, especially on fungus, microorganisms that can be better controlled by natural products.

The essential oil of eucalypt, according to Piatti et al. (2011), does not present toxic activity towards human being nor the environment, what makes that oil viable to be applied on food cultivation, avoiding pathogenic microorganism activity and use of chemical defensives harmful to the environment.

### Essential oils activity towards *Rhizopus spp.* fungus

The study with *Rhizopus spp.* fungus showed this microorganism has great resistance towards the essential oils, even when used at high concentration, as it can be observed in Figure 2.

**Figure 2.** Comparative of mycelial growth for *Rhizopus spp.* towards the essential oil of *Eucalyptus globulus*, *Cymbopogon winterianus*, *Tetradenia riparia* and *Callistemon viminalis* with different concentration when compared with the fungus control, average with different letters are significantly different, according to Tukey test ( $P > 0,05$ )



**Source:** Elaborated by the authors (2015)

All the concentrations of *Tetradenia riparia* and *Callistemon viminalis* essential oils have been incapable of reducing the mycelial growth of *Rhizopus spp.* On the other hand, the concentration of

50 and 100 % for *Cymbopogon winterianus* e *Eucalyptus globulus* oils have had a meaningful growth reduction.

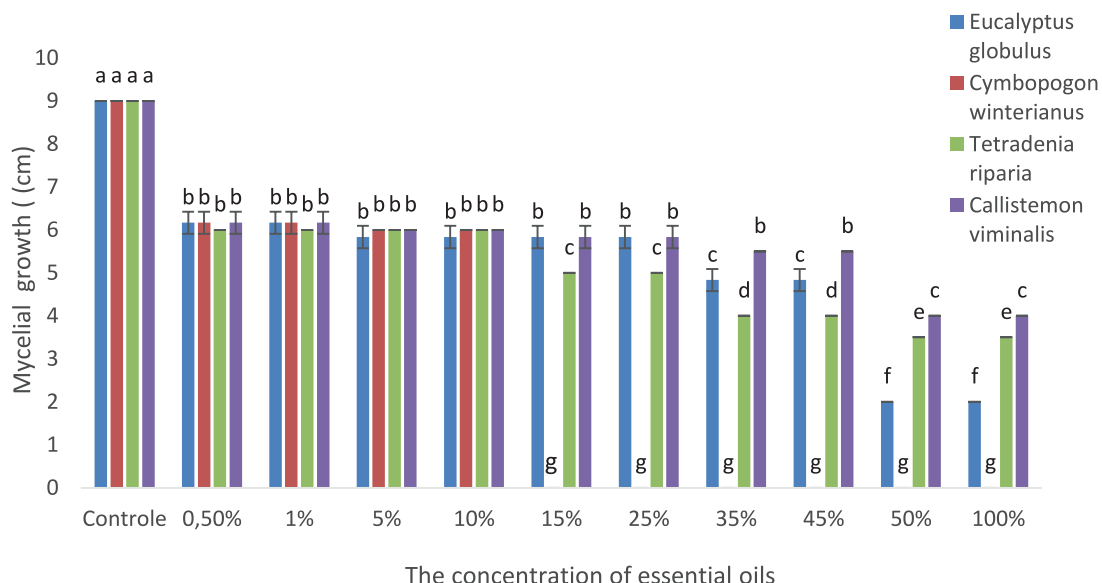
According to Andrade et al. (2012), citronellal and linalol substances contribute a lot to the antifungal activities from the *Cymbopogon winterianus* (citronella). Different specimens have different concentrations of oil, but it was found that an average of 47,12 % of citronella oil correspond to citronellal and linalool.

The essential oil from many plants have different chemical compounds according to the weather along the year, what affects the amount of compound available in the plants. Studies appoint results are not different for *T. Riparia*, since this plant has more chemical compounds during seasons with a higher frequency of precipitation, alternating its antimicrobial potential (GAZIM et al., 2010). That could explain the inefficient fungicide effects on *Rhizopus spp.*

### Essential oils activity towards *Monilinia sp.* fungus

*Monilinia sp.* fungus showed susceptibility towards high concentrations of the essential oils tested, as it can be observed in Figure 3.

**Figure 3.** Comparative of mycelial growth for *Monilinia sp.* towards the essential oil of *Eucalyptus globulus*, *Cymbopogon winterianus*, *Tetradenia riparia* and *Callistemon viminalis* with different concentration when compared with the fungus control, average with different letters are significantly different, according to Tukey test (P > 0,05)



**Source:** Elaborated by the authors (2015)

The essential oils from *Callistemon vimilanis*, *Tetradenia riparia* and *Eucalyptus globulus* have demonstrated greater fungistatic activity at 50 and 100 % concentrations.

According to Pieters and Vlietinck (2005), *T. riparia* has different compounds, including the diterpenes. During tests with this plant, Coopoosamy e Naidoo (2011) observed its efficiency to combat many human pathogens, such as the gram-positive bacteria, gram-negative bacteria and also tests with fungus (*Aspergillus flavus*, *Aspergillus glaucus*, *Candida albicans* and *Candida tropicalis*).

Plants from *Callistemon* genus are frequently used in traditional treatments because they have unique sources of many chemicals compounds with therapeutic activity. However, only a few studies about the biological activities and possible medicine uses of phytochemicals from *C. viminalis* have been conducted until today (GOHAR et al., 2013).

According to Oyedeji et al. (2009), leaves from *C. viminalis* have 20 chemical compounds, among them: 1,8cineol (83,2 %),  $\alpha$ pineno (6,4 %)  $\alpha$ terpineol (4,9 %). 1,8cineol (eucalyptol) has proven to have anti-inflammatory action (BASTOS, et al., 2010).

The essential oil from *Cymbopogon winterianus* has shown an efficient fungicide action, starting at 15 % concentration. In the study conducted by Souza et al. (2006), the *C. winterianus* also presented itself as an efficient fungicide at high doses to control the *Monilinia fructicola* and a great mycelial inhibition similar to Triazole fungicide and superior to Azoxystrobin and the *Eucalyptus* oil.

## Conclusion

From the information presented in this study, it has been concluded that the essential oil from *Cymbopogon winterianus* at 50 and 100 % concentration have had the most efficient fungistatic and fungicide activity on all the fungi tested. The essential oil of *Eucalyptus globulus*, *Tetradenia riparia* and *Callistemon viminalis* have shown only fungistatic activity on the fungi.

## Acknowledgment

To professor Rogério Lobo Sáber, to Laboratory technicians Renan Neves and Eliakim Lopes and to José Donizeti Reis.

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#### **Histórico editorial:**

Submetido em: 11/04/2016

Aceito em: 05/07/2016

Como citar:

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COUTO, D. A.; DIAS, F. S.; SÁBER, M. L. Antimicrobial activity of essential oils on pathogenic fungi. **Revista Agrogeoambiental**, Pouso Alegre, v. 9, n. 3, p. 37-46, jul./set.  
Doi: <http://dx.doi.org/10.18406/2316-1817v9n32017979>

#### APA

COUTO, D. A., DIAS, F. S. & SÁBER, M. L. (2017). Antimicrobial activity of essential oils on pathogenic fungi. *Revista Agrogeoambiental*, Pouso Alegre, 9 (3), 37-46.  
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COUTO, D. A.; DIAS, F. S. e SÁBER, M. L. Antimicrobial activity of essential oils on pathogenic fungi. *Revista Agrogeoambiental*, 2017, vol. 9, n. 3, pp. 37-46. Eissn 2316-1817.  
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Couto DA, Dias FS, Sáber ML. Antimicrobial activity of essential oils on pathogenic fungi. *Rev agrogeoambiental*. 2017 jul/set; 9(3): 37-46.  
Doi: <http://dx.doi.org/10.18406/2316-1817v9n32017979>