

Essential Oils in the Control of *Fusarium solani*

Rafaella Azevedo Aguiar¹, Sinara Patrícia Mendes da Costa², Isabela Oliveira Santos², Maria Josiane Martins²,
Marcilio Nunes Moreira², Isabelle Carolyne Cardoso Batista², Thais de Castro Moreira²,
Regina Cássia Ferreira Ribeiro², Adelica Aparecida Xavier², Viviane Aparecida Costa Campos³,
Lorena Gracielly de Almeida Souza³, Carlos Augusto Rodrigues Matrangolo², Renato Martins Alves²,
Natan Cantuária Nunes², Max Pereira Gonçalves¹, Patrícia Nirlane da Costa Souza¹, Silas Silva Santana¹,
Denílson Ferreira Oliveira³, Polyana Danyelle dos Santos Silva², Mary Ingrid Alves e Santos²,
Raquel Venceslau Santos², Sindy Natany Martins Barbosa², Mariany de Jesus Limas²,
Wander Guilherme da Silva Leles², Helena Souza Nascimento Santos², Nathalia Lélis Faria²,
Marcelo Ramos de Jesus², Taylor Johnny Patricio Silva², Irisléia Pereira Soares de Sousa²,
Sabrina Gonçalves Vieira de Castro², Geisla Garcia Leal²

¹ Federal University of the Jequitinhonha and Mucuri Valleys, Campus Janaúba, Minas Gerais, Brazil

² Montes Claros State University, Campus Janaúba, Minas Gerais, Brazil

³ Federal University of Lavras, Lavras, Minas Gerais, Brazil

Correspondence: Maria Josiane Martins, State University of Minas Gerais, Avenue Reinaldo Viana, 2630, Morada do Sol, Divinópolis, Minas Gerais, Brazil. E-mail: josianemartins102012@hotmail.com

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Abstract

Fusarium solani is a soil pathogen and causes disease in several economically important crops. The development of research with essential oils is important. These present a diversity of active substances, with fungicidal properties. This study aimed to evaluate the effect of essential oils from *Copaifera reticulata*, *Cymbopogon nardus*, *Origanum majorana*, *Allium sativum*, *Rosmarinus officinalis*, *Piper nigrum* and *Cymbopogon citratus* on *Fusarium solani*. The oils were extracted using the technique of steam dragging, in a Clevenger apparatus. In Petri dishes containing essential oils and PDA medium, a fungal mycelium disc was added. After seven days, the mycelial growth, germination and sporulation of the fungus were evaluated. The essential oils of *C. nardus*, *C. citratus* and *A. sativum* were more efficient in controlling *F. solani*.

Keywords: biological control, soil-dwelling pathogen, sustainability

1. Introduction

The passion fruit is a plant of tropical climate, of the Passifloraceae family. The largest and most important genus of this family is Passiflora to which passion fruit (*Passiflora* spp.) belongs (Ramaiya et al., 2020). The genus Passiflora has more than 500 species, most of them producing fruits for fresh consumption (Faleiro et al., 2019). No Brasil a produção chega a cerca de 602.651 toneladas (IBGE, 2019), sendo considerado o maior produtor mundial de maracujá (FAO, 2019).

However, the productive potential of the culture is much greater. In addition, this low production is usually associated with inadequate management of the orchard, low-quality seedlings or seeds, lack of adequate technologies, and mainly phytosanitary problems (Moreira et al., 2017). Among these phytosanitary problems are soil-dwelling fungi. *Fusarium solani* (*Nectria haematococca*), considered the main causal agent of collar rot in passion fruit (Preisigke et al., 2015).

F. solani produces cylindrical microconidia and spindle-shaped macroconidia, in addition to numerous resistance structures, namely chlamydospores, which are difficult to eradicate from infested areas (Gupta & Meenu, 2019). The infection starts at the taproot and progresses to the neck (Ambrósio et al., 2018) as the rot progresses, the bark lesion darkens, and then the tissue at the bark site crumbles (Buritcá et al., 2017).

Tissues below the shell rupture at various points lengthwise; meanwhile, the canker advances laterally, encircling the root and neck and, more deeply, destroys the vessels, which causes the reflex symptoms of wilting, yellowing, and drying of the foliage (Freitas et al., 2016; Preisigke et al., 2015).

Some soil-dwelling fungi management measures have been proposed (Cruz et al., 2015; Sameza et al., 2016). Due to difficulties related to its control and increasing pressure from society to use techniques that harm the environment less, many types of research have been carried out with alternative means of control (Sampaio et al., 2016; Araújo et al., 2017). Among these measures, the use of essential oils has been gaining ground in research within universities and showing promising results (Silva et al., 2019; Habbadi et al., 2018).

Essential oils are substances from the secondary metabolism of plants, and these are liquid, hydrophobic, highly concentrated volatiles (Sharma et al., 2017). Essential oils are known to have fungicidal (Avanço et al., 2017; Kumar et al., 2016), antimicrobial (Bagy and Abo-Elyousr, 2019; Najafgholi et al., 2017), nematocidal (Jardim et al., 2017), and insecticides (Polatoglu et al., 2017; Oliveira et al., 2018).

The use of essential oils as fungicides has numerous advantages: they may contain compounds that fungi cannot inactivate (Ghalem, 2016), they are less aggressive to the environment, they can be biodegraded, and have multiple modes of action, which expands the spectrum of action over time while acting selectively (Nazzaro et al., 2017). To understand the fungicidal activity of essential oils on *F. solani*, this study extracted essential oils from seven different plant species and evaluated their action on mycelial growth, sporulation, and germination of *F. solani*.

2. Method

The experiments were set up at the State University of Montes Claros (Unimontes) in the Plant Physiology and Phytopathology laboratories.

2.1 Obtaining Essential Oils

Seven different plant species were have used *Copaifera reticulata*, *Cymbopogon nardus*, *Origanum majorana*, *Allium sativum*, *Rosmarinus officinalis*, *Piper nigrum*, and *Cymbopogon citratus*. For the extraction of essential oils, we used the technique of dragging by water vapor, in a Clevenger apparatus. The fresh leaves of the seven plant species were have added to distilled water, and they were crushed in a blender.

Then transferred to round-bottomed balloons. The extraction process took about four hours. The extracted oil was transferred to glass containers with the aid of a Pasteur pipette. To remove excess moisture, anhydrous sodium sulfate (Na_2SO_4) was added to the glass vessels. The oils were stored in a freezer at -18°C .

2.2 Obtaining the Fungal Isolate

The MR-35 isolate of *F. solani* was obtained from the fungal collection of the Laboratory of Phytopathology of Unimontes. This was cultivated in Petri dishes with a diameter of nine centimeters containing 20 mL of PDA (Potato-Dextrose-Agar) medium, in the continuous dark at 25°C for seven days. After that, 5 mm disks were removed from the edge of the colony to be used in the antibiosis test.

2.3 Effect of Essential Oils on the in vitro Mycelial Growth of *F. solani*

We added 200 μL of each essential oil to nine-centimeter Petri dishes containing 20 mL of BDA at a temperature of 45°C . Next, 5 mm discs containing *F. solani* mycelium were placed in the center of the Petri dishes, which were sealed and conditioned in BOD in the continuous dark for seven days at 25°C . The treatment contained only BDA. After seven days, the mycelial growth of *F. solani* was evaluated with the aid of a millimeter ruler.

2.4 Effect of Essential Oils on *F. solani* Germination

To estimate the germination of *F. solani*, sterile distilled water plus tween 80 were added to the colonies with seven days of growth and, with the aid of a glass slide, the conidia were disaggregated. The suspension was filtered through sterile gauze. A 100 μL aliquot of this suspension was added to the center of a seven-centimeter-diameter Petri dish with agar medium, which were incubated at room temperature for 12 hours. After this period, 100 germinated and non-germinated conidia were quantified, and the germinated ones were those with a germ tube greater than or equal to the length of the conidia.

2.5 Effect of Essential Oils on Sporulation of *F. solani*

To evaluate the sporulation of *F. solani*, the suspension described above was used. About 500 μL of this suspension was taken to a Neubauer chamber under an optical microscope for spore quantification. The trial was set up in a completely randomized design, with eight treatments (control and seven oils) and four replications. The variables were submitted to analysis of variance and the means were compared by the Scott Knott test at 5% significance. The percentage of reduction of variables was calculated using the methodology proposed by Mourão et al. (2003). Statistical analyzes were performed using R version 5.3 software.

3. Results and Discussion

In the Results There was a significant effect of different essential oils on mycelial growth, germination and sporulation of *F. solani* ($p < 0.05$). By means of the Scott-Knott test, it was possible to verify that the essential oils of *C. nardus*, *A. sativum*, *C. citratus*, *O. majorana*, *C. reticulata* and *P. nigrum*, were statistically different from the control ($p < 0.05$) for the response variable colony diameter (Table 1). As for the variables twinning conidia and sporulation, the best results were found in *C. nardus*, *A. sativum*, *C. citratus* and *O. majorana* oils (Table 1).

Table 1. Diameter, germinated conidia and sporulation of *Fusarium solani* as a function of different essential oils in PDA culture medium

Treatment	Colony Diameter (cm)	Germinated Spores	Sporulation ($\times 10^5$)
<i>Cymbopogon nardus</i>	0a	0a	0a
<i>Allium sativum</i>	0a	0a	0a
<i>Cymbopogon citratus</i>	0a	0a	0a
<i>Origanum majorana</i>	0.90a	10.33a	11.67a
<i>Copaifera reticulata</i>	6.30b	62.67b	87.33b
<i>Piper nigrum</i>	6.60b	81.67c	73.67b
<i>Rosmarinus officinalis</i>	7.43c	78.33c	75.67b
Control	8.26c	100d	124.67c
Coefficient of variation (%)	16.43	22.77	25.19

Note. Means followed by the same letter in the column do not differ from each other by the Scott Knott test at 5% probability.

The diameter of the *F. solani* colony reduced by up to 100% when it was in the presence of the essential oils of *C. nardus*, *A. sativum*, *C. citratus* (Figures 1 and 2). The essential oil of *O. majorana* reduced the colony diameter by 89.1%. The smallest reductions were verified by the oils *C. reticulata*, *P. nigrum*, *R. officinalis* (Figure 1). The effectiveness of essential oils against the diameter of *F. solani* was on the order of that of *C. nardus* > *A. sativum* > *C. citratus* > *O. Majorana* > *C. reticulata* > *P. nigrum* > *R. officinalis* (Figure 2).

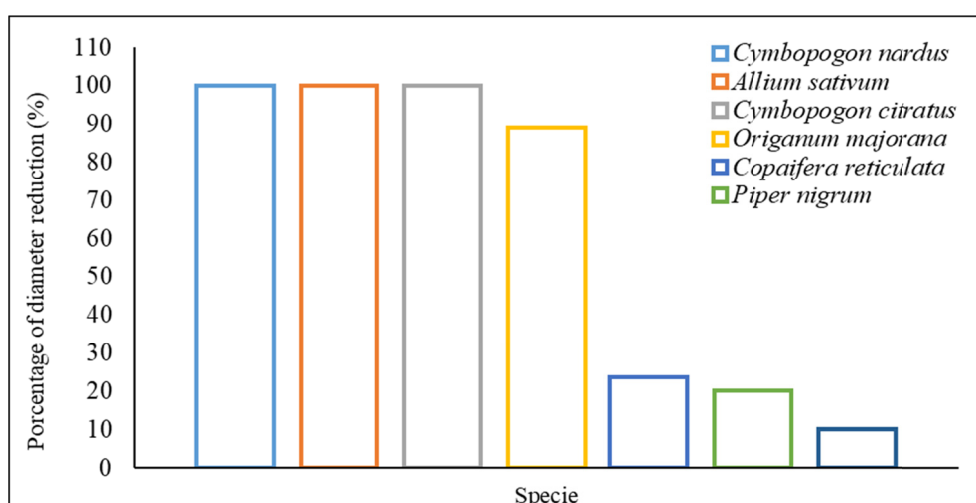


Figure 1. Percentage of reduction in colony diameter of *F. solani* as a function of different essential oils

The fat-soluble nature of essential oils enables interaction with cellular structures that have a lipid constitution, resulting in increased membrane permeability and consequent electrolyte imbalance and cell death (Steffen et al., 2019). Damage to the cytoplasmic membrane of lipoproteins causes cytoplasmic leakage, as well as the death of hyphae (Santos et al., 2013), thus reducing the mycelial growth of the fungal colony (Figure 2).

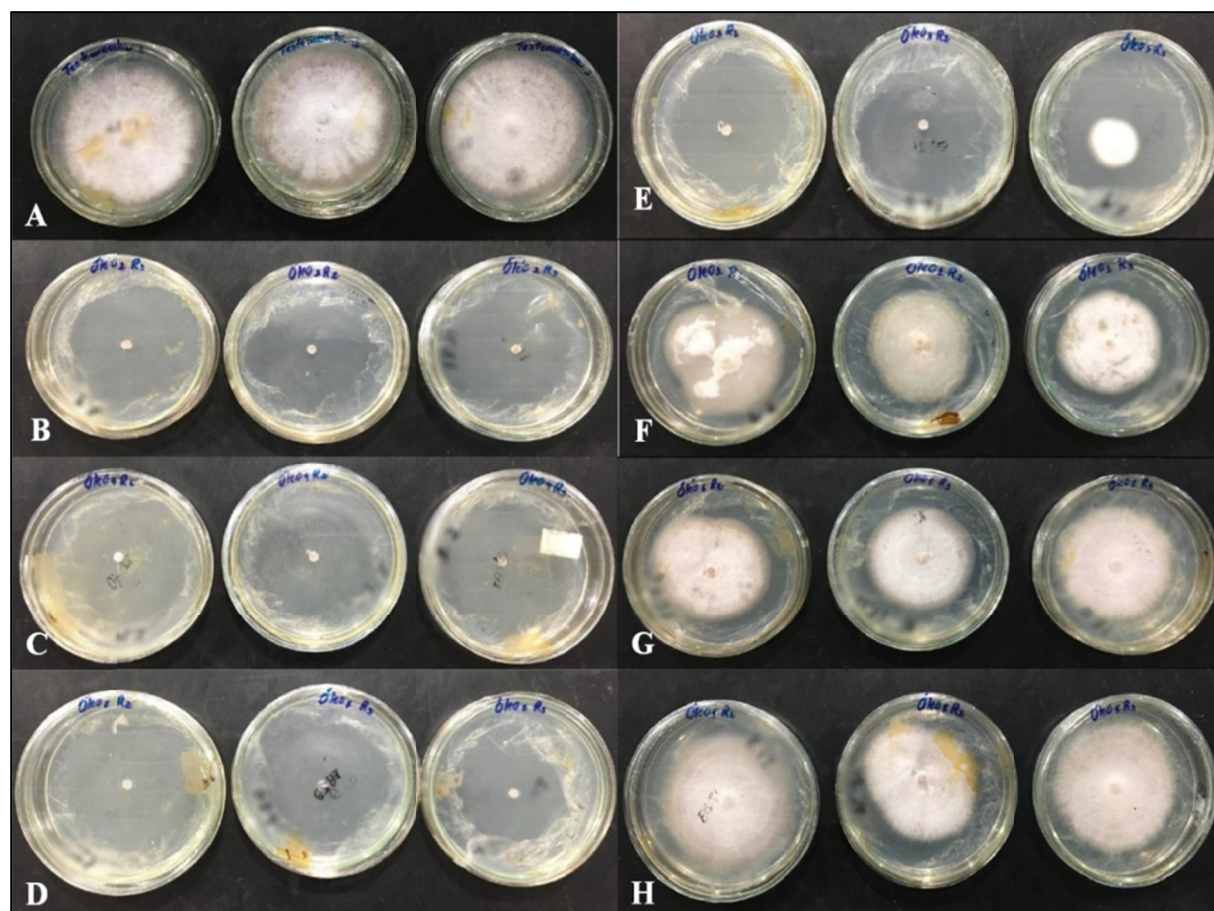


Figure 2. Diameter of the colony of *F. solani*. Control (A), *C. nardus* (B), *A. sativum* (C), *C. citratus* (D), *O. majorana* (E), *C. reticulata* (F), *P. nigrum* (G), *R. officinalis* (H)

The reductions in conidia germination reached up to 100% for the essential oils *C. nardus*, *A. sativum* and *C. citratus* (Figure 3). The smallest reduction was observed with *P. nigrum* and *R. officinalis* oils. Several studies have proven the antifungal effects of essential oils from the Cymbopogon genus (Mohajeri et al., 2018). The chemical constituents of essential oils have a variety of targets, including the membrane and cytoplasm, and in certain situations, they completely change the morphology of cells (Salas et al., 2016). Products derived from secondary metabolism plants are potentially useful for the development of new products capable of circumventing the problems caused by *F. solani* (Araújo et al., 2017).

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Authors Contributions

RAA, SPAMC, IOS, MJM, MNM, TJPS, IPSS, SGVC and GGL were responsible for study design and revising. ICCB, TCM, RCFR, AAX, VACC, LGAS, DFO, PDSS, MIAS, RVS and SNMB was responsible for data collection. CARM, RMA, NCN, MPG, PNCS, SSS, MJL, WGSL, HSNS, NLF and MRJ drafted the manuscript revised it. All authors read and approved the final manuscript.

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Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Informed Consent

Obtained.

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Data Sharing Statement

No additional data are available.

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