Antifungal action of essential oils on *Colletotrichum musae in vitro*

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Abstract

Anthracnose is a phytosanitary problem affecting banana producers. It is a major postharvest disease and is mainly controlled by the use of fungicides. As the search for healthy foods free of chemical residues has increased among consumers, the objective of this study was to evaluate the *in vitro* antifungal efficacy of different concentrations of essential oils on mycelial growth of *Colletotrichum musae*, the causal agent of anthracnose in banana fruits. The essential oils used were citronella grass (*Cymbopogon nardus*), lemongrass (*Cymbopogon citrates*), clove (*Syzygium aromaticum*), and basil (*Ocimum basilicum*). The essential oils were added to a potato dextrose agar medium at concentrations of 0, 0.5, 1.0, 1.5, 2.0,and 2.5 μLmL^{-1} and distributed in 90 mm diameter Petri dishes. Mycelium discs from the fungal colony, grown for seven days, were transferred to Petri dishes containing the oils and incubated at 25°C with a photoperiod of 12 h (light/dark) cycle for eight days. Colony growth was monitored daily to determine the percentage, index of inhibition of mycelial growth, and average diameter of the colonies. The experimental design consisted of randomized blocks with six different extract concentrations with replicates (n = 4). Each oil extract was then evaluated separately. The essential oils from citronella grass , lemongrass, and basil inhibited mycelial growth to the proportion that the oil concentration increased. The total inhibition of the pathogen occurred at a concentration of 1.5 μLmL^{-1} using citronella grass and lemongrass and 2.5 μLmL^{-1} using basil. However, essential oils from clove completely inhibited the mycelial growth of *C. musae* at a concentration of 0.5 μLmL^{-1}. The tested essential oils showed *in vitro* antifungal activity against *C. musae* at different concentrations. However, essential oils from clove are unique in exhibiting a better inhibitory effect on mycelial growth at lower concentrations.

Keywords: Banana; Postharvest; Alternative control; Anthracnose; Mycelial growth.

Introduction

Bananas are one of the most popular tropical fruits worldwide. The commercialization of the fruit and growth of banana trees (*Musa spp*.) are responsible for economic development and employment and income generation. In the 2021 harvest, Brazil produced 7,018,879 tons of bananas [Instituto Brasileiro de Geografia e Estatistica (IBGE), 2021]. However, a large part of this production supplies the domestic market, characterizing the country as a small exporter. Lack of export is attributed to the increased number of pests, diseases, precarious commercial structure, and preference of the Brazilian domestic market for common bananas and demand of the international market for the Cavendish variety (RANGEL *et al*., 2002; AMORIM *et al*., 2011).

Several diseases can affect banana trees. One of them is anthracnose, caused by the fungus *Colletotrichum musae* (Berk. & Curt.), which is spread throughout the banana-producing regions and is considered a problem for postharvest fruits. This disease in bananas limits the internal market as well as exports, since the fruits look undesirable for consumption, with spots varying from dark brown to black reducing their shelf life and causing rot in a short time (CORDEIRO; MATOS; MEISSNER FILHO, 2004).

Several techniques are used to control the disease, from cultural practices to chemical control and the adoption of adequate packaging and transport systems (BASTOS; ALBUQUERQUE, 2004; BARBOSA; VIEIRA; TEIXEIRA, 2015). Although effective, incorrectly applied fungicides, mainly in postharvest fruits, can leave residues and lose effectiveness against populations resistant to pathogens (NEGREIROS *et al*., 2013). In addition, the consumer market is increasingly demanding sustainable agriculture to avoid the indiscriminate use of chemical products. Therefore, integrated and alternative management measures are safe and ecologically appropriate options (BONETT *et al*., 2013). In this context, alternative control of plant diseases with essential oils derived from plants exhibiting antifungal potential is an important strategy for the phytosanitary management of conventional, agroecological, and organic crops.

Essential oils are volatile, oily liquids obtained from plants (MORAIS *et al*., 2006). They are composed of many secondary metabolites, such as terpenes and steroids, that can be used to control phytopathogens (SILVA *et al*., 2016). They present either an elicitor fungitoxic action, acting on plant defense mechanisms, or a direct fungitoxic action, inhibiting mycelial growth and conidia germination (STANGARLIN *et al*., 1999, cited by CRUZ *et al*., 2013). In addition, the use of essential oils in the food and pharmaceutical industries to control pathogenic microorganisms, which is less harmful to human health and the environment, has been studied (SANTOS *et al*., 2022; SANTOS *et al*., 2021).

The effectiveness of such oils in managing plant diseases has been demonstrated in several studies, raising hopes for their incorporation into agricultural systems. Souza, Pinto, and Carvalho (2016) reported that the essential oils of chamomile (*Chamomilla recutita*), mint (*Mentha sp*.), and neem (*Azadirachta indica*) could inhibit the *in vitro* development of *Colletotrichum gloeosporioides*, the causal agent of anthracnose, in several plant cultures. Oliveira Júnior *et al*. (2013) found that the essential oil extracted from the fruits of *Schinus terebinthifolius* (aroeira) exhibited fungitoxic activity against the same phytopathogen *in vitro*. Negreiros *et al*. (2013) tested neem and garlic oil at 10 μLmL^{-1} and verified the reduction in anthracnose intensity in banana fruits, cv. 'Silver'. Along with these authors, Nobre *et al*. (2021) demonstrated that neem and clove oils effectively control *C. gloeosporioides in vitro*, with neem oil showing a more pronounced effect.

Therefore, the current *in vitro* study aimed to evaluate the efficacy of different concentrations of essential oils extracted from various plants, including citronella grass (*Cymbopogon nardus*), lemongrass (*Cymbopogon citratus*), clove (*Syzygiumaromaticum*), and basil (*Ocimum basilicum*), on mycelial growth of *C. musae*, the causal agent of anthracnose in banana fruits.

Material e métodos

This study was conducted at the Phytopathology Laboratory of the Federal Institute of Education, Science, and Technology of the South of Minas Gerais – *Campus* Inconfidentes -Brazil.

The *C. musae* isolate used in the experiments was obtained and provided by the Phytopathology Laboratory of the Federal Institute of Education, Science, and Technology of the South of Minas Gerais – *Campus* Machado - Brazil.

The essential oil used in the experiments were purchased from Ferquima Indústria e Comércio Ltd – São Paulo - Brazil. Citronella (*Cymbopogon winterianus*) is made up of citronellal, citronellol, and geraniol. Lemongrass (*Citrus flexuosus*) is composed of 72% citral (percentage = 43% andneral = 29%); basil (*O. basilicum*) comprises 85% methyl chavicol (estragole), 3% eucalyptol, 2.7% bergamotene, 2% transocement, 0.8%

linalol, and 0.6% eugenol; and clove flower buds (*Eugenia caryophyllus*) contain 84% eugenol, 6% beta-caryophyllene, and 8% eugenol acetate.

The essential oils were filtered using a 0.22 mm Millipore membrane and embedded in the Potato-dextrose-agar (PDA) melting culture medium (pH 5.7) at concentrations of 0, 0.5, 1.0, 1.5, 2.0, and 2.5 μLmL^{-1} (values in percent proportions were 0, 0.0025, 0.0050, 0.0075, 0.0100, and 0.015%). Culture media with different extract concentrations were poured into 90 mm Petri dishes at a volume of 20 mL per dish. After seven days of cultivation and solidification, 10 mm wide discs were removed from the edges of the *C. musae* isolate colony and inserted in the center of each treatment dish. The dishes were sealed, labeled , incubated in a BOD-type chamber at 25°C, and exposed to 12h light/dark photoperiod cycles.

The colonies were assessed every 24 h, and their diameters were measured in two opposite directions using a digital pachymeter for eight days,till the mycelial growth of the control treatment (0 μLmL^{-1}) covered the entire surface of the culture medium. Based on the evaluation results, the colony diameter (CD), percentage of mycelial growth inhibition (MGI), and mycelial growth rate (MGSR) were determined.

The MGI was estimated using the formula:

$$MGI = \frac{CAD - TAD \times 100}{CAD}$$

The MGSR was obtained using the formula:

$$MGSR = \frac{\sum D - Da}{N}$$

Where:
CAD = control average diameter
TAD = treatment average diameter
D = current colony mean diameter (mm);
Da = previous Day colony mean diameter (mm);
N = numbers of days after subculture

Each essential oil was tested separately. The experimental design consisted of randomized blocks with six treatments according to the concentrations and four replicates. The data obtained were analyzed using the lack-of-fit test and analysis of variance ($p \leq 0.01$). Significant variables were evaluated by means of regression analysis (MAFRA et al., 2020; NAVES et al., 2021) using the SISVAR software (FERREIRA, 2019), and the graphs were created using MS Excel.

Results and discussion

Citronella essential oils inhibited the growth of pathogenic colonies at all tested concentrations. At a concentration of 0.5 μLmL^{-1}, the percentage of inhibition was 56%; however, at all other concentrations, it was above 80% (FIGURE 1A). Furthermore, MGSR decreased as the oil concentration increased. At concentrations of 0.5 and 1.0 μLmL^{-1}, MGSR was 7.1 and 0.91 mm/day, respectively, and complete inhibition was observed from 1.5 μLmL^{-1} of citronella oil (FIGURE 1B). The average diameter of colonies was 43.4% at a concentration of 0.5 μLmL^{-1}, 11.7% at a concentration of 1.0 μLmL^{-1}, and no growth was observed at a concentration of 1.5 μLmL^{-1} (FIGURE 1C).

Domingos, Carvalho, and Pacheco (2019) also found that citronella oil inhibited the mycelial growth of *C. musae* at a concentration of 1.0 μLmL^{-1}, with a pronounced effect on the mycelial growth at a concentration of 5.0 μLmL^{-1}, reaching an inhibition percentage of 28%. In the current study, a concentration of 1.5 μLmL^{-1} of citronella oil, lower than the concentration mentioned in the above work, inhibited the mycelial growth of *C. musae* by 100%.

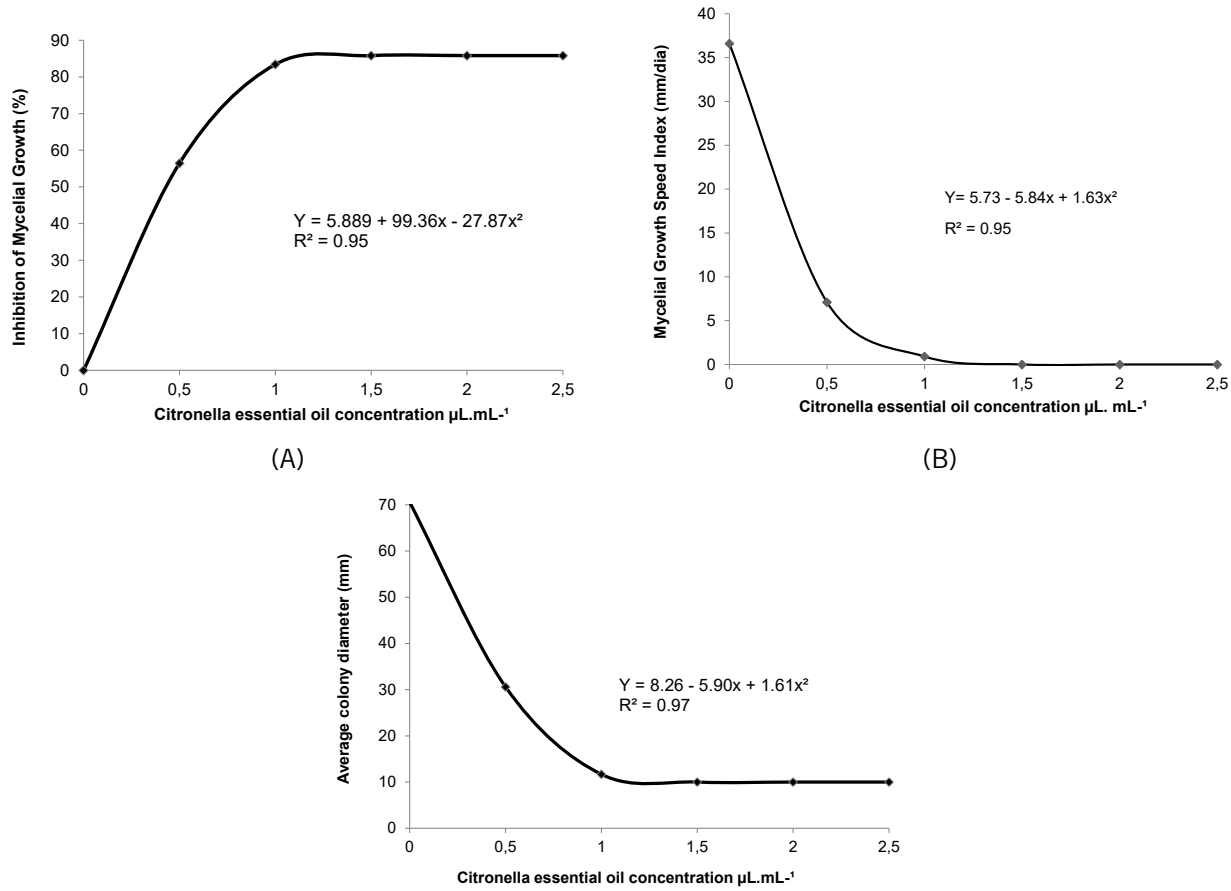
Other authors have also reported the efficacy of citronella essential oils in inhibiting the mycelial growth of different pathogens. Lima *et al*. (2010) reported a reduction in the mycelial growth of *Colletotrichum gossypii* var. *cephalosporioides* similar to the concentrations of most of the oils analyzed in our study. It

was observed that citronella oil inhibited mycelial growth at concentrations higher than 2,000 μLmL^{-1}. Peixinho *et al*. (2019) reported that citronella essential oil at a concentration of 0.25% could completely inhibit the growth of *Lasiodiplodia theobromae*, an important pathogen in postharvest infections.

Lemongrass essential oils inhibited the mycelial growth of *C. musae* at all tested concentrations. At a concentration of 0.5 μLmL^{-1}, the inhibition was 85.73%, and at other concentrations, it was more than 86% (FIGURE 2A). The colony growth speed rate (MGSR) was low, close to zero for all tested concentrations, with a small MGSR of 0.26 and 0.12 mm/day at concentrations of 0.5 and 1.0 μLmL^{-1}, respectively (FIGURE 2B). The average colony diameter decreased by 14.3% at a concentration of 0.5 μLmL^{-1} and 14% at 1.0 μLmL^{-1}, while no growth of pathogenic colonies was observed at a concentration of 1.5 μLmL^{-1} in response to lemongrass oils (FIGURE 2C).

Andrade and Vieira (2016) found that lemongrass essential oils have a fungitoxic effect on the fungus *C. gloeosporioides* at a concentration of 10 μL as measured by the *in vitro* colony diameter and lesion diameter of the inoculated papaya fruits. Similar results were observed by Carnelossi *et al*. (2009), who reported the efficacy of 10 μL of lemongrass

Figure 1 – Means of mycelial growth inhibition percentage (PIC) (A), mycelial growth speed index (IVCM) (B) and mean colony diameter (DC) (C) of *Colletotrichum musae* at different concentrations of citronella essential oil (*Cymbopogon nardus*).



Dates transformed $\sqrt{x} + 1$ for IVCM and DC.

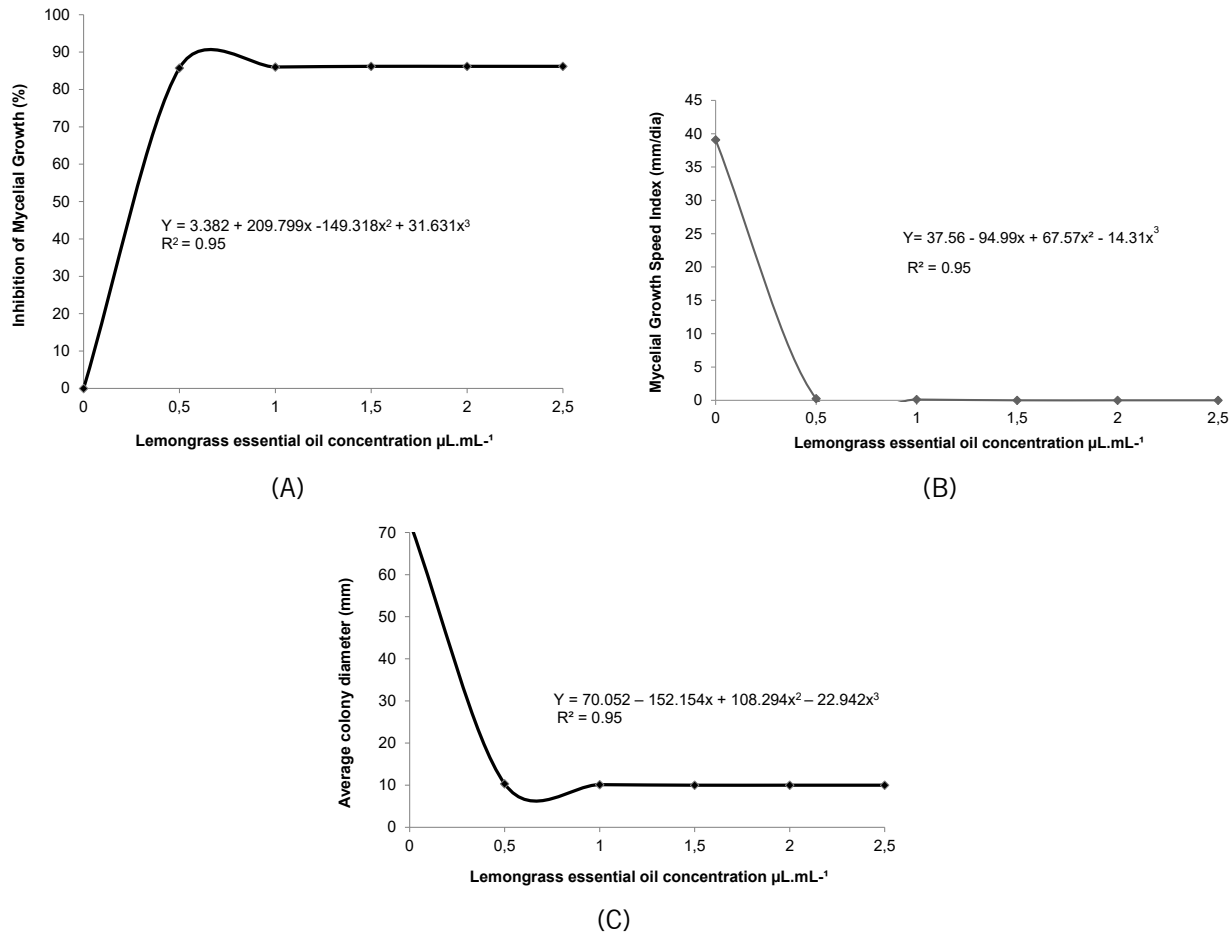
Source: prepared by the authors (2022).

essential oil as demonstrated by the total inhibition of the mycelial growth of *C. gloeosporioides*, the causal agent of anthracnose in papaya. A high antifungal efficacy of this essential oil was also reported by Santos *et al*. (2013) for the fungus *Helminthosporium sp*., causal agent of leaf spots on important crops.

At a concentration of 0.5 μLmL^{-1}, the essential oil from clove flower buds completely inhibited the mycelial growth of *C. musae* (FIGURE 3A). The growth rate was null at all tested concentrations, and no mycelial growth was observed (FIGURE 3B). Only the control treatment (0μLmL^{-1}) showed colony growth, with an average colony diameter of 70.5 mm (FIGURE 3C).

A study conducted by Barbosa, Vieira, and Teixeira (2015) corroborated the results obtained in the present work. The authors reported that clove essential oils inhibited the mycelial growth of *C. musae* at all tested concentrations, even after fungicidal treatment. The fungicidal action of clove oils has already been determined on other phytopathogenic fungi, such as *Rhizoctonia solani, Fusarium solani, Fusarium oxysporum,* and *Macrophomina phaseolina*, and only the latter was not affected by clove essential oils at a concentration of 0.15%. The authors reported that the high efficacy observed with clove oils is mainly due to the presence of eugenol, which represents 83.6% of the substances found in this oil. The hydrophobicity of the essential

Figure 2 – Means of mycelial growth inhibition percentage (PIC) (A), mycelial growth velocity index (IVCM) (B) and mean colony diameter (DC) (C) of *Colletotrichum musae* at different concentrations of Lemongrass (*Cymbopogon citratus*) essential oil.



Source: prepared by the authors (2022).

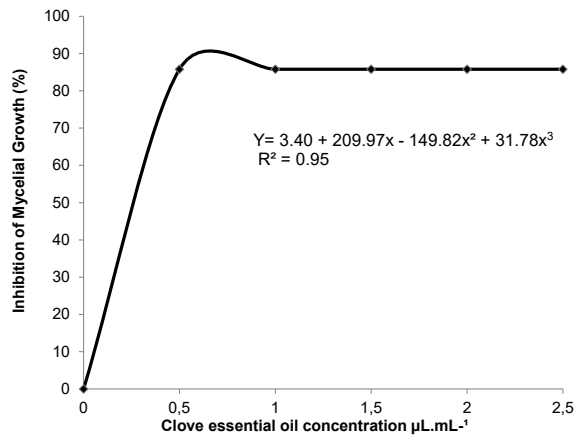
oils allows them to interact with the lipids of the cell wall, cell membrane, and mitochondria of fungus, altering permeability and causing disruptions in these structures, thus providing efficient fungicidal activity (COSTA *et al*., 2011).

Basil essential oils inhibited the mycelial growth of *C. musae* demonstrated an increase in the percentage of inhibition and a decrease in the growth rate and size of the fungal colony with increasing concentrations. The percentage of inhibition of mycelial growth was 49.8, 55.1, 77.4, 85.1, and 86.2% at concentrations of 0.5, 1.0, 1.5, 2.0, and 2.5 μLmL^{-1}, respectively (FIGURE 4A). The MGSR was found to be 13.1, 10.7, 4.6, 0.1, and 0.00 mm/day at concentrations of 0.5, 1.0,1.5, 2.0, and 2.5 μLmL^{-1}, respectively (FIGURE 4B). Regarding the average colony diameter, no growth was observed only at the concentration of 2.5 μLmL^{-1}.

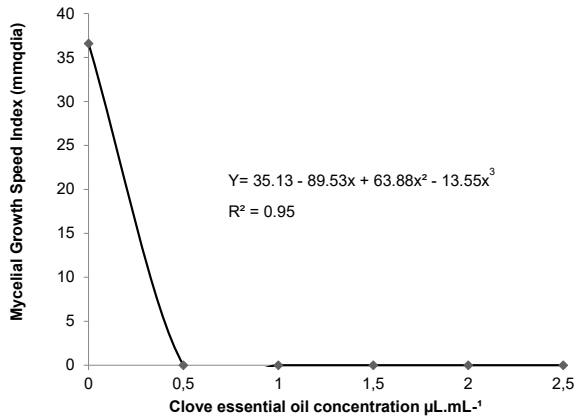
According to Ramos, Andreani Junior, and Kozusny-Andreani (2016), increasing the concentration of basil essential oil improved its antifungal effect on *C. gloeosporioides*, and the total inhibition of the fungus was observed at a concentration of 100% of this oil.

Almeida (2017) observed that as the concentration of basil essential oils increased, the growth rate of *Colletotrichum lindemuthianum* colonies, which causes anthracnose in beans,

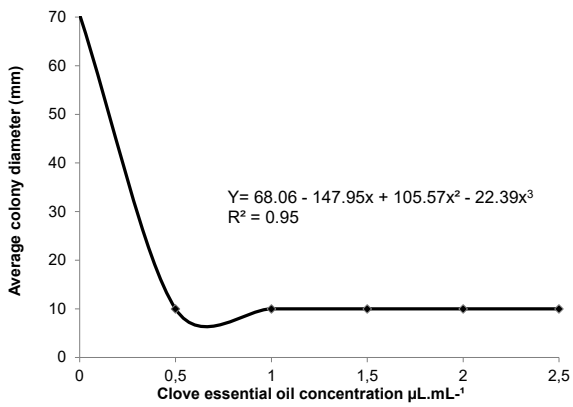
Figure 3 – Means of mycelial growth inhibition percentage (PIC) (A), mycelial growth velocity index (IVCM) (B) and mean colony diameter (DC) of *Colletotrichum musae*, and different concentrations of clove (*Syzygium aromaticum*) essential oil.



(A)



(B)



(C)

Source: prepared by the authors (2022).

decreased. At a concentration of 1.0 μLmL^{-1}, no fungal growth was observed indicating complete inhibition of this pathogen.

Conclusions

The tested essential oils showed *in vitro* antifungal activity against *C. musae* at different concentrations, with the highest efficiency at 1.5 μLmL^{-1} for citronella grass and lemongrass essential oils, 0.5 μLmL^{-1} for clove essential oil, and 2.5 μLmL^{-1} for basil essential oil.

Overall, clove essential oil showed the highest inhibition of the mycelial growth of *C. musae* at low concentrations.

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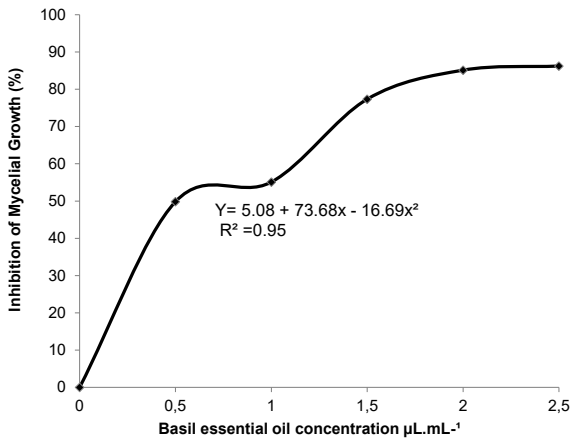
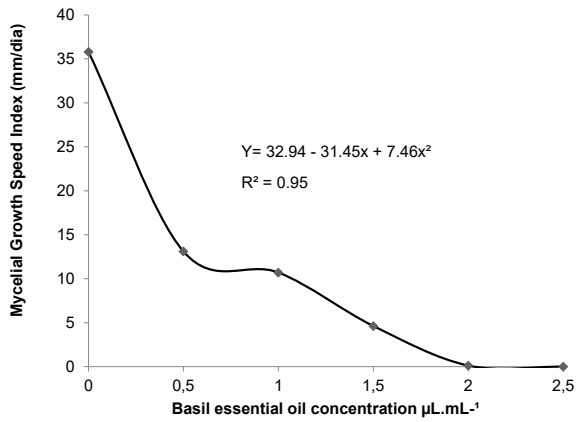
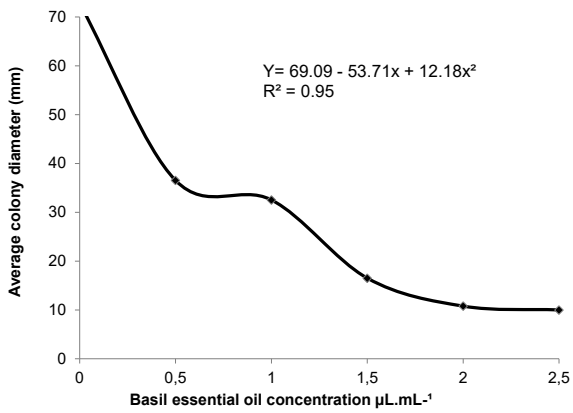
Figure 4 – Means of mycelial growth inhibition percentage (PIC) (A), mycelial growth velocity index (IVCM) (B) and mean colony diameter (DC) (C) of *Colletotrichum musae* at different concentrations of basil (*Ocimum basilicum*) essential oil.



(A)



(B)



(C)

Source: prepared by the authors (2022).

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