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Potential use of lemongrass essential oil as fungicide against *Aspergillus brasiliensis* and as post-harvest protectant of wheat

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ABSTRACT. Fungi are among the main responsible for damage and loss in stored grains, its control has been done through synthetic substances, which are harmful to man and the environment. Brazil, one of the leading countries in agriculture, has optimal environmental conditions for the development of mycotoxigenic fungi. Most of the synthetic chemicals used as preservatives have often been realized to be toxic to humans and also cause adverse environmental effects. Thus, it is necessary to search for alternative methods of controlling. In this study the aimed was to evaluate the efficacy of lemongrass essential oil in the control of the *Aspergillus brasiliensis*. *In vitro* and serial microdilution tests were carried out at different concentrations of essential oil and citral, which corresponds to 72% of the total oil composition. Inhibition of fungal growth on contaminated wheat grain was evaluated. The *in vitro* test results showed that the essential oil has fungicidal potential at concentrations from 0.6 µL mL^{-1}, the minimum inhibitory concentration was determined at 0.8 µL mL^{-1}. The tests with citral showed fungal control at concentrations from 0.6 µL mL^{-1} onwards. For wheat grain, fungal growth inhibition was obtained at the concentration of 1.6 µL mL^{-1}. The essential oil of *Cymbopogon flexuosus* showed fungicidal activity against the fungus *Aspergillus brasiliensis*.

Keyword: antifungal; microorganism; mycotoxin; storage grain.

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Introduction

Fungi are considered one of the major causes of seed and grain deterioration in storage. Fungal and mycotoxin contamination of stored food items is of utmost concern throughout the world due to their hazardous effects on mammalian systems. It has been estimated that of the world crop production, one million tonnes of food is being compromised because of contamination with some type of mycotoxin, with recognized harmful effects on human and animal health. Brazil, one of the leading countries in agriculture, has optimal environmental conditions for the development of mycotoxigenic fungi. Most of the synthetic chemicals used as preservatives have often been realised to be toxic to humans and also cause adverse environmental effects (Freire, Vieira, Guedes, & Mendes 2007; Prakash, Kedia, Mishra, & Dubey 2015; Chaudhari et al., 2019).

Ingestion of mycotoxin-contaminated food may cause hepatotoxic, nephrotoxic, mutagenic, estrogenic, neurotoxic, immunosuppressive, and carcinogenic effects in both humans and animals. Contamination of grains and seeds with mycotoxins occurs widely and affects cereals of economic and food importance such as wheat, which is a source of raw material for the production of various foodstuffs, fundamental in human diet. *Triticum aestivum*, known as common wheat, is the most widely planted species on the planet and the most common grain used for bread-making (Mallmann et al., 2018).

The fungal species *Aspergillus brasiliensis* was first observed in a study by Pařenicová et al. (2001), which aimed to develop methods for identifying and characterizing the genus *Aspergillus*. *Aspergillus brasiliensis* showed different characteristics from *A. niger* and *A. tubingensis*, suggesting the classification of a new species, which was described by Varga et al. (2007). It was isolated from Brazilian soils, in the region of São Paulo, which gave rise to its name. Later, *A. brasiliensis* was also found in soils of Australia, the United States,

and Holland. It produces colonies that are white, dark brown, and later change to black, and the optimal temperature for its growth and sporulation is 37 ºC. To control fungal contamination in foods, alternative measures to synthetic pesticides have been sought using natural agents such as essential oils that cause no environmental pollution, side effects and are safe for human use (Bhatt & Kale, 2019)

Among the essential-oil producing species from several regions of Brazil, lemongrass [*Cymbopogon flexuosus* (Nees ex Steudel) Watson] is widely known and used. Native to India, lemongrass is a perennial aromatic herb grown to obtain its essential oil, which is rich in citral, a substance used by the perfumery, cosmetics and pharmaceutical industries (May et al., 2008). Studies such as Pandey, Rai, and Acharya (2003), Jaramillo-Colorado, Palacio-Herrera, and Duarte-Restrepo (2020), Lima et al. (2020), Lee, Garcia, Matinazzo, and Teodoro (2020), have demonstrated intense antimicrobial, antifungal, antiviral, and insecticidal activity of the essential oils. The objective in this study was to analyze the antifungal activity of lemongrass (*Cymbopogon flexuosus*) essential oil and citral for the control of the fungus *Aspergillus flavus* in *in vitro* tests and its proliferation in contaminated grains of wheat (*Triticum aestivum*).

Material and methods

Fungal strain and production of conidia

Aspergillus brasiliensis (CCCD No. AA002) was purchased from Didática sp.® (Ind. And Com. Ltda). Cultures were grown in PDA medium (potato, dextrose and agar) in Petri dishes at 30 ºC for seven days. For spore collection, the plates were flooded with 15 mL sterile distilled, and conidia were harvested with a pipette. The spore suspension was adjusted with sterile distilled water to give the final concentration of 4.5 x 10^6 spores mL^{-1} using a Neubauer chamber. The suspension was stored at 4 ºC until use.

Analysis of essential oils and gas chromatography

The essential oil of *C. flexuosus* was purchased from an industry company. Analysis of essential oil constituents was performed by Gas Chromatography Mass Spectrometry (GC-MS). The compounds were separated in a fused-silica capillary column with DB-5 stationary phase (30 m long x 0.25 mm internal diameter x 0.25 µM inner film thickness). Helium was used as carrier gas at a flow rate of 1.0 mL min^{-1}. The temperature of the injector was maintained at 220 °C and the detector at 240 °C. The initial oven temperature was maintained at 60 °C for 2 min and programmed with a heating rate of 3 °C min^{-1} to 240 °C and held for 30 min, in a total analysis time of 91 minutes. The split ratio was 1:20 and the solvent cut-off time was 5 minutes. The sample injection volume was 1 µL, at a concentration of 10,000 ppm, using hexane as solvent (Martinazzo, Oliveira, & Teodoro, 2019). Compounds were identified by comparing the mass spectra obtained with those of the apparatus database and by the Kovats Retention Index (IK) of each component (Lanças, 1993). The quantitative analysis of the main components of the essential oil, expressed as a percentage, was performed by the peak area integration normalization method, as described by Zhang, Chen, Wang, and Yao (2006).

Tests for antifungal activity

In the *in vitro* antifungal assay, the following concentrations of lemongrass essential oil were tested: 0.05; 0.1; 0.2; 0.4; 0.6; 0.8; 1.6; 3.2; 6.4 and 12.8 µL mL^{-1}. These concentrations were based on the results obtained in the initial screening and based on the study of Martinazzo et al. (2019).

To facilitate the dispersion of the essential oil through the culture medium, 1% of the surfactant DMSO (dimethyl sulfoxide) was supplemented to the melted PDA (45 °C) for addition of the essential oil concentrations. After homogenization, the medium was poured into Petri dishes and incubated with a 7 mm mycelial disk. Control plates, without essential oil, were inoculated following the same procedure.

The plates were incubated at 30 °C until the fungus covered the entire control plate, then the incubation time was completed. The colony diameter was recorded daily with a digital caliper. The results were expressed in terms of the diameter of the halo of microbial growth. The percentage of colony inhibition (PI) was calculated according to Billerbeck, Roques, Bessière, Fonvieille, and Dargent (2001):

$$PI = \frac{\emptyset_0 - \emptyset_T}{\emptyset_0} 100 \qquad \text{(Equation 01)}$$

where: $\emptyset_0$ is the mean diameter of the mycelium in the control; $\emptyset_T$ is the mean diameter of the mycelium in the treatment.

From the results obtained in the essential oil test, an *in vitro* test was performed with its major component, Citral, purchased from Sigma-Aldrich®. The concentrations tested were: 0; 0.2; 0.4; 0.6; 0.8; and 1.6 µL mL^{-1}. The percentage of inhibition was determined by Equation 01.

Determination of the minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of the essential oil on *A. brasiliensis* was determined by serial microdilution in a 96-well microtiter plate. The screened concentrations were based on the *in vitro* test results and the following were tested: 1.0; 1.2; 1.4; 1.6, and 1.8 µL mL^{-1}. Each concentration was replicated four times in PD medium (potato and dextrose containing essential oil, DMSO, and spore suspension (10^7) and a control treatment without essential oil. The plates were kept in a B.O.D. chamber at 35°C for 72 hours. After the incubation time, the results were analyzed visually. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of essential oil in which no fungal growth occurred (Billerbeck et al., 2001; Pandey et al., 2003; Dellavalle et al., 2011).

Wheat grain samples and experimentation

Wheat grains were supplied by EMBRAPA Trigo, Passo Fundo, Rio Grande do Sul, Brazil. The analysis of the effect of lemongrass essential oil on the development of fungi was carried out with the three concentrations of the essential oil that presented the highest percentage of *in vitro* inhibition of mycelial growth (PI), that is, 0.6, 0.8, and 1.6 µL mL^{-1} plus control without essential oil. These concentrations were tested over different storage periods (10, 20, and 30 days) in a controlled environment (30°C).

Samples of 200 g of wheat grains were placed in glass jars and were pre-sterilized by autoclaving at 120°C for 15 min. Then, each jar was inoculated with 2 mL of the *A. brasiliensis* spore suspension. The jars were kept at controlled temperature (30°C) in B.O.D. chamber for fungal growth. After 48 hours, sachets with the different essential oil concentrations were placed into the jars containing the fungus-inoculated grains. The control treatment was carried out without essential oil and was analyzed over the different periods as same as the treatments. Each concentration was tested with three replicates. Colony Forming Units (CFUs) were counted under the effects of each treatment and percentage of growth inhibition (PI) was calculated according to Tatsadjieu, Yaouba, Nukenine, Ngassoum, and Mbofung (2010):

$$PI = \frac{C_0 - C_T}{C_0} 100 \qquad \text{(Equation 02)}$$

where: C_0 is the number of colonies without treatment; C_T is the number of colonies with essential oil treatment.

Statistical analysis

The experiment was arranged in a completely randomized design, data were analyzed by analysis of variance and means compared by the Scott-Knott test at 5% of significance, using the SISVAR® statistical program.

Results and discussion

Identification of the chemical components of lemongrass (*Cymbopogon flexuosus*) essential oil

Figure 1 shows the chromatogram obtained in the identification of the main components of lemongrass (*C. flexuosus*) essential oil.

Table 1 shows the mean retention time and the Kovats index of the components identified by the chromatogram in Figure 1.

Results show that the main component of lemongrass essential oil used in this study is citral, a mixture of neral and geranial. The citral concentration was quantified in 72% of the oil composition, in which 40% is geranial and 32% is neral. Citral has been identified as the main component of some plants such as *Lippia alba* (Barbosa, Barbosa, Melo, & Botelho, 2006), *Pectis brevipedunculata* (Oliveira, Berbert, Matos, Mathias, & Moreira 2011), and *Cymbopogon citratus* (Martinazzo, Melo, Demuner, & Berbert, 2013).

Kakarla and Ganjewala (2009) evaluated the control of bacteria and fungi by the essential oils of four *C. flexuosus* varieties from different regions of India and found that all evaluated oils had strong antifungal activity with small variations of control. According to the authors, the anti-microbial potential of essential

oils depends on their chemical composition and the proportion of their different constituents. And citral showed the best results against almost all microorganisms tested, including the fungus *A. flavus*.

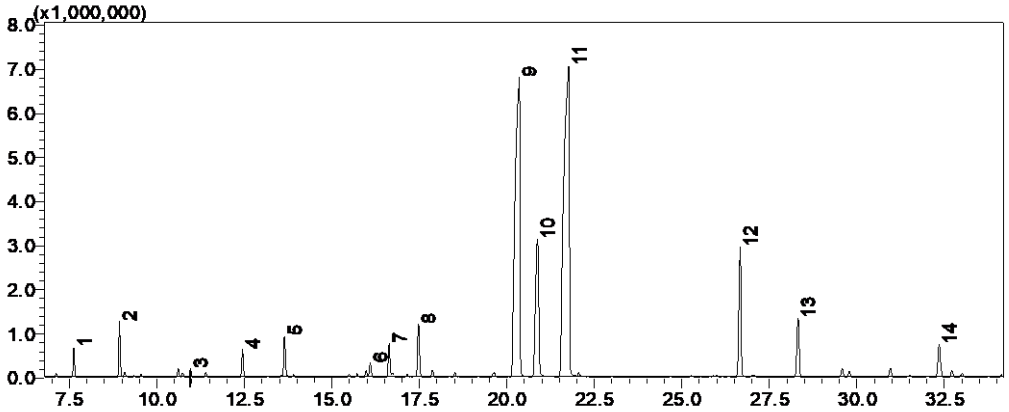

Figure 1. Chromatogram of lemongrass (*Cymbopogon flexuosus*) essential oil used in the experiment.

Table 1. Major components of lemongrass (*Cymbopogon flexuosus*) essential oil identified by GC-MS.

Peak	Component	Retention time* (min)	Kovats Index		
			This study	Adams (2012)	Choi (2003)
02	6-methyl-5-hepten-2-one	8.927	994	992	
05	Linalool	13.647	1093	1098	
06	Citronellal	16.097	1150	1153	
09	Neral	20.353	1245	1244	
10	Geraniol	20.873	1257	1257	
11	Geranial	21.768	1272	1270	
12	Geranyl Acetate	26.664	1388	1383	
13	Caryophyllene	28.315	1430	-	1428[(1)]

* DB-5 column.

Evaluation of the in vitro antifungal activity of lemongrass (*Cymbopogon flexuosus*) essential oil against *Aspergillus brasiliensis*

Figure 2 shows the inhibitory effect of the lemongrass essential oil on the mycelial growth of the fungus *A. brasiliensis*, in the different concentrations tested during the incubation time.

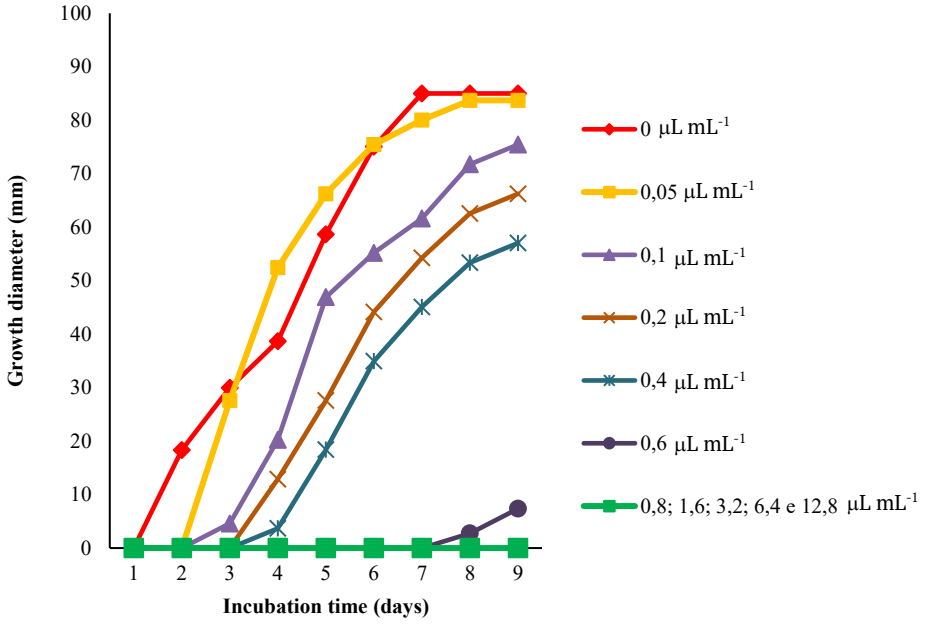

Figure 2. Effect of different concentrations of lemongrass (*Cymbopogon flexuosus*) essential oil on the growth of *Aspergillus brasiliensis*.

For the essential oil concentrations of 0.05 and 0.4 µL mL^{-1}, the diameter of the fungal growth varied between 84 and 57 mm during the incubation time (Figure 2). At the concentration of 0.6 µL mL^{-1} there was 7 mm growth of fungus diameter from day 7. For the other concentrations, the radial growth of fungal colonies was totally inhibited. It is noteworthy that the end of the incubation time was determined by the time that the mycelial growth in the control treatments (concentration zero) covered the entire plate surface in the Petri dish of 92 mm in diameter.

To evaluate the effect of the concentrations tested, the Percentage of Inhibition (Equation 01) of each concentration on *A. brasiliensis* was calculated and the statistical analysis was performed as described below:

The analysis of variance of the effect of lemongrass essential oil and incubation time on the mycelial growth of the fungus *A. brasiliensis* showed a significant effect for the different concentrations of lemongrass essential oil (D), for the incubation time (t), and the interaction (D x t). This indicates that the inhibition of *A. brasiliensis* growth by lemongrass essential oil depends on the interaction between the concentration of the essential oil applied and the incubation time. In this way, the interaction was unfolded to study the behavior of the microorganism control within each factor studied, as described in Table 2.

Table 2. Mean percentage of *in vitro* inhibition of mycelial growth of *Aspergillus brasiliensis* at different concentrations (µL mL^{-1}) of lemongrass (*Cymbopogon flexuosus*) essential oil during the incubation time.

Essential oil (µL mL^{-1})	Percentage of inhibition of mycelial growth **								
	Incubation time (days)								
	1	2	3	4	5	6	7	8	9
0.05	100aA	100aA	70bB	43cC	28dD	18dE	13dE	9dE	9cE
0.1	100aA	100aA	95aA	78bB	49cC	40cD	33cD	22dE	18cE
0.2	100aA	100aA	100aA	96aA	80bB	62bC	51bD	42bE	38bE
0.4	100aA	100aA	100aA	96aA	80bB	62bC	51bD	42bE	38bE
0.6	100aA	100aA	100aA	100aA	100aA	100aA	100aA	97aA	92aA
*	100aA	100aA	100aA	100aA	100aA	100aA	100aA	100aA	100aA
CV: 19.03%									

*0.8 1.6; 3.2; 6.4; 12.8 µL mL^{-1} essential oil; **Means followed by the same small letters in the columns and capital letters in the rows are not significantly different by the Scott-Knott's test at 5% probability.

Table 2 shows that in the first 48 hours all the concentrations provided fungal growth control, from 72 hours onwards the concentration of 0.05 µL mL-1 differed statistically from the others, which occurred successively over the days with the concentrations of 0.1, 0.2, and 0.4. µL mL^{-1}. Only the concentrations from 0.6 to 12.8 µL mL^{-1} maintained, without significant statistical difference, the control of fungal mycelial growth during the nine days of observation. There was a significant effect of the different doses of lemongrass essential oil, for the incubation time, as well as for the interaction (dose x time), indicating that the growth of the fungus *A. brasiliensis*, with the essential oil of lemongrass, depends on the interaction between the applied oil dose and the fungus incubation time.

These results differed from those found by Sarma, Saikia, Sarma, and Boruah (2004), who tested the fungicidal activity of *C. flexuosus* essential oil against the species *Aspergillus niger* at concentrations of 10, 20, and 30 µL mL^{-1} and found that there was fungal growth after 72 hours of incubation. This difference can be explained by the distinction between the fungal species and the amount citral present in the essential oils used, 25.9% in Sarma et al.'s work and 72% in this work, being that this component is likely responsible for the fungal control at lower concentrations of essential oil.

The concentration of 0.6 µL mL^{-1}, which showed control over *A. brasiliensis* growth, is close to the results reported by Shahi, Patra, Shukla, and Dikshit (2003). They evaluated the antifungal activity of lemongrass essential oils at concentrations from 0.1 to 0.8 µL mL^{-1} against four species of the genus *Aspergillus* (*A. flavus*, *A. fumigatus*, *A. niger* and *A. parasiticus*) and found 100% inhibition of fungal growth with seven days of incubation at the concentration of 0.4 µL mL^{-1}. According to the authors, citral was the main component identified, with amounts varying from 68% to 80% of the essential oil composition.

Analysis of antifungal activity of lemongrass (*Cymbopogon flexuosus*) essential oil against fungi of the genus *Aspergillus* by the microdilution method

Serial microdilution in microtiter plate was performed to determine the minimum inhibitory concentration (MIC) of the essential oil to inhibit fungal growth. The results are presented in Table 3. The concentrations tested were based on the results of the *in vitro* tests.

Table 3. Indication of fungal growth * in serial microdilution of *Aspergillus brasiliensis* at different concentrations (µL mL^{-1}) of lemongrass (*Cymbopogon flexuosus*) essential oil.

Essential oil concentration (µL mL^{-1})	*A.*	*b.*	Essential oil concentration (µL mL^{-1})	*A.*	*b.*
1.8	-		0.45	+	
1.6	-		0.4	+	
1.4	-		0.35	+	
1.2	-		0.3	+	
1.0	-		0.25	+	
0.9	-		0.22	+	
0.8	-		0.2	+	
0.7	+		0.17	+	
0.6	+		0.15	+	
0.5	+		0.12	+	

* + indicates fungal growth; - no growth. *A.b* = *Aspergillus brasiliensis*.

Higher values of MIC were found by Kumar, Shukla, Singh, and Dubey (2009), for fungi of the genus *Aspergillus* in the evaluation of *Cymbopogon flexuosus* essential oil and its components as antifungal and inhibitor of aflatoxin production, with MICs of 1.2, 1.3, and 1.9 µL mL^{-1} for the species *A. fumigatus*, *A. terreus*, and *A. niger*, respectively. Citral was quantified in 63.17%, below the value found in this work, which may have been one of the causes for the higher concentrations of essential oil required for fungal growth inhibition, besides the specificity of each species. In their research Dong and Thuy (2019) found the MIC of 5 µL mL^{-1} of lemongrass oil against *Aspergillus niger*.

Considering that the essential oil of *C. flexuosus* showed antifungal activity against *A. brasiliensis* and its major component is citral, accounting for 72% of its composition, *in vitro* tests with citral were carried out to assess whether the growth of the fungus is inhibited by this component, as described below. According Bhatt and Kale (2019), the antimicrobial activity of essential oil is directly dependent on its chemical composition.

Evaluation of the in vitro antifungal activity of citral against the fungus *Aspergillus brasiliensis*

Figure 3 shows the inhibitory effect of citral on the mycelial growth of *A. brasiliensis* at the different concentrations tested during the incubation time of seven days.

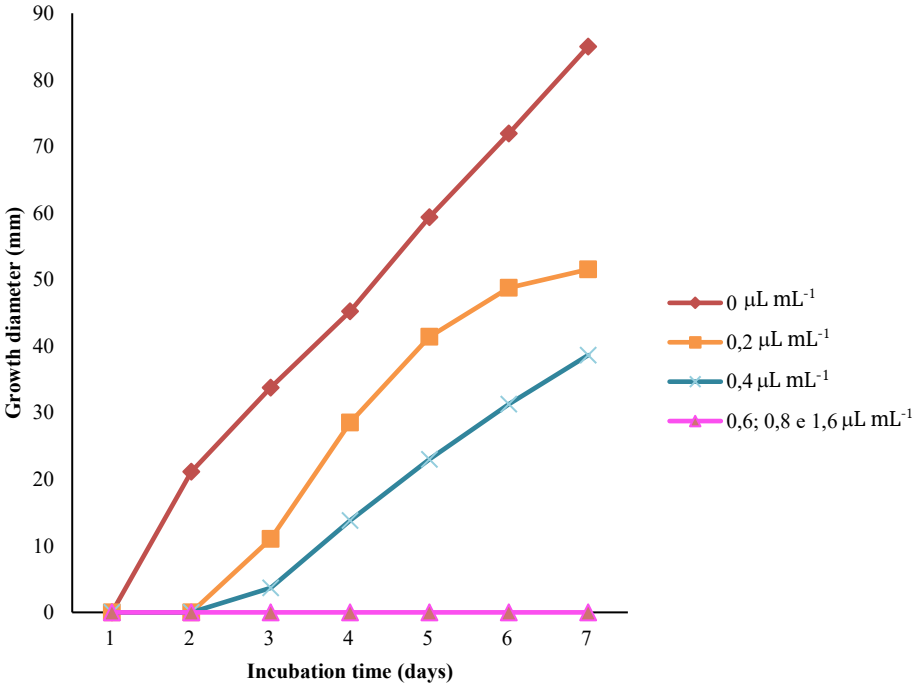

Figure 3. Effect of different concentrations of citral on the growth of the fungus *Aspergillus brasiliensis*.

Figure 3 shows that for the concentrations of 0.6 µL mL^{-1} onwards there was no fungal growth, as it occurred in the *in vitro* tests, with a result close to that of the serial microdilution, which shows the efficacy of citral as an antifungal component against *A. brasiliensis*. Growth was observed at the lowest concentrations, 0.2 and 0.4 µL mL^{-1}, with mean growth diameters of 52 and 39 mm, respectively.

The statistical analysis revealed significant effect of the different citral (D) concentrations for the incubation time (t) and the interaction (D x t), indicating that the inhibition of *A. brasiliensis* by citral depends on the interaction between the concentration of the essential oil and the incubation time, as was the case for the control of *A. brasiliensis* in the *in vitro* test. The behavior of the fungal growth control within each factor is presented in Table 4.

Table 4. *In vitro* mean percentage inhibition of mycelial growth of the fungus *Aspergillus brasiliensis* at different concentrations (µL mL^{-1}) of citral during the incubation time

Citral concentration	Inhibition of mycelial growth** in days (%)						
(µL mL^{-1})	1	2	3	4	5	6	7
0.2	100aA	100aA	88bB	69cC	55cD	47cD	44cD
0.4	100aA	100aA	96aA	85bB	75bC	66bD	58bD
*	100aA	100aA	100aA	100aA	100aA	100aA	100aA
CV: 9.60%							

*0.6; 0.8; 1.6 µL mL^{-1} essential oil; **Means followed by the same small letters in the columns and capital letters in the rows are not significantly different by the Scott-Knott's test at 5% probability.

The results in Table 04 demonstrate the complete inhibition of fungal growth with application of citral from the concentration of 0.6 µL mL^{-1} onwards over the incubation time, which agrees with the results of tests performed for lemongrass essential oil and demonstrates the fungicidal action is related to the citral. The concentration of 0.4 µL mL^{-1} was statistically different from the highest concentrations only from day 4 of incubation, when its antifungal activity begins to decrease. The concentration of 0.2 µL mL^{-1} showed control only in the first 48 hours.

In the first 48 hours, all the concentrations showed 100% inhibition, results that are close to those obtained by Moleyar and Narasimham (1987), who evaluated the fungicide potential of citral against the fungus *A. niger* at concentrations of 0.06, 0.12, and 0.24 µL mL^{-1} and found an inhibition percentage of 83% at the concentration of 0.06 µL mL^{-1} and 100% at 0.12 and 0.24 µL mL^{-1}, in the same period of observation. Other studies, including Stevens, Jurd, King Jr, and Mihara (1971), Batt, Solberg, and Ceponis (1983), Kakarla and Ganjewala (2009), confirmed the antimicrobial action of citral and reported its fungitoxic potential toward the genus *Aspergillus*, but with higher concentrations than the present study. Results from Kaur, Ganjewala, Bist, and Verma (2019) revealed that citral exhibited antifungal activity effect at concentrations above 0.2 µL mL^{-1} against some pathogenic fungi.

According to Natu and Tatke (2019) the aldehydes like citral and cinnamaldehyde, phenolic compounds like thymol, carvacrol, eugenol are abundantly found in some essential oils and show potent antifungal activity. The terpenes have an ability to cross plasma membrane of fungi and interact with enzymes and proteins of the membrane. Interactions of terpenes with membrane proteins results in morphological changes in hyphae and plasma membrane leading to fungal cell death (Nazzaro, Fratianni, Coppola, & Feo, 2017).

Evaluation of the efficacy of lemongrass (*Cymbopogon flexuosus*) essential oil in the control of the fungus *Aspergillus brasiliensis* in infected wheat (*Triticum aestivum*) grains

The final concentrations of lemongrass essential oil to test the effectiveness of the oil in controlling the fungus in infected wheat grains were based on the best results found in the previous experiments. At the end of each storage period (10, 20, and 30 days) the colony forming units were counted and the percentage of inhibition of fungal growth in the infected grains was determined by applying the best control concentrations (0.6, 0.8, and 1.6 µL mL^{-1}).

The analysis of variance for each incubation time at the different concentrations, in each time evaluated, showed significant differences of the concentrations, then the percentage of inhibition was compared by the mean test (Table 5).

The percentage inhibition was above 80% in all treatments. At 10 and 20 days of storage, the percentage of inhibition was higher and at the concentration of 1.6 µL mL^{-1}, which showed increased control over the period, unlike the other concentrations that showed variation in inhibition. Tatsadjieu et al. (2010) evaluated the percentage inhibition of *A. flavus* growth in maize grains using essential oils (*O. gratissimum*, *L. rugosa,*

and *X. aethiopica*) and found that after two weeks, the percentage of inhibition was 93.1%; then at 6 weeks this percentage fell to 34.6%. The authors suggested that this might result from a relatively long incubation time, in which some volatile components of the oils may evaporate, decreasing their concentration and control over the microorganism.

Table 5. Mean percentage inhibition of the growth of the fungus *Aspergillus brasiliensis* in wheat grains (*Triticum aestivum*) at different concentrations of lemongrass (*Cymbopogon flexuosus*) essential oil and storage periods.

Cymbopogon flexuosus essential oil concentration (μL mL^{-1})	Percentage inhibition of fungal growth during storage period (days) *		
	10	20	30
0.6	88.69aA	83.51aB	88.44aA
0.8	88.11aA	86.41aA	96.82bB
1.6	92.00 aA	92.28bA	96.04bB

* Means followed by the same small letters in the columns and capital letters in the rows are not significantly different by the Scott-Knott's test at 5% probability.

In this study, we found that the percentage of inhibition remained high for all concentrations at the end of 30 days, indicating that the components of the *Cymbopogon flexuosus* oil, mostly citral (72%), have a greater antifungal effect for longer periods compared with the species studied by Tatsadjieu et al. (2010).

The grain storage period generally varies with the market demand, and the findings of this study indicate, initially, that the highest concentration tested showed more consistent results in the control of fungal growth as a function of storage time. Isman (2006) pointed out that studies on botanical compounds and essential oils can control pests that attack grains. Because essential oils are volatile, their persistence in field conditions is limited, although natural enemies are susceptible to direct contact, a long-term effect is unlikely. In the context of agricultural pest management, botanical insecticides are best suited for use in organic food production in industrialized countries but can play a much greater role in the production and postharvest protection of food in developing countries.

Conclusion

The essential oil of *Cymbopogon flexuosus* showed *in vitro* fungicidal activity against the fungus *Aspergillus brasiliensis*.

The essential oil of *Cymbopogon flexuosus* proved to be efficient in the test with infected wheat grains, maintaining the fungicidal capacity even after 30 days of storage. The concentration of 1.6 μL mL^{-1} was shown to be the most recommended.

The essential oil used and the major component citral proved to be efficient in controlling the mycelial growth of the fungus *Aspergillus brasiliensis*, presenting itself as possible non-synthetic alternatives for the development of a fungicide in the control of this species. Therefore, additional research can be developed with other species and individually assessing the main components of essential oil.

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