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Use Of Cymbopogon Citratus Leaf Essential Oil For Management Of Anthracnosis In Yam-Grops

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Abstract

The essential oil of Cymbopogon citratus (DC.) Stapf. (Lemongrass) has been positioned as a phytosanitary alternative to counteract the effects caused by phytopathogenic fungi. The aim of the present study was to evaluate in vitro the inhibitory activity of essential oils from Cymbopogon citratus (lemongrass) leaves against Colletotrichum gloeosporioides. Leaves of the silver species Cymbopogon citratus were collected in the sub-region of the savannahs of the department of Sucre; for the extraction of essential oils (EO), fresh leaves were weighed and EO was obtained using the microwave-assisted hydrodistillation method. The antifungal activity of the EOs was evaluated using the surface seeding technique on solid medium, the activity was expressed as percentage inhibition index. Chemical characterisation of the essential oils was performed by gas chromatography coupled to mass spectrometry. The highest antifungal index efficiency of lemongrass was observed at concentrations of 5,000, 8,000 and 10,000 ppm. The main constituent identified in lemongrass EOs was citral, which is possibly related to the inhibitory activity of the fungus causing anthracnose disease in yam crop.

Keywords: essential oils, Cymbopogon citratus, inhibitory activity, Colletotrichum gloeosporioides.

1. Introduction

Colombia is considered one of the 17 megadiverse countries, hosting 70% of the world's biodiversity in only 10% of its territory [1], where more than 54,000 species have been recorded [2], sharing first place with Brazil in terms of world biodiversity and second in terms of plant diversity [3]. Although all this great diversity of plants is present in the national territory, there is still little ethnobotanical and phytochemical research, which is why this raw material constitutes a great phytosanitary alternative to counteract the diseases that afflict the health of people and animals, and mainly crops of economic importance.

In the Colombian Caribbean region, many crops are affected by these pathogens, as is the case of yam (Dioscoreas pp.), where the main production is centred and where Colombia is among the 12 countries with the highest yam production worldwide with 395,374 tons and ranked first in yield with 28.30 tons per hectare sown [4]. There are many agricultural products cultivated in Sucre, among which the varieties of creole yam (Dioscorea alata L.) and spiny yam (Dioscorea rotundata Poir) [5] are sown, generating direct and indirect sources of employment in its productive chain, but this tuber has been the target of numerous phytopathogens such as the fungus Colletotrichum gloeosporioides, which causes anthracnose, causing a reduction of up to 85% of the production [6].

For the management of anthracnose in yam, growers use continuous doses of fungicides such as benomyl to counteract the effects of the phytopathogen on the crop. Studies published in the literature show that these compounds cause problems for the environment, human health, animal health and microbial diversity [7]. Furthermore, varieties tolerant to this phytopathogen have been improved without obtaining satisfactory results in the field.

Essential oils are seen as a great agronomic alternative to replace synthetic pesticides on the market today, as they have antifungal, antibacterial, antiviral, etc. properties. In addition to this, they can replace synthetic additives in food, favouring food stability and protection against lipid alterations due to their antioxidant activity [8]. It has been shown that essential oils abundant in citral are well known for their bactericidal and fungicidal properties [9]. This study evaluated the antifungal activity of C. citratus essential oil against C. gloeosporioides (Penz.) Penz. & Sacc. causing anthracnose in yam crops in the department of Sucre.

2. Methodology

2.1 Plant material.

Samples of Cymbopogon citratus were collected in the morning hours between 60 and 213 m asl at the following coordinates: 8° 51' 26" N, 75° 16' 36" W and 9° 17' 58" N, 75° 23' 45" W. The plant material was packed in an expanded polystyrene (Styrofoam) container and preserved at 25ºC [10]. The taxonomic identity of the specimens collected in the different municipalities corresponds to the species Cymbopogon citratus (DC. ex Ness) Stapf, belonging to the family Poaceae, collection of the Herbarium of the University of Sucre under registration N°000831.

2.2 Extraction of essential oils.

This was carried out by microwave-assisted hydrodistillation (MWHD). Hydrodistillation equipment with a capacity of 2 L (distillation balloon) was used. Approximately 500 g of plant material was weighed, washed with water and sorted to ensure good condition, then chopped and subjected to the extraction process in the extraction balloon, which contained 300 mL of distilled water. In the steam extraction process using heating, the extraction time was 30 minutes divided into three cycles of 10 minutes each. A conventional oven (SAMSUNG AME9114ST) was used as a source of microwave radiation. The essential oils (EO) obtained were collected in a DeanStark container. The EO was separated by decanting and immediately stored in a 4mL amber vial.

2.3 Yield of the essential oil.

The following expression was used to determine the essential oil yield: R=(V/M)*100; where R: yield (%), V: volume of essential oil (mL) and M: mass of plant material (g) [11].

2.4 In vitro evaluation of the antifungal activity of the essential oil.

The fungus used for the antimicrobial assays corresponds to the strain identified by the Universidad de los Andes as Colletotrichum gloeosporioides (Penz.) Penz. and Sacc., which causes anthracnose in yam crops in the department of Sucre.

The inhibitory activity test of lemongrass essential oils was carried out against the phytopathogenic fungus C. gloeosporioides, causal agent of the disease known as yam anthracnose, by preparing concentrations of each essential oil of 5,000, 8,000 and 10,000 ppm, previously dehydrated with sodium sulphate anhydride.

For the inhibitory activity test, the direct surface seeding method was used with pure growth of the isolates. Seedings of each isolate of approximately 6 mm diameter growth área [12] were sown on the surface of potato dextrose agar (PDA) medium enriched with the antibiotics chloramphenicol, ampicillin and rifampicin. The inhibition test was performed as follows: Isolates seeded on PDA were spiked with 30µL of each essential oil at different concentrations in ppm dissolved in acetone. A positive control with benomyl (1g/L) was used, which corresponds to the doses of this antifungal used by yam growers in the department of Sucre, and an absolute control without any type of treatment. The tests were incubated at 30°C for 8 days in light and dark intervals. The antifungal activity of the essential oil was evaluated by measuring the radial growth of each isolate with the different concentrations after day 8. The result was interpreted as percentage antifungal index: %I.A= [1- (Da/Db)] x 100, where Da corresponds to the growth of each treatment and Db to the growth of the absolute control [13]. To determine the efficiency of each oil, the %I.A of the negative control (acetone) is subtracted from the %I.A of each essential oil and compared with the positive control used in this experiment.

2.5 Analysis by gas chromatography/mass spectrometry (GC/MS).

The determination of the chemical components of the essential oils was carried out by the instrumental technique of Gas Chromatography coupled to Mass Spectrometry (GC/MS), using an Agilent 6890 N gas chromatography apparatus coupled to an Agilent 5973N mass selective detector. The Kovats indices were determined on a slightly polar capillary column DB_5MS 30 m x 320 µm x 0.5 µm long. Helium was used as carrier gas with a pressure of 0.27 psi and an average flow velocity of 40 cm/sec. The initial furnace temperature was 150 °C and the final temperature was 350 °C. The injector temperature was 250 °C and the detector temperature was 300 °C. The identity of the components was assigned by comparison of the experimentally obtained mass spectra for each component with those reported in the NIST98.L, NIST02.L and NIST5a.L databases.

2.6 Statistical análisis.

All assays were performed in triplicate. Results were expressed as mean ± SD (standard deviation). A Shapiro-Wilk test was performed to corroborate the normality of the data, significant differences were determined by analysis of variance (ANOVA) and Tukey's test to establish the correlation of antifungal activity against the oils according to the concentration used. Statistical analysis was performed using R software (The R Project for Statistical Computing) [14].

3. RESULTS

In vitro evaluation of the antifungal activity of lemongrass essential oils. The results of the in vitro test of antifungal activity against C. gloeosporioides (figure 1) for the lemongrass specimen collected in the city of Sincelejo, showed the highest inhibitory activity at a concentration of 5,000 ppm, obtaining an inhibition index percentage (%I. A) of 97.77 % (Figure 2). The analysis of variance shows significant differences (p-value= 0.0003689) between the lemongrass treatments used with respect to inhibition index against C. gloeosporioides. The Shapiro-Wilk normality test yielded a p-value= 0.149, which indicates that the data are normally distributed and with Tukey's test it was found that the highest % I.A (97.77 %), were observed for lemongrass at 5,000 ppm collected in Sincelejo, and no significant differences were found with respect to the positive control with benomyl (Figura 1D). The lowest inhibitory activity rates against C. gloeosporioides were reported for lemongrass EO collected in Sampués at 5,000 ppm (Figure, 1A and 2). The chemical characterisation of lemongrass essential oil collected in the department of Sucre-Colombia, showed Citral as the main secondary metabolite type compound with concentrations of 40.60 %, 37.40 % and 38.34 %, respectively (Figure 3).

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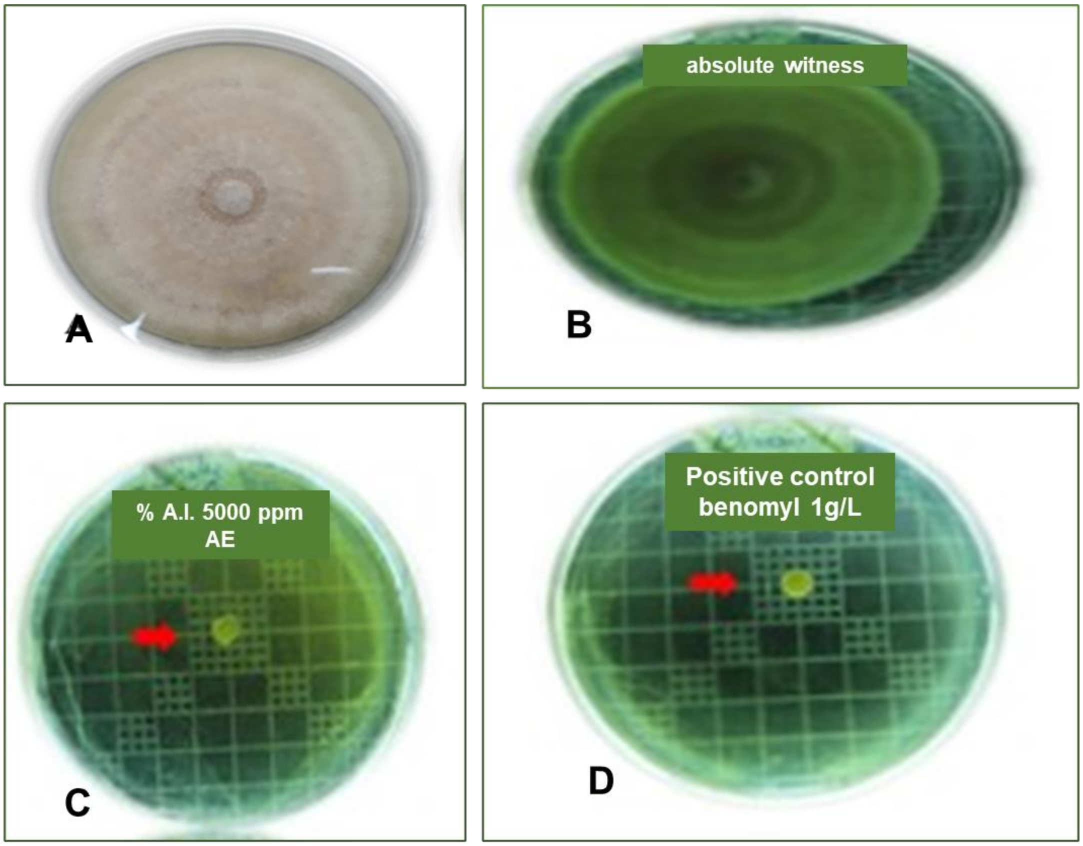
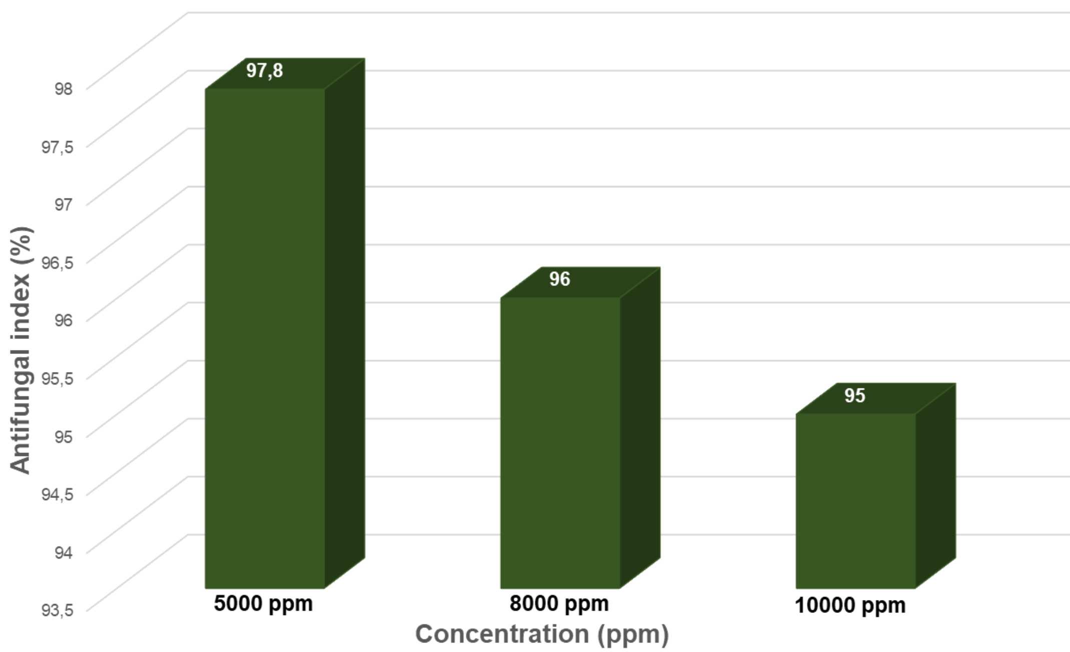


Figure 1. (A): Growth of the fungus C. gloeosporioides on potato-dextrose-agar-PDA medium. (B): Absolute witness; (C): % A.I 500 ppm and (D): Positive control benomy 1g/L.



(B)

Figure 2. Results of the inhibitory activity of essential oil of Cymbopogon citratus against C. gloeosporioides.

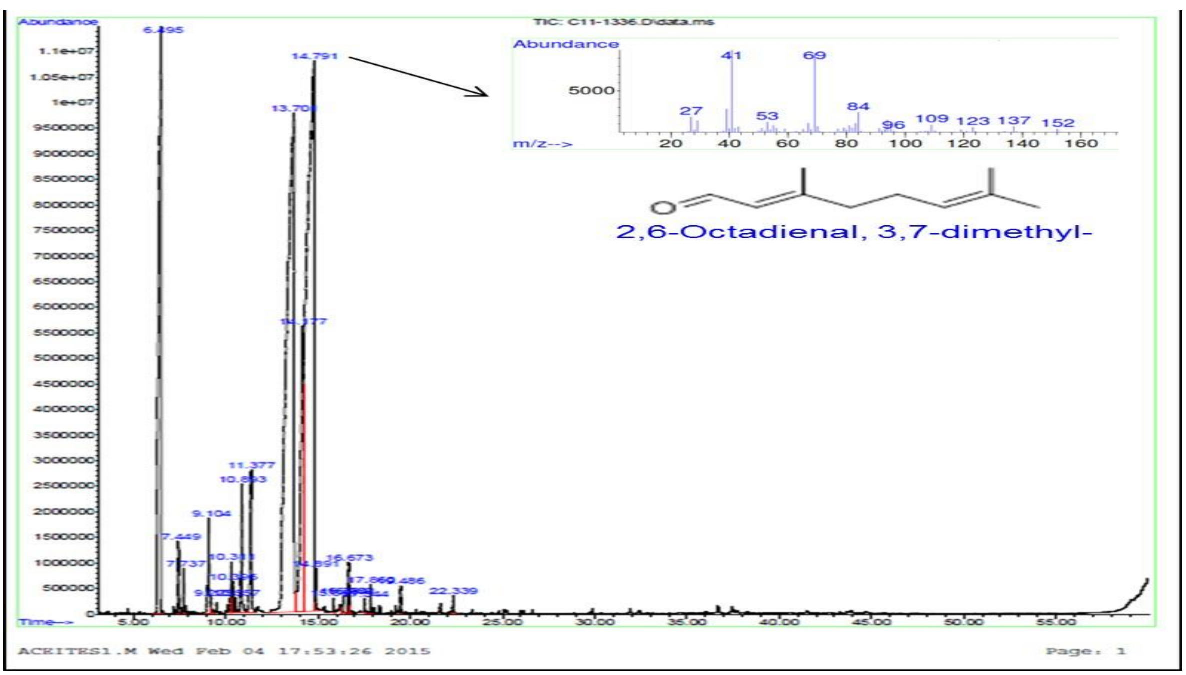


Figure 3. Chromatographic profile of the essential oil of C. citratus collected in the department of Sucre-Colombia.

4. DISCUSSION

The yield of the essential oil of C. citratus collected in the department of Sucre is between 0.29 % and 0.68 % (v/p), which indicates that the commercial exploitation of the essential oil of this plant species can be proposed, since its yield is higher than 0.1%, which is the minimum yield limit value [15]. The percentage yield found in this plant species is close to the values reported in the scientific literature, where percentages of 0.645 %(16), 1.06 % (17), 1.22 % (18) have been reported.

When comparing the highest inhibition indices of the different essential oils collected in the three municipalities with the % I.A obtained for the positive control with benomyl at 1g/L, it was found that at concentrations (5,000, 8,000 and 10,000 ppm) the efficiency of these essential oils was similar to that of the chemical control used by yam farmers in the field against C. gloeosporioides (Figure 1D).

The results of the chromatographic profile, carried out on the lemongrass species collected in the three municipalities show the same secondary metabolites with small differences such as the presence of Spiro (5.5) undec-1-ene found in the Sincelejo lemongrass, while in La Unión it presented Eucarvone and in Sampués 3-5-Heptadienal, 2-ethylidene-6-methyl- with very similar retention times. In addition, Caryophyllene was found in the latter two essential oils and not in the former.

Infusions of lemongrass leaves are used in folk medicine as an antimicrobial compound against various pathogenic bacteria and fungi, as an anti-inflammatory and sedative [9] This plant species has also been used in the food, perfume, cosmetics, pharmaceutical and insecticide industries.

However, comparisons of chromatographic profiles carried out on the essential oil of Cymbopogon citratus showed that the main secondary metabolite was Geranial with 25.50%19 and Geranial with 46.3% [19].

Studies carried out with essential oil of C. citratus collected in Sao Paulo, Brazil, showed a minimum inhibitory concentration of between 250 and 300 ppm (20). It has also been shown that this essential oil delays sporulation and the length of the germ tube of C. coccodes [21]. On the other hand, it has been shown that between 350 and 400 ppm the germination of C. acutatum spores is prevented by 100.00% with EO from fresh leaves of C. citratus collected in Antioquia-Colombia24. Concentrations between 0.25 and 0.3 mg/mL of C. citratus essential oil showed antagonistic activity against C. gloeosporioides [22]. It was also corroborated that 100 % of the mycelial growth of C. gloeosporioides is inhibited with lemongrass essential oil [23].

The area cultivated with yam in Colombia was drastically reduced by 77.6 %, i.e. from 20100 to 4500 ha cultivated in the second half of the 80's due to anthracnose caused by the phytopathogenic fungus C. gloeosporioides [24].

Conclusion

The implementation of products such as essential oil derived from plant biodiversity represent a great sanitary and economic alternative to minimise these effects. Based on the chromatographic analysis carried out, it was determined that the main component of the lemongrass harvested in the department of Sucre corresponds to Citral.

Author contribution: Alexander Pérez Cordero: research, conceptualization, writing - revision and editing. Donicer E. Montes Vergara: data analysis, revision and editing. Yelitza Aguas Mendoza: research, data analysis, writing - original draft.

Conflict of interest: The information contained in this manuscript is original, reviewed and prepared by the persons involved. We declare that there is no conflict of interest that could compromise the validity of the results.

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