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Ethanolic extract of *Copaifera*, *Croton* and *Lippia* on the control of phytopathogenic fungi[1]

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ABSTRACT

The search for vegetable extracts for phytosanitary control has been expanded to find new active ingredients to control plant diseases. This study aimed to evaluate the *in vitro* effect of the fixed constituents of *Copaifera luetzelburgii*, *Croton zehntneri* and *Lippia lasiocalycina*, at the concentrations of 2, 20, 200 and 2,000 μg mL^{-1}, on the percentage of mycelial growth inhibition of *Colletotrichum siamense*, *C. truncatum*, *Fusarium sacchari*, *F. udum*, *Lasiodiplodia theobromae* and *Thielaviopsis ethacetica*, as well as the conidium concentration of *C. siamense*, *F. sacchari* and *F. udum* produced in culture medium with all the extracts. The tested ethanolic extract, especially at the highest concentration, inhibited the percentage of mycelial growth and/or conidium concentration of the evaluated fungi. The other concentrations showed low inhibitory effects or no activity against the fungi. The average values for percentage of mycelial growth inhibition of the ethanolic extract from *L. lasiocalycina*, *C. zehntneri* and *C. luetzelburgii* against the six fungi were 62.5, 53.4 and 51.0 %, respectively. The ethanolic extract of *L. lasiocalycina* showed the most significant effect on the percentage of mycelial growth inhibition and conidia concentration. The fixed constituents of *C. luetzelburgii*, *C. zehntneri* and *L. lasiocalycina* at 2,000 μg mL^{-1} showed to be efficient in inhibiting the mycelial growth of *C. siamense*, *C. truncatum*, *F. sacchari*, *F. udum*, *L. theobromae* and *T. ethacetica*, and inhibit the conidia production of *C. siamense*, *F. sacchari* and *F. udum.*

KEYWORDS: Antifungal activity, bioactive botanical compounds, fungitoxicity, Brazilian Savanna plants.

RESUMO

Extrato etanólico de *Copaifera*, *Croton* e *Lippia* no controle de fungos fitopatogênicos

A busca por extratos vegetais para serem utilizados no controle fitossanitário tem sido ampliada, visando encontrar novos ingredientes ativos para o controle de doenças de plantas. Objetivou-se avaliar o efeito *in vitro* dos constituintes fixos de *Copaifera luetzelburgii*, *Croton zehntneri* e *Lippia lasiocalycina*, nas concentrações de 2; 20; 200; e 2.000 μg mL^{-1}, sobre o percentual de inibição de crescimento micelial de *Colletotrichum siamense*, *C. truncatum*, *Fusarium sacchari*, *F. udum*, *Lasiodiplodia theobromae* e *Thielaviopsis ethacetica*, bem como a concentração de conídios de *C. siamense*, *F. sacchari* e *F. udum* produzidos em meio de cultura com todos os extratos. O extrato etanólico testado, principalmente na maior concentração, inibiu o percentual de crescimento micelial e/ou concentração de conídios dos fungos avaliados. As demais concentrações apresentaram baixos efeitos inibitórios ou nenhuma atividade contra os fungos. Os valores médios para percentual de inibição de crescimento micelial do extrato etanólico de *L. lasiocalycina*, *C. zehntneri* e *C. luetzelburgii* contra os seis fungos foram de 62,5; 53,4; e 51,0 %, respectivamente. O extrato etanólico de *L. lasiocalycina* apresentou o efeito mais significativo sobre o percentual de inibição de crescimento micelial e concentração de conídios. Os constituintes fixos de *C. luetzelburgii*, *C. zehntneri* e *L. lasiocalycina* a 2.000 μg mL^{-1} mostraram-se eficientes na inibição do crescimento micelial de *C. siamense*, *C. truncatum*, *F. sacchari*, *F. udum*, *L. theobromae* e *T. ethacetica*, e inibem a produção de conídios de *C. siamense*, *F. sacchari* e *F. udum.*

PALAVRAS-CHAVE: Atividade antifúngica, compostos botânicos bioativos, fungitoxicidade, plantas do Cerrado.

INTRODUCTION

Plant diseases caused by fungi can affect the yield and quality of agricultural products, resulting in economic losses to the producer. *Colletotrichum*, *Fusarium*, *Lasiodiplodia* and *Thielaviopsis* are important pathogens of crops such as Fabaceae, Cucurbitaceae and fruit and palm trees in Brazil, causing severe damage (Cannon et al. 2012, Borges et al. 2019, Nikitin et al. 2023). Several measures can be taken to control the diseases. For example, chemical fungicides usually show a high efficiency (Molina

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et al. 2019). However, the continued use of these products leads to environmental and human health problems and selection of insensitive pathogens, since many of them are highly toxic and persistent (Carvalho 2017). The inherent disadvantages of chemical fungicides drive the search for alternative products to control fungal diseases (Zubrod et al. 2019).

Plants bioactive compounds have been successfully tested and used to control phytopathogenic fungi (Lengai et al. 2020, Pham et al. 2021). Their action can be proven by inhibiting mycelial growth and spore production (Saravanakumar et al. 2015, Mourão et al. 2017). The advantages of these compounds include the low cost and toxicity, ease of use, high efficiency and biodegradability (Kumar et al. 2019). Consequently, farmers are increasingly using products formulated with bioactive compounds extracted from plants due to the growing demand for organic products in the market.

Fixed oils (natural non-volatile oils) are among the bioactive compounds extracted from plant seeds, bark and fruit pulp. These oils are complex mixtures and water-insoluble (hydrophobic), what means they are soluble in nonpolar compounds (organic, predominantly formed by triglycerides) (Saravanakumar et al. 2015).

Several plants present antifungal properties. Therefore, they are excellent sources for oils or extracts with such activity. In this context, Cerrado (Brazilian Savanna) plants stand out (Colli et al. 2020). This biome covers about 23 % of the Brazilian territory and presents approximately 12,000 species of angiosperms (Zappi et al. 2015). Some native species of this biome (e.g.: *Copaifera* spp., *Croton* spp. and *Lippia* spp.) have demonstrated antimicrobial activity and can be used against plant pathogens (Carvalho et al. 2013, Peixoto et al. 2018, Andrade et al. 2020).

Thus, this study aimed to evaluate the *in vitro* effect of the ethanolic extract of *Copaifera luetzelburgii*, *Croton zehntneri* and *Lippia lasiocalycina* on the mycelial growth of *Colletotrichum siamense*, *Colletotrichum truncatum*, *Fusarium sacchari*, *Fusarium udum*, *Lasiodiplodia theobromae* and *Thielaviopsis ethacetica*, in addition to evaluating the concentration of conidia of *C. siamense*, *F. sacchari* and *F. udum* produced in culture medium with the three extracts.

MATERIAL AND METHODS

The experiment was conducted under laboratory conditions at the Universidade Federal do Piauí (UFPI), in Teresina, Piauí state, Brazil, from February 2019 to February 2020.

The plant material and extracts preparation are described as it follows: *L. lasiocalycina* (Verbenaceae): made available by the Embrapa Meio-Norte (Teresina, Piauí state), with voucher specimen deposited at the Embrapa Recursos Genéticos e Biotecnologia herbarium (number CEN92437); *C. zehntneri* (Euphorbiaceae): collected in Simões (Piauí state), being deposited at the Graziela Barroso herbarium of the UFPI (number 27.273); *C. luetzelburgii* (Fabaceae): quilombola community, São Miguel do Tapuio, Piauí state. An exsiccation of the species was deposited at the Graziela Barroso herbarium under the number TEPB 26235. More details about the plant material, extraction process and analysis can be seen in previous publications (Almeida et al. 2018, Fonseca et al. 2019, Almeida et al. 2021, Lima et al. 2021).

The organic geochemistry research group at the UFPI provided the ethanolic extract of *C. zehntneri*, *L. lasiocalycina* and *C. luetzelburgii*. The collected material (*C. zehntneri* stem bark and *C. zehntneri* and *L. lasiocalycina* leaves) was dried in an oven with humid air draught at the temperature of 40 ºC, for 48 h, and stored away from light and moisture. Then, the material was subjected to extraction in ethanol. After the extraction, a simple filtration was performed, and the extracts were concentrated at a rotary evaporator under reduced pressure.

Six isolates belonging to the phytopathogenic fungi collection of the UFPI (COUFPI) were used. The isolates were previously identified by morphological markers and multilocus phylogenetic analysis (data not shown): *Colletotrichum siamense* (COUFPI 233), *C. truncatum* (COUFPI 227), *Fusarium sacchari* (COUFPI 72), *F. udum* (COUFPI 34), *Lasiodiplodia theobromae* (COUFPI 264) and *Thielaviopsis ethacetica* (COUFPI 01). All isolates were cultivated in potato-dextrose-agar (PDA) medium and kept at 26 ± 2 ºC in a growth chamber, with a photoperiod of 12 h, until the experiments were carried out.

The extracts of *C. luetzelburgii*, *C. zehntneri* and *L. lasiocalycina* were solubilized in P.A. ethanol and homogenized using a vortex mixer. Then, they

were filtered on a 0.22 µm PES membrane and stored in sterile 15 mL conical polypropylene tubes. Concentrations of 2, 20, 200 and 2,000 µg mL^{-1} were used for all the extracts. The extracts were added separately to the PDA culture medium, melted at a maximum temperature of 45 ºC, containing the antibiotic streptomycin at a concentration of 0.2 g L^{-1}, and then poured into sterile Petri dishes with 90 mm diameter. Each plate was inoculated, in the center, with a 5 mm diameter disk of culture medium containing mycelium from pure cultures of the fungal isolates. The plates were kept at 26 ± 2 ºC, with a photoperiod of 12 h, in a growth chamber. The control comprised Petri dishes containing only PDA culture medium with the addition of streptomycin and inoculated with a 5 mm fungal disc in the center (Cruz et al. 2018).

Assessments were performed daily at the same time, starting at 24 h after the beginning of incubation. The diameters of the colonies were measured on the orthogonal axis (average of two diametrically opposite measurements) until the control treatment reached the total diameter of the Petri dish. After the evaluations, the percentage of mycelial growth inhibition (PMGI) was calculated in comparison to the control sample, without the addition of extract, where: PMGI = [(diameter of the control treatment - diameter of the treatment)/mean diameter of the control] x 100 (Salgado et al. 2003).

The experimental design was completely randomized in a factorial scheme, with five replications. The obtained data were submitted to analysis of variance with the "F" test and regression analysis when significant, evaluating the trend line and "R^2". The data analysis was performed using the R software v. 3.5.1.

To each colony's surface were added 10 mL of sterilized distilled water and Tween 20 (1 %), followed by scraping with the aid of a Drigalski loop. The conidia from each plate were filtered with gauze and resuspended in 50 mL of sterilized distilled water. Three 100 µL aliquots of each conidia suspension were transferred separately to a Neubauer chamber. The conidia suspension was shaken to homogenize the sample. Next, the slide was covered with a coverslip, and the suspension was transferred with the aid of a micropipette. A drop was applied to one of the coverslip vertices, and the slide was tilted little by little to fill the compartments with liquid. After preparing the slide with the suspension, a perfect spore distribution was waited for 2 to 3 min before counting. Then, the spores were counted under an optical microscope (Carvalho et al. 2013).

The "C" compartment was used to count the six fungi in all the fixed oils. Individual counts were carried out in the four sub-compartments "c" in the corners and the center. Then, the average of five counts was calculated, and the following formula was used (Alfenas & Mafia 2016): inoculum concentration = (average number of conidia in the "C" compartment) x (2.5×10^5).

The obtained data were subjected to analysis of variance and the means compared by the Tukey test at 5 % of significance, using the R software v. 3.5.1. The Kruskal-Wallis test was applied in the absence of data normality.

RESULTS AND DISCUSSION

The highest concentration (2,000 µg mL^{-1}) of *C. luetzelburgii*, *C. zehntneri* and *L. lasiocalycina* extract inhibited the mycelial growth of all the evaluated fungi. The other concentrations demonstrated low inhibitory effects. Therefore, the following results refer only to the highest concentration of the extracts tested.

The extracts' average percentages of mycelial growth inhibition from *L. lasiocalycina*, *C. zehntneri* and *C. luetzelburgii* were 62.5, 53.4 and 51.0 %, respectively, against the six fungi species. The extract that best inhibited the mycelial growth of the six fungi was *L. lasiocalycina*, reaching percentages of 100, 86.1 and 43.4 % on *T. ethacetica*, *L. theobromae* and *C. truncatum*, respectively (Figures 1B, 1E and 1F).

The highest percentage of mycelial growth inhibition (100 %) was observed for the *L. lasiocalycina* extract on *T. ethacetica* (Figure 1E), while *C. zehntneri* was the lowest one (34 %) on *F. sacchari* (Figure 1C). The most significant inhibitions among all the fungal species were observed on *T. ethacetica*, as the extracts from *L. lasiocalycina*, *C. zehntneri* and *C. luetzelburgii* presented mycelial growth inhibition of 100, 87.3 and 72.4 %, respectively (Figure 1E). The extracts from *L. lasiocalycina*, *C. zehntneri* and *C. luetzelburgii* showed mycelial growth inhibition of 86.1, 66.5 and 45.4 %, respectively, on *L. theobromae* (Figure 1F).

Thus, the highest concentration of all fixed extracts was the one that exerted the most significant

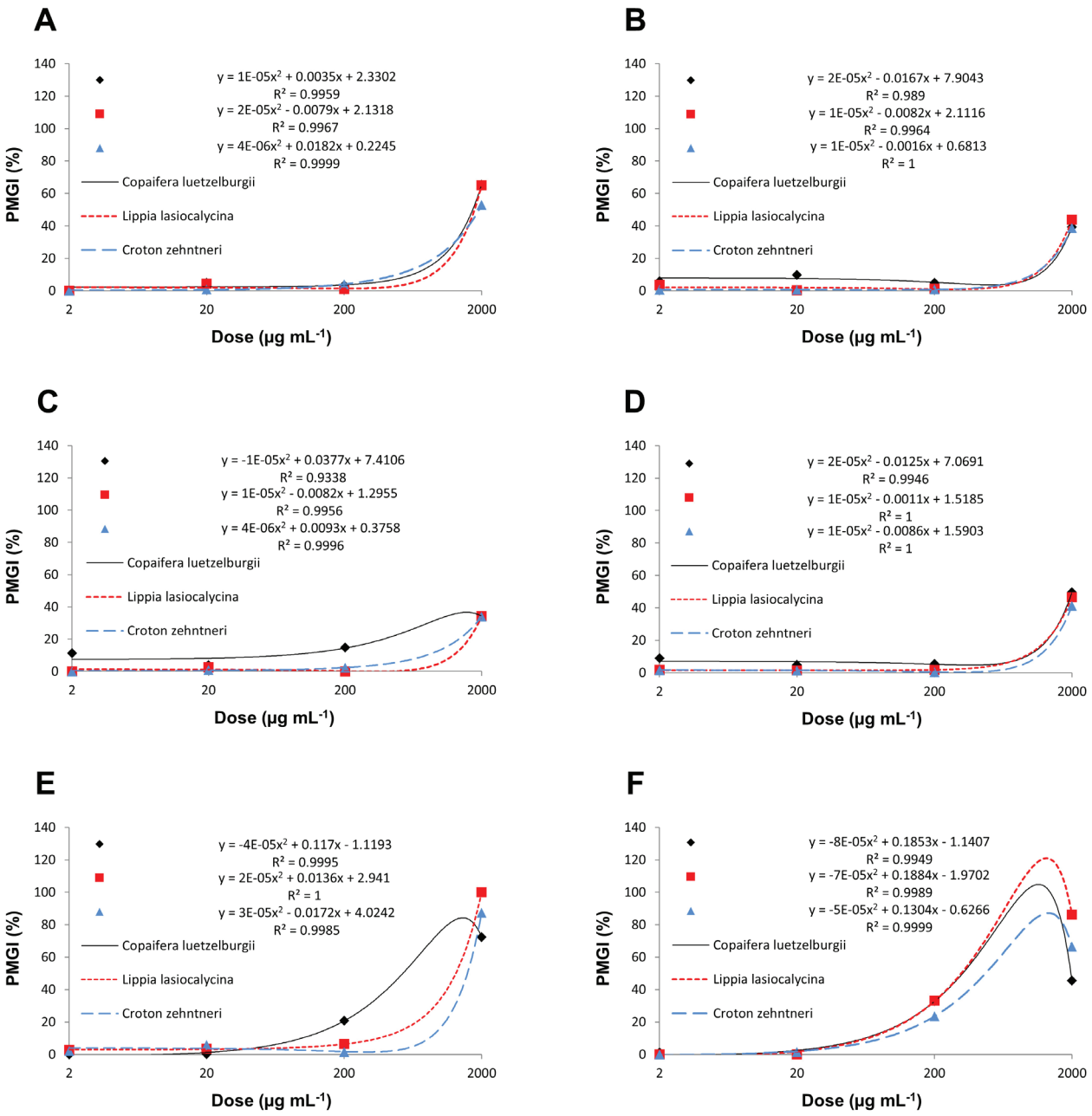

Figure 1. Effect of *Copaifera luetzelburgii*, *Lippia lasiocalycina* and *Croton zehntneri* ethanolic extracts on the percentage of mycelial growth inhibition (PMGI) of *Colletotrichum siamense* (A), *Colletotrichum truncatum* (B), *Fusarium sacchari* (C), *Fusarium udum* (D), *Thielaviopsis ethacetica* (E) and *Lasiodiplodia theobromae* (F).

percentages of mycelial growth inhibition on the fungi tested. A mycelial growth inhibition above 50 % was exerted by the three extracts on *C. siamense* and *T. ethacetica* and by *C. zehntneri* and *L. lasiocalycina* on *L. theobromae*.

The conidia production was determined at the end of the mycelial growth assessment. Only the *C. siamense*, *F. sacchari* and *F. udum* fungi produced conidia. The others (i.e., *C. truncatum*, *L. theobromae* and *T. ethacetica*) were not evaluated, since they did not produce conidia in any of the treatments.

The conidia production of *C. siamense* (Table 1) was completely inhibited by 2,000 µg mL^{-1} of *C. luetzelburgii* and *L. lasiocalycina* extracts. The other concentrations tested for the three extracts were inefficient in inhibiting the conidia production. The

Table 1. Concentration of *Colletotrichum siamense* conidia in doses of *Copaifera luetzelburgii*, *Lippia lasiocalycina* and *Croton zehntneri* extracts.

Dose (µg mL^{-1})	Concentration (conidia x 10^5 mL^{-1})		
	Copaifera luetzelburgii	*Lippia lasiocalycina*	*Croton zehntneri*
2	3.50 a	3.50 a	2.17 a
20	2.67 ab	1.50 ab	2.83 a
200	2.17 ab	0.67 abc	3.00 a
2,000	0.00 b	0.00 c	0.00 a
Control	4.50 a	0.17 bc	4.00 a

* Means followed by the same letter do not differ statistically from each other by the Tukey test (p > 0.05).

Table 3. Concentration of *Fusarium udum* conidia in doses of *Copaifera luetzelburgii*, *Lippia lasiocalycina* and *Croton zehntneri* extracts.

Dose (µg mL^{-1})	Concentration (conidia x 10^5 mL^{-1})		
	Copaifera luetzelburgii	*Lippia lasiocalycina*	*Croton zehntneri*
2	9.66 a	31.33 a	20.33 a
20	4.33 a	26.83 a	23.16 a
200	5.83 a	16.66 b	27.83 a
2,000	3.50 a	15.00 b	9.16 b
Control	8.50 a	30.33 a	27.83 a

* Means followed by the same letter do not differ statistically from each other by the Tukey test (p > 0.05).

extract from *C. zehntneri* did not differ from the control in any of the concentrations. There is already a report of the volatile composition of *C. zehntneri* oils, whose main component is trazol, with known bactericidal activity (Andrade et al. 2015). This compound is also present in the fixed constituents. Apparently, this compound (and others present in the extract) does not show antifungal activity against *C. zehntneri*.

In their highest concentration, the extracts from *C. luetzelburgii*, *C. zehntneri* and *L. lasiocalycina* were the most efficient in reducing the *F. sacchari* conidia production (74, 62 and 83 %, respectively) (Table 2). Furthermore, lower concentrations of extracts from *C. luetzelburgii* and *L. lasiocalycina* had less pronounced effects on the inhibition of conidia germination.

The most efficient concentration in reducing the *F. udum* conidia production was 2,000 µg mL^{-1} of the extracts from *C. zehntneri* and *L. lasiocalycina* (67 and 50 %, respectively) (Table 3). The extracts from *C. luetzelburgii* did not differ from the control.

Table 2. Concentration of *Fusarium sacchari* conidia in doses of *Copaifera luetzelburgii*, *Lippia lasiocalycina* and *Croton zehntneri* extracts.

Dose (µg mL^{-1})	Concentration (conidia x 10^5 mL^{-1})		
	Copaifera luetzelburgii	*Lippia lasiocalycina*	*Croton zehntneri*
2	18.33 a	43.00 b	26.50 a
20	17.00 b	29.83 c	25.33 a
200	18.50 b	26.00 c	27.50 a
2,000	7.67 c	8.83 d	8.50 b
Control	29.50 a	53.33 a	22.83 a

* Means followed by the same letter do not differ statistically from each other by the Tukey test (p > 0.05).

The ethanolic extracts yields were 4.9, 8.8 and 11.2 % for *C. luetzelburgii*, *C. zehntneri* and *L. lasiocalycina*, respectively. Phytochemical tests for the fixed extract of *C. luetzelburgii* stem bark indicated the substantial presence of compounds such as anthocyanins, anthocyanidins, aurones, chalcones, flavanones, flavanonols, flavonols, leucoanthocyanidins and xanthones, all of which have a potential antioxidant activity (Lima et al. 2021). Furthermore, compounds such as trans-docosanil, ferulate, acetyl aleuritolic acid, 3-O-methylquercetin, E-anethole, 2-hydroxy-4,6-dimethoxyacetophenonan, 3-O-methylquercetin, β-sitosterol, stigmasterol (Santos et al. 2017), crototropone (Bracher et al. 2008) and triterpene acetyl aleuritolic acid (Bezerra et al. 2021) have been identified in fixed extracts of *C. zehntneri*. Moreover, in the ethanolic extract of leaves and stem of *L. lasiocalycina*, phenylpropanoid (Forsitoside B) and verbacoside were detected (Funari et al. 2012). Overall, volatile constituents have been widely used since ancient times, and their role and action have been discussed regarding their bioactivity as antibacterial, antiviral, antioxidant and antidiabetic by Tanu & Harpreet (2016).

It is known that several species from the *Lippia* genus have antifungal properties in their oils/extracts (Pandey et al. 2016, Peixoto et al. 2018). However, until the time that this study was carried out, the effects of the ethanolic extract of *L. lasiocalycina* against phytopathogenic fungi were not known. According to the results, the ethanolic extract of *L. lasiocalycina* presented fungicide activity against a wide variety of fungi, such as *Colletotrichum*, *Fusarium*, *Lasiodiplodia* and *Thielaviopsis*, showing antifungal properties at different levels. This activity

can be attributed to the piperitenone oxide, which represents about 58 % of the essential oil composition. Piperitenone oxide has several industrial applications and has shown potential properties against herpes and *Aedes aegypti*, vasodilatory effects in hypertensive rats and antiparasitic effect (Almeida et al. 2018).

The hydrophobic monoterpenes (e.g., limonene and piperitenone oxide) and other compounds in the *L. lasiocalycina* essential oil act on the plasma membrane of *Candida albicans*, which is a yeast capable of causing mycosis in humans. The antifungal property can be attributed to the disruption of the plasma membrane (Almeida et al. 2018).

There are more than 20 *Copaifera* spp. already described, some of which have had their antimicrobial activity evaluated on bacteria and dermatophyte fungi (Santos et al. 2008, Zimmermmam-Franco et al. 2013). However, this is the first time that the effect of the ethanolic extract of *C. luetzelburgii* is assessed on the mycelial growth and conidium concentration of phytopathogenic fungi. The main compounds in the essential oils of *Copaifera* spp. are sesquiterpenes and diterpenes, which seem responsible for their antifungal properties (Veiga-Júnior et al. 2001).

The larvicidal and antifungal properties of *Croton* spp. oils, including *C. zehntneri*, have already been demonstrated (Sing et al. 2006, Fontenelle et al. 2008). The main compounds in the *C. zehntneri* oil are estragole and anethole (Fontenelle et al. 2008), which may be responsible for inhibiting the growth of dermatophyte and saprophyte fungi. However, the inhibitory concentrations against fungi have been high, i.e., between 25,000 and 100,000 μg mL^{-1} (Pereira et al. 2021). In this study, the concentration of 2,000 μg mL^{-1} was moderately effective in inhibiting the growth of the fungi evaluated, showing an excellent potential in controlling phytopathogenic fungi.

Only one qualitative study presents the composition of the fixed oil of *C. luetzelburgii* through chemical tests (Araújo et al. 2021). Its preliminary results indicate the presence of flavanones, flavanonols and flavonols. It also studied its antioxidant effect and toxicity on the reproductive system in rats. The subchronic toxicity was evaluated through the number, morphology and functional viability of spermatozoa and histopathology.

The findings of the present study emphasize the potential of these plant extracts as effective agents for phytosanitary control, suggesting their possible application as environmentally friendly alternatives to synthetic pesticides. However, further research is necessary to explore their full potential and ensure their safe and practical use in agricultural practices.

CONCLUSIONS

The fixed constituents of *Copaifera luetzelburgii*, *Croton zehntneri* and *Lippia lasiocalycina* at 2,000 μg mL^{-1} are efficient in inhibiting the mycelial growth of *Colletotrichum siamense*, *C. truncatum*, *Fusarium sacchari*, *F. udum*, *Lasiodiplodia theobromae* and *Thielaviopsis ethacetica*. Furthermore, they inhibit the conidia production of *C. siamense*, *F. sacchari* and *F. udum*.

REFERENCES

ALFENAS, A. C.; MAFIA, R. G. (ed). *Métodos em fitopatologia*. Viçosa: Ed. UFV, 2016.

ALMEIDA, W. S.; LIMA, S. G.; BARRETO, H. M.; ANDRADE, L. M. S.; FONSECA, L.; ATHAYDE SOBRINHO, C.; SANTOS, A. R. B.; MURATORI, M. C. S. Chemical composition and antimicrobial activity of the essential oil of *Lippia lasiocalycina* Cham. (Verbenaceae). *Industrial Crops and Products*, v. 125, n. 1, p. 236-240, 2018.

ALMEIDA, W. S.; LIMA, S. G.; SANTOS, M. C.; ATHAYDE SOBRINHO, C.; BACELAR, R. G. A.; MURATORI, M. C. S. Determination of antifungal and anti-aflatoxigenic activity of the essential oil of *Lippia lasiocalycina* Cham (Verbenacea). *Current Aspects in Pharmaceutical Research and Development*, v. 2, n. 1, p. 37-51, 2021.

ANDRADE, G.; ORLANDO, H. C. S.; SCORZONI, L.; PEDROSO, R. S.; ABRÃO, F.; CARVALHO, M. T. M.; VENEZIANI, R. C. S.; AMBRÓSIO, S. R.; BASTOS, J. K.; MENDES-GIANNINI, M. J. S.; MARTINS, C. H. G.; PIRES, R. H. Brazilian *Copaifera* species: antifungal activity against clinically relevant candida species, cellular target, and *in vivo* toxicity. *Journal of Fungi*, v. 6, n. 3, e153, 2020.

ANDRADE, T. C.; LIMA, S. G.; FREITAS, R. M.; ROCHA, M. S.; ISLAM, T.; SILVA, T. G.; MILITÃO, G. C. Isolation, characterization and evaluation of antimicrobial and cytotoxic activity of estragole, obtained from the essential oil of *Croton zehntneri* (Euphorbiaceae). *Anais da Academia Brasileira de Ciências*, v. 87, n. 1, p. 173-82, 2015.

ARAÚJO, J. L. A.; FERNANDES, M. Z. L. C. M.; LIMA, S. G.; SANTOS, M. C.; SILVA, S. M. M. S.;

BRANCO, M. A. C.; FERNANDES, R. M. Study on *Copaifera luetzelburgii* Harms: phytochemistry evaluation, antioxidant activity and toxicological ethanol extract on the reproductive system of rats. *Current Aspects in Pharmaceutical Research and Development*, v. 3, n. 1, p. 73-87, 2021.

BEZERRA, A. J. N.; SILVA, F. C. O.; SILVA, A. W.; FERREIRA, M. K. A.; MARINHO, E. M.; MARINHO, M. M.; MAGALHÃES, F. E. A.; BANDEIRA, P. N.; RODRIGUES, T. A. M.; MARINHO, E. S.; MENEZES, J.; SANTOS, H. S. Antinociceptive effect of triterpene acetyl aleuritolic acid isolated from *Croton zehntneri* in adult zebrafish (*Danio rerio*). *Biochemical and Biophysical Research Communications*, v. 534, n. 1, p. 478-484, 2021.

BORGES, A. F.; ALCÂNTARA NETO, F.; MATOS, K. S.; BESERRA JÚNIOR, J. E. A.; MASSOLA JÚNIOR, N. S.; MOREIRA, S. I.; MELO, M. P. *Thielaviopsis ethacetica*: the etiological agent of sugarcane pineapple sett rot disease in Brazil. *Tropical Plant Pathology*, v. 44, n. 5, p. 460-467, 2019.

BRACHER, F.; RANDAU, K. P.; LERCHER, H. Crototropone, a new tropone derivative from *Croton zehntneri*. *Fitoterapia*, v. 79, n. 3, p. 236-237, 2008.

CANNON, P. F.; DAMM, U.; JOHNSTON, P. R.; WEIR, B. S. *Colletotrichum*: current status and future directions. *Studies in Mycology*, v. 73, n. 1, p. 181-213, 2012.

CARVALHO, F. P. Pesticides, environment, and food safety. *Food and Energy Security*, v. 6, n. 2, p. 48-60, 2017.

CARVALHO, R. R. C.; LARANJEIRA, D.; CARVALHO FILHO, J. L. S.; SOUZA, P. E.; BLANK, A. F.; ALVES, P. B.; JESUS, H. C. R.; WARWICK, D. R. *In vitro* activity of essential oils of *Lippia sidoides* and *Lippia gracilis* and their major chemical components against *Thielaviopsis paradoxa*, causal agent of stem bleeding in coconut palms. *Química Nova*, v. 36, n. 2, p. 241-244, 2013.

COLLI, G.; VIEIRA, C. R.; DIANESE, J. D. Biodiversity and conservation of the Cerrado: recent advances and old challenges. *Biodiversity and Conservation*, v. 29, n. 5, p. 1465-1475, 2020.

CRUZ, E. M. O.; MENDONÇA, M. C.; BLANK, A. F.; SAMPAIO, T. S.; PINTO, J. A. O.; GAGLIARDI, P. R.; OLIVEIRA JÚNIOR, L. F. G.; LIMA, R. S. N.; NUNES, R. S.; WARWICK, D. R. *Lippia gracilis* Schauer essential oil nanoformulation prototype for the control of *Thielaviopis paradoxa*. *Industrial Crops and Products*, v. 117, n. 1, p. 245-251, 2018.

FONSECA, L.; ROCHA, M. S.; BRITO, L. C. F.; SOUSA, E. S.; REINALDO, F. S.; PEREIRA, F. M. M.; SANTOS, F. E. P.; LIMA, S. G. Characterization of inclusion complex of *Croton zehntneri* essential oil and β-cyclodextrin prepared by spray drying and freeze drying. *Revista Virtual de Química*, v. 11, n. 2, p. 529-542, 2019.

FONTENELLE, R. O. S.; MORAIS, S. M.; BRITO, E. H.; BRILHANTE, R. S.; CORDEIRO, R. A.; NASCIMENTO, N. R.; KERNTOPF, M. R.; SIDRIM, J. J.; ROCHA, M. F. Antifungal activity of essential oils of *Croton* species from the Brazilian Caatinga biome. *Journal of Applied Microbiology*, v. 104, n. 5, p. 1383-1390, 2008.

FUNARI, C. S.; GULLO, F. P.; NAPOLITANO, A.; CARNEIRO, R. L.; MENDES-GIANNINI, M. J.; FUSCO-ALMEIDA, A. M.; PIACENTE, S.; PIZZA, C.; SILVA, D. H. Chemical and antifungal investigations of six *Lippia* species (Verbenaceae) from Brazil. *Food Chemistry*, v. 135, n. 3, p. 2086-2094, 2012.

KUMAR, A.; AHMAD, F.; ZAIDI, S. Importance of bioactive compounds present in plant products and their extraction: a review. *Agricultural Reviews*, v. 40, n. 4, p. 249-260, 2019.

LENGAI, G. M. W.; MUTHOMI, J. W.; MBEGA, E. R. Phytochemical activity and role of botanical pesticides in pest management for sustainable agricultural crop production. *Scientific African*, v. 7, e00239, 2020.

LIMA, S. G.; FIGUEREDO, J. S.; SANTOS, M. C.; MENESES, A. K. S.; ROCHA, M. S.; COSTA, J. G. M.; FERNANDES, R. M. Study on volatile constituents, cytotoxic activity and antioxidant potential of fixed extracts of *Copaifera luetzelburgii* Harms. *Current Aspects in Pharmaceutical Research and Development*, v. 2, n. 1, p. 22-36, 2021.

MOLINA, J. P. E.; PAUL, P. A.; AMORIM, L.; SILVA, L. H. C. P.; SIQUERI, F. V.; BORGES, E. P.; CAMPOS, H. D.; NUNES JÚNIOR, J.; MEYER, M. C.; MARTINS, M. C.; BALARDIN, R. S.; CARLIN, V. J.; GRIGOLLI, J. F. J.; BELUFI, L. M. R.; GODOY, C. V. Meta-analysis of fungicide efficacy on soybean target spot and cost-benefit assessment. *Plant Pathology*, v. 68, n. 1, p. 94-106, 2019.

MOURÃO, D. S. C.; PEREIRA, T. F. S.; SOUZA, D. J.; CHAGAS JÚNIOR, A. F.; DALCIN, M. S.; VELOSO, R. A.; LEÃO, E. U.; SANTOS, G. R. D. Essential oil of *Cymbopogon citratus* on the control of the *Curvularia* leaf spot disease on maize. *Medicines*, v. 4, n. 3, e62, 2017.

NIKITIN, D. A.; IVANOVA, E. A.; SEMENOV, M. V.; ZHELEZOVA, A. D.; KSENOFONTOVA, N. A.; TKHAKAKHOVA, A. K.; KHOLODOV, V. A. Diversity, ecological characteristics and identification of some problematic phytopathogenic *Fusarium* in soil: a review. *Diversity*, v. 15, n. 1, e49, 2023.

PANDEY, A. K.; SONKER, N.; SINGH, P. Efficacy of some essential oils against *Aspergillus flavus* with special reference to *Lippia alba* oil, an inhibitor of fungal proliferation and aflatoxin B1 production in green gram

seeds during storage. *Journal of Food Science*, v. 81, n. 4, p. 928-934, 2016.

PEIXOTO, M. G.; BLANK, A. F.; ARRIGONI-BLANK, M. F.; GAGLIARDI, P. R.; MELO, J. O.; NIZIO, D. A. C.; PINTO, V. S. Activity of essential oils of *Lippia alba* chemotypes and their major monoterpenes against phytopathogenic fungi. *Bioscience Journal*, v. 34, n. 5, p. 1136-1146, 2018.

PEREIRA, F. O.; TEIXEIRA, A. P. C.; MEDEIROS, F. D. Do essential oils from plants occurring in the Brazilian Caatinga biome present antifungal potential against dermatophytoses?: a systematic review. *Applied Microbiology and Biotechnology*, v. 105, n. 18, p. 6559-6578, 2021.

PHAM, D. Q.; PHAM, H. T.; HAN, J. W.; NGUYEN, T. H.; NGUYEN, H. T.; NGUYEN, T. D.; NGUYEN, T. T. T.; HO, C. T.; PHAM, H. M.; VU, H. D.; CHOI, G. J.; DANG, Q. L. Extracts and metabolites derived from the leaves of *Cassia alata* L. exhibit *in vitro* and *in vivo* antimicrobial activities against fungal and bacterial plant pathogens. *Industrial Crops and Products*, v. 166, e113465, 2021.

SALGADO, A. P. S. P.; CARDOSO, M. G.; SOUZA, P. E.; SOUZA, J. A.; ABREU, C. M. P.; PINTO, J. E. B. P. Avaliação da atividade fungitóxica de óleos essenciais de folhas de *Eucalyptus* sobre *Fusarium oxysporum*, *Botrytis cinerea* e *Bipolares sorokiniana*. *Ciência e Agrotecnologia*, v. 27, n. 2, p. 249-254, 2003.

SANTOS, A. O.; UEDA-NAKAMURA, T.; DIAS FILHO, B. P.; VEIGA JUNIOR, V. F.; PINTO, A. C.; NAKAMURA, C. V. Antimicrobial activity of Brazilian copaiba oils obtained from different species of the *Copaifera* genus. *Memória do Instituto Oswaldo Cruz*, v. 103, n. 3, p. 277-281, 2008.

SANTOS, H. S.; FURTADO, E.; RODRIGUES, A. S.; BANDEIRA, P. N.; LEMOS, T. L. G.; BEZERRA, A. M. C.; BRAZ-FILHO, R. Chemical composition and antioxidant activity of chemical constituents from *Croton zehntneri* (Euphorbiaceae). *Journal of Pharmacognosy and Phytochemistry*, v. 6, n. 4, p. 1146-1149, 2017.

SARAVANAKUMAR, D.; KARTHIBA, L.; RAMJEGATHESH, R.; PRABAKAR, K.; RAGUCHANDER, T. Characterization of bioactive compounds from botanicals for the management of plant diseases. *In*: GANESANM, S.; VADIVEL, K.; JAYARAMAN, J. *Sustainable crop disease management using natural products*. Wallingford: CAB International, 2015. p. 1-18.

SINGH, G.; MAURYA, S.; LAMPASONA, M. P.; CATALAN, C. Chemical constituents, antifungal and antioxidative potential of *Foeniculum vulgare* volatile oil and its acetone extract. *Food Control*, v. 17, n. 9, p. 745-752, 2006.

TANU, B.; HARPREET, K. Benefits of essential oil. *Journal of Chemical and Pharmaceutical Research*, v. 8, n. 6, p. 143-149, 2016.

VEIGA-JÚNIOR, V. F.; ZUNINO, L.; CALIXTO, J. B.; PATITUCCI, M. L.; PINTO, A. C. Phytochemical and antioedematogenic studies of commercial copaiba oils available in Brazil. *Phytotherapy Research*, v. 15, n. 6, p. 476-480, 2001.

ZAPPI, D. C.; FILARDI, F. L. R.; LEITMAN, P. Growing knowledge: an overview of seed plant diversity in Brazil. *Rodriguésia*, v. 66, n. 4, p. 1085-1113, 2015.

ZIMMERMMAM-FRANCO, D. C.; BOLUTARI, E. B.; POLONINI, H. C.; CARMO, A. M.; CHAVES, M. D.; RAPOSO, N. R. Antifungal activity of *Copaifera langsdorffii* Desf oleoresin against dermatophytes. *Molecules*, v. 18, n. 10, p. 12561-12570, 2013.

ZUBROD, J. P.; BUNDSCHUH, M.; ARTS, G.; BRÜHL, C. A.; IMFELD, G.; KNÄBEL, A.; PAYRAUDEAU, S.; RASMUSSEN, J. J.; ROHR, J.; SCHARMÜLLER, A.; SMALLING, K.; STEHLE, S.; SCHULZ, R.; SCHÄFER, R. B. Fungicides: an overlooked pesticide class? *Environmental Science & Technology*, v. 53, n. 7, p. 3347-3365, 2019.