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EVALUATION OF THE ANTIMICROBIAL POTENTIAL OF ENDOPHYTIC MICROORGANISMS ISOLATED FROM *Piper hispidinervum*

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Abstract: Resistance to antimicrobial drugs has become a serious public health problem due to indiscriminate use by humans. Therefore, several alternatives have been studied to contain super microorganisms that cause diseases, among them the use of secondary compounds produced by plants together with endophytic microorganisms. Therefore, this work aimed to isolate, identify and analyze the antimicrobial potential of endophytic microorganisms of *Piper hispidinervum* C. DC. against pathogens that affect humans and different animals. First, the leaf and inflorescence were collected and the plant material was identified. The material collected went through the process of asepsis and inoculation, which resulted in the growth of endophytes. After their growth, several replications were performed to purify the colonies. Regarding the macromorphology of endophytic fungi, different textures and colors were observed. Were obtained 27 isolates of fungal cultures, among them the most found genera are *Colletotrichum* sp., and *Fusarium* sp., we also identified a species of *Curvularia* sp. The rest of the isolates were classified as *Mycelia sterilia.* For the antimicrobial susceptibility tests, *the inoculum of the test pathogens was carried out in Mueller-* Hinton culture medium and the suspension was adjusted to the MacFarland scale. To obtain the fungal extract, colony fragments were deposited in Erlenmeyer flasks with Potato-Dextrose medium, after 14 days the culture medium was filtered, then Ethyl Acetate was added to extract metabolites. The fungal extract obtained by Ethyl Acetate was used in the sensitivity test by the well diffusion method. The *Fusarium* Extract sp. esp07 was the most effective against the microorganisms tested, being able to inhibit *S. aureus, E. coli* and *C. albicans,* resulting in halos with an average of 23 mm, 21 mm and 14 mm, respectively. The *Fusarium* sp. esp04 and *Curvularia* sp. fol50 have also been shown to be effective against *E. coli.*

Keywords: *Piper* sp.; Fungal extracts; antimicrobial potential.

INTRODUCTION

Microbial resistance is a serious public health problem resulting from the indiscriminate use of antibiotics, therefore, investigations into new compounds of pharmaceutical applicability have been carried out, especially those of natural origin. The mechanism of resistance occurs through the selective pressure caused by the use of drugs on a large scale, as a consequence, infections previously controlled by these drugs can no longer be contained (SERRA VALDÉS, 2017).

In order to find a cure, treatment and prevention for various diseases, ancient civilizations already used flora for medicinal use, due to the presence of biologically active components. For millennia, human beings obtained knowledge empirically, teaching subsequent generations how to survive using plants taken from the environment in which they lived (ANGELO and RIBEIRO, 2014; FIRMO, 2011). From this perspective, natural products, which until then were predominantly extracted from plants, can now also be obtained from microorganisms that inhabit the interior of plant tissues. bioactive molecules with the most diverse activities including antimicrobial (MARTINEZ-KLIMOVA et al., 2017).

Endophytic microorganisms are living beings that inhabit the internal tissues of plants for some part or all of their life cycle without causing visible symptoms of disease. These have become of great technological interest, as they can produce antimicrobial, anticancer and antitumor compounds, in addition to providing defense against insects, helping plant growth and tolerance to ecological changes. Biotechnological studies

report the potential applicability in medicine, agriculture and industry (ASSUNÇÃO, 2010).

The symbiotic association between endophytic microorganisms and plant tissues is beneficial for both beings, as they help each other to survive, either by providing food or protection against invaders. In addition, some produce secondary metabolites that are widely used as antimicrobials, including flavonoids, alkaloids, phenols, sterols and terpenoids, however, studies show that endophytes are unable to produce them independently (FADIJI and BABALOLA, 2020).

Brazil has several species of plants that are used for their medicinal properties, among which are those belonging to the Piperaceae family, with more than 700 species distributed throughout the country, and about 300 of these are present in the rainforest of the Amazon. The genus *Piper* is the most abundant, mainly in the neotropics, occurring from Mexico to southern Brazil. They correspond to shrubs, herbs and vines, known to be used in teas and infusions for the treatment of diseases by traditional medicine (DOS SANTOS, 2018; GONÇALVES, 2022).

Long pepper (*Piper hispidinervum* Candolle, De Candolle) is a shrub that can reach about 7 meters in height, has elliptical leaves and inflorescence in the form of elongated spikes with tiny flowers. It is native to the Amazon, found mainly in pasture fields, and easily adapts to nutrient-poor soils. This species contains safrole, a compound extracted from the essential oil that is widely used by the industry in the composition of fragrances for beverages, cosmetics, insecticides and cleaning products (DE SANTIAGO, 2003).

For LIMA (2019), ethanolic extracts of *Piper callosum* and *Piper cocorvadensis* were effective against the microorganisms *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Candida krusei* and *Candida utilis.*

From studies with different species of the genus, and also the lack of content on the antimicrobial potential of endophytes present in *P. hispidinervum,* it is of paramount importance to obtain new information about this species. Therefore, this work aimed to both identify, isolate and evaluate whether the endophytic microorganisms of long pepper (*Piper hispidinervum*) have antimicrobial activity against pathogenic microorganisms.

MATERIALS AND METHODS

COLLECTION OF PLANT MATERIAL

Long pepper (*Piper hispidinervum*) samples were collected in the southern sector of ‘’Universidade Federal do Amazonas”, located in Manaus at 3°06’03.0”S 59°58’29.3”W.

The collected material was taken to the Microbiology Research Laboratory located at ICB 01 (Institute of Biological Sciences), UFAM. Samples of leaves and inflorescences, without any type of damage or disease, were collected and carefully washed under running water and rinsed with distilled water. After this procedure, median portions of the leaves were cut, fragments of 1.5 cm with the aid of sterilized scissors, the same was done for the inflorescence. Subsequently, all samples were submitted to previous sterilization treatment, according to PETRINI and FISHER (1986).

STERILIZATION OF PLANT MATERIAL

For surface disinfection, aiming at eliminating the epiphytic microbiota, successive immersions in: i) 70% alcohol (v/v) for 1 minute were used; ii) commercial bleach at 3% (v/v) for 3 minutes; iii) alcohol 70% (v/v) for 30 seconds; iv) autoclaved distilled water. This last immersion was performed in two sequential repetitions. 20 µL of the distilled water used for the 2nd repetition of immersion, consisting of the negative control, were seeded in a Petri dish containing specific

medium (Araújo et al., 2010).

After serial washings, the samples were deposited on an autoclaved Petri dish and, with the aid of a scalpel, were fragmented into squares of approximately 1.5 cm, which were deposited in specific culture media for the isolation of fungi.

All materials used in sample preparation and sterilization were manipulated in laminar flow to avoid contamination by other microorganisms.

ISOLATION OF MICROORGANISMS

After asepsis of the plant material, 3 fragments were placed in each Petri dish containing PDA culture medium (Potato, Dextrose and Agar) added 50 µL in Tetracycline antibiotic (50 mg/mL). Plates were incubated in BOD (Biological oxygen Demand) at +/- 35°C, and were examined over 14 consecutive days or longer, depending on the growth of the microorganisms.

For fungi, as the mycelia appeared on the culture medium, they were picked and isolated in Petri dishes containing PDA culture medium. Then, the plates were incubated again for mycelium growth and sporulation. Analyzes were performed in quadruplicate. The identification of endophytic fungi of *P. hispidinervum* was carried out by viewing the macro and micromorphology according to ARAUJO *et al.*, 2010.

STANDARDIZATION OF TEST MICROORGANISMS

Cultures of test microorganisms were maintained at 4°C in nutrient agar (NA). Samples were recovered in Mueller- Hinton broth (MH) for bacteria and Sabouraud Dextrose broth (SAB) for yeast, and incubated without agitation for 24 hours at 36°C. Subsequently, the inoculums were spread on Mueller Hinton agar and Sabouraud agar plates, 24 hours before the test. Culture suspensions were prepared, diluted in 0.85% saline solution using MacFarland 's 0.5 scale until approximately 1.5×10^6 Colony Forming Units ($CFU.mL^{-1}$) were obtained.

PREPARATION OF FUNGAL EXTRACTS

To obtain the fungal extracts from the endophytic microorganisms obtained in the isolation process, 50% potato-dextrose broth was used, where 30 mL of the medium was distributed in autoclaved Falcon tubes. When the medium temperature was between 36°C and 38°C, 3 fragments of 2 cm^2 of each fungal culture were placed in the flasks, closed and incubated in a BOD-type oven (25°C) for the growth of microorganisms for 14 days. After this period, the fermented culture medium was filtered using sterile filter paper, obtaining between 20-25 mL of each isolate. To carry out the liquid-liquid extraction, 25 mL of the solvent Ethyl Acetate was added to each filtrate, both were placed in a separating funnel in which it was shaken so that the filtrate had contact with the solvent. Then the less dense layer where the solvent was present was removed. After this procedure, the samples were placed in a forced ventilation oven at 50°C for total solvent evaporation.

SENSITIVITY TEST BY WELL DIFFUSION METHOD

Tests were performed with standard strains of *Pseudomonas aeruginosa* (ATCC27853), *Escherichia coli* (ATCC25922), *Staphylococcus aureus* (ATCC25923), *Klebsiella pneumoniae* (ATCC13899) and the yeast *Candida albicans* (FIOCRUZ). The extracts were weighed and diluted at concentrations of 20 $mg.mL^{-1}$ in Dimethylsulfoxide. The concentrations were homogenized with the aid of Vortex.

A sterile cotton swab was dipped in the suspensions of the test microorganisms and rubbed across the entire surface of the plates

containing Mueller- Hinton Agar culture medium.

For the diffusion test per well, holes of 10 mm in diameter were made with the aid of tips in the culture medium in a Petri dish, these were filled with 100 µL of extract at a concentration of 20 mg/mL. As a positive control, 50 µL was used in Tetracycline antibiotic (50mg/mL), and for *Candida albicans* was used 50 µL of Fluconazole (50mg/mL). After this procedure, the Petri dishes were kept refrigerated at 4°C for two hours for complete penetration of the extract into the culture medium. Then they were removed and placed in a BOD oven at 25°C.

MORPHOLOGICAL CHARACTERIZATION AND IDENTIFICATION OF ISOLATES

To visualize the microscopic structures, the microculture technique was performed (Shirling and Gottlieb, 1966), which consists of transferring two to four small fragments of the fungal mycelium into a Petri dish containing Nutrient Agar (NA) medium. Autoclaved microscopy coverslips measuring 22x22mm were deposited on these small inoculums. Light pressure was applied to the cover slip, so that it remained adhered to the surface of the culture medium. The Petri dishes were transferred to the BOD oven, where they were monitored, day by day, until the mycelium of the developing fungus reached the edge of the cover slip and began to grow over it. At that moment, the coverslip was carefully removed with tweezers and placed on a microscopy slide with lactophenol blue cotton. The observation of the microstructures and the photo documentation were carried out in an optical microscope coupled to an image capture system. For visualization in optical microscopy, a magnification of 400X was used.

Fungal isolates were grouped into morphotypes, based on common morphological characteristics.

STATISTICAL ANALYSIS

For the comparison of the expressed results, as average of the halos obtained in each concentration, the Analysis of Variance (ANOVA) was carried out, and then the Tukey test at the 5% level of significance. Statistical analyzes were performed using the Sisvar software, version 5.6, according to FERREIRA, 2014.

RESULTS AND DISCUSSIONS

In isolation, 27 fungal isolates were obtained, 18 (66.6%) belonging to *Colletotrichum* sp., 2 (7.4%) to *Fusarium* sp., 1 (3.7%) to *Curvularia* sp. and 6 (22.3%) classified as *Mycellia sterilia,* sterile mycelium without specific genus and species. Among the 27 isolates, 3 showed antimicrobial activity against the strains used, 2 belonging to *Fusarium* sp., 1 to *Curvularia* sp.

IDENTIFICATION OF ISOLATES

- *Fusarium* sp.

Colony morphology: Initially white in color, becoming slightly pink with a cottony to woolly texture. The background of some species of the genus presented red, orange and lilac colors.

Microscopic characteristics: The hyphae are light colored, septate, in which they produced conidiophores and conidia. Small, cylindrical microconidia without septum were observed. Macroconidia were large, multiseptate, sickle -shaped.

- *Curvularia* sp.

Colony morphology: Showed a dark brown color with a woolly appearance. The background was black.

Microscopic characteristics: Septate hyphae, brown in color. The conidia had four cells and were curved.

- *Colletotrichum* sp.

Colony morphology: It was velvety with a beige and brown color. The background was beige and brown like the surface of the board.

Microscopic characteristics: Septate hyphae in which they presented hyaline and septate conidiophores. The presence of appressoria was observed, which are specialized brownish structures for fixation in the host tissue, this type of structure is common in the genus.

SENSITIVITY TEST

Statistically, analysis of variance showed a significant difference between all means of halos for *S. aureus, E. coli* and *Candida albicans* for the *Fusarium* esp07 extract, with the highest mean inhibition occurring in *S. aureus* with 23 mm. for *Fusarium* esp04 there was an inhibition halo only in the *S. aureus strain* with an average of 10 mm. Regarding *Curvularia* fol50, there was an inhibition halo only for *E. coli,* with an average of 10 mm (Figure 7). *P. aeruginosa* and *K. pneumoniae* did not show inhibition zones for any fungal extract. The positive control for *S. aureus* showed a halo of inhibition of 42 mm, 24 mm for *E. coli* and 24 mm for *C. albicans*. The halo means are shown in Table 1.

Sensitivity tests and statistical analysis show that *Fusarium* esp07 extract was effective against *S. aureus bacteria,* classified as Gram-positive, against *E. coli,* Gram-negative bacteria, and *Candida albicans,* a yeast-like fungus. Thus, the antimicrobial action of this extract was of a broad spectrum, acting on bacteria with cell walls with different structures, in addition, it acted as an antifungal.

	Diameter of inhibition halos (mm)		
Strains	*Fusarium* **esp07**	*Fusarium* **esp04**	*folio* **sheet 50**
s. aureus	23.00 to	10.00 d	-
E coli	21.00 b	-	10.00 d
candida albicans	14.00 c	-	-
P. aeruginosa	-	-	-
K. pneumoniae	-	-	-

(-) did not show an inhibition halo against the extract.

Table 1: Average of the growth inhibition zones obtained by the fungal extract.

Among the broad-spectrum antibiotics, the beta-lactams are the most used, acting by inhibiting the synthesis of the bacterial cell wall, mainly affecting the peptidoglycan, resulting in the destruction of the cell wall, which is why they are effective against Gram-positive, Gram- negative and strict anaerobes. This is perhaps one of the mechanisms used by the fungal extract of *Fusarium* esp07 against *S. aureus* and *E. coli* in which it was effective for both bacteria and fungus (ARAUJO and AZEVEDO, 2020; GONÇALVES, 2019).

As for antifungals used to treat *Candida* sp., azoles and triazoles, such as fluconazole, for example, act by inhibiting the fungal enzyme lanosine-14α- demethylase, which is responsible for converting lanosterol into ergosterol, directly affecting the fluidity of the plasmatic membrane and the enzymes linked to it (VIEIRA and SANTOS, 2017).

K. pneumoniae and *P. aeruginosa are* among the most antibiotic-resistant Gram-negative bacteria, which are classified as a critical priority for the production of new antibiotics. Among the mechanisms used by these bacteria is the production of beta-lactam enzymes, which inhibit the action of broad-spectrum beta- lactam antibiotics (MOTA and OLIVEIRA, 2018).

Antimicrobial drugs are of great

importance to public health as they help to reduce mortality and increase life expectancy, however, misuse causes resistance of microorganisms to these drugs, resulting in the race for new drugs capable of fighting diseases. previously controlled (DA COSTA *et al,* 2022).

Brazilian biodiversity is considered one of the largest in the world. Interest by researchers has grown in recent decades, even more so by plants that can generate compounds that can be used in industry. Extracts from plants are the best known and most used in the production of new drugs, mainly antibiotics, however, endophytic microorganisms are still poorly studied in relation to the pharmacological potential they can provide (SILVA *et al.*, 2020; TORRES *et al.*, 2022).

Despite being considered plant pathogenic fungi, *Colletrotrichum* sp. and *Fusarium* sp., found in greater quantity in this work, can coexist harmoniously within plant tissues, producing molecules capable of benefiting the plant against other disease-causing beings.

According to ALEXANDRE-POLIDO (2019), some species of *Fusarium* sp. have been shown to be able to inhibit cancer cells in addition to being effective antioxidants. Most species of this genus have the ability to produce secondary metabolites, including bioactive ones. Li *et al* 2020 demonstrated 272 extracted compounds. Therefore, many species are placed in confrontation with other microorganisms in order to observe inhibitory properties, as observed in the present work, in which this genus showed a high inhibition capacity against pathogenic microorganisms. Among the metabolites present in *Fusarium* sp., there are flavonoids, terpenes, alkaloids, phenolic acids, and quinones, bioactives widely used in the pharmaceutical industry in the production of antibiotic drugs, however, still little applied when referring to this genus (BATISTA *et al..*, 2022).

Therefore, it is necessary to know in more detail the endophytic microorganisms, such as those present in *Piper* sp. and their use in the treatment of diseases, in view of the diversity of essential compounds produced by them to protect the environment in which they live, the plants. Thus, the identification of new species of endophytes plays an important role in the discovery of new antimicrobial drugs.

FINAL CONSIDERATIONS

The extracts of endophytic microorganisms present in the leaves and inflorescences of *Piper hispidinervum* C. DC. are a source of compounds capable of inhibiting the growth of pathogenic microorganisms such as *S. aureus, E. coli* and *Candida albicans.* Among these extracts, the most efficient against the test microorganisms was that obtained from *Fusarium* esp 07. However, further studies are still needed on the antimicrobial potential of molecules from this genre, which in the future may compose new drugs.

REFERENCES

ALEXANDRE-POLIDO, S. M. A. Caracterização química e avaliação citotóxica de heteropolissacarídeos dos fungos *Colletotrichum gloeosporiodes* e *Fusarium* sp., Repositório Unesp, 2019.

ÂNGELO, T.; RIBEIRO, C. C. Utilização de plantas medicinais e medicamentos fitoterápicos por idosos. Ciência & Desenvolvimento-Revista Eletrônica da FAINOR, v. 7, n. 1, 2014.

ARAÚJO, L. L. C.; AZEVEDO, F. H. C. Estudo da enzima beta-lactamase e sua relação com a resistência aos antibióticos. Research, Society and Development, v. 9, n. 7, p. e663974594-e663974594, 2020.

ARAÚJO, W. L., LACAVA, P. T., MARCO, J., LIMA, A. O. D. S., SOBRAL, J. K., AZEVEDO, J. L. D., & PIZZIRANI-KLEINER, A. A. Guia prático: isolamento e caracterização de microrganismos endofíticos. Piracicaba: Copiadora "Luiz de Queiroz", 2010.

ASSUNÇÃO, M. M. C. Fungos endofíticos isolados de folhas de bananeira (*Musa* spp.) e seleção de antagonistas a fitopatógenos dessa cultura. Tese (Doutorado), Programa de Pós-graduação Biologia de Fungos, Universidade Federal de Pernambuco, Recife, 2010.

BATISTA, G. R. Metabólitos fúngicos com potencial antimicrobiano isolados e caracterizados produzidos por gênero *Fusarium*: uma revisão sistemática da literatura. Dissertação (Mestrado em Ciência e Tecnologia para Recursos Amazônicos), UFAM, 2022.

DA COSTA, J. M., DE PÁDUA, C. A. M., VEGI, A. S. F., CINTRA, L. P., DE SOUZA, G. C., DE ARAGÃO, B. A. D.,... & RIBEIRO, A. Q. Utilização de antimicrobianos conforme resistência microbiana: qual a influência da medida restritiva implementada no Brasil?. Research, Society and Development, v. 11, n. 9, p. e38111931902-e38111931902, 2022.

DE SANTIAGO, E. J. A. Caracterização morfológica e bioquímica de calos de pimenta longa (*Piper hispidinervium* Candolle, De Candolle). Embrapa Amazônia Oriental-Tese/dissertação (ALICE), 2003.

DOS SANTOS, W. B., MAJOLO, C., DOS SANTOS, D. S., ROSA, M. C., MONTEIRO, P. C., ROCHA, M. J. S.,... & CHAGAS, E. C. Eficácia in vitro de óleos essenciais de espécies de Piperaceae no controle do acantocéfalo *Neoechinorhynchus buttnerae*. Embrapa Amazônia Ocidental-Artigo em periódico indexado (ALICE), 2018.

FADIJI, A. E.; BABALOLA, O. O. Elucidating mechanisms of endophytes used in plant protection and other bioactivities with multifunctional prospects. Frontiers in Bioengineering and Biotechnology, v. 8, p. 467, 2020.

FAZOLIN, M., ESTRELA, J. L. V., CATANI, V., ALÉCIO, M. R., & LIMA, M. S. D. Propriedade inseticida dos óleos essenciais de *Piper hispidinervum* C. DC.; *Piper aduncum* L. e *Tanaecium nocturnum* (Barb. Rodr.) Bur. & K. Shum sobre *Tenebrio molitor* L., 1758. Ciência e Agrotecnologia, v. 31, n. 1, p. 113-120, 2007.

FERREIRA, D. F. Sisvar: a Guide for Bootstrap produces in multiple comparisions. Ciência e Agrotecnologia, Lavras, v.38, n.2, 2014.

FIRMO, W. D. C. A., DE MENEZES, V. D. J. M., DE CASTRO PASSOS, C. E., DIAS, C. N., ALVES, L. P. L., DIAS, I. C. L.,... & OLEA, R. S. G. Contexto histórico, uso popular e concepção científica sobre plantas medicinais. Cadernos de pesquisa, v. 8, UFMA, 2011.

GONÇALVES, C. M. A. A importância clínica dos antibióticos β-lactâmicos. Tese de mestrado. Mestrado Integrado em Ciências Farmacêuticas, p. 67, Repositório Institucional da Universidade Fernando Pessoa, Porto, 2019.

GONÇALVES, V. L., ESMERINO, L. A., DE SOUZA, V. M., BATISTA, B. D., TORRES, A. L., ARMSTRONG, L., & DE ARAÚJO ÁVILA, G. M. Atividade antimicrobiana e inseticida do óleo essencial de *Piper amalago* L.(Piperaceae). Brazilian Journal of Development, v. 8, n. 1, p. 998-1013, 2022.

LI, M.; YU R.; BAI, X.: WANG H.; ZHANG, H. *Fusarium*: a treasure trove of bioactive secondary metabolites. Natural Product Reports, v. 10, n. 1, p. 1039-1060, 2020.

LIMA, J. B. Estudo químico e atividade antimicrobiana das folhas de *Piper callosum* e *Piper corcovadensis* (Piperaceae). Trabalho de Conclusão de Curso (Licenciatura em Química) - Departamento de Química, Universidade Federal Rural de Pernambuco, 58 f. Recife, 2019.

MARTINEZ-KLIMOVA, E.; RODRÍGUEZ-PEÑA, K.; SÁNCHEZ, S. Endophytes as sources of antibiotics. Biochemical Pharmacology, v. 134, p. 1–17, 2017.

MOTA, F. S.; OLIVEIRA, H. A.; SOUTO, R. C. F. Perfil e prevalência de resistência aos antimicrobianos de bactérias Gram-negativas isoladas de pacientes de uma unidade de terapia intensiva. RBAC, v. 50, n. 3, p. 270-277, 2018.

PETRINI, O.; FISHER, P. J. Fungal endophytes in *Salicornia perennis*. Transactions of the British Mycological Society, v. 87, n. 4, p. 647-651, 1986.

SCALDAFERRI, L. G., TAMEIRÃO, E. R., FLORES, S. A., NEVES, R. A. S. C., CORREIA, T. S., DO CARMO, J. R.,... & FERRANTE, M. Formas de resistência microbiana e estratégias para minimizar sua ocorrência na terapia antimicrobiana: Revisão. Pubvet, v. 14, p. 163, 2020.

SERRA VALDÉS, M. Á. La resistencia microbiana en el contexto actual y la importancia del conocimiento y aplicación en la política antimicrobiana. Revista Habanera de Ciencias Médicas, v. 16, n. 3, p. 402-419, 2017.

SILVA, M. V. S., BRITO, M. C. A., SALES, J. S., DOS SANTOS MONTEIRO, O., MENDES, S. J. F., FERRO, T. A. F.,... & COUTINHO, D. F. Plantas Medicinais: potencial para o desenvolvimento de medicamentos antimicrobianos. Tópicos em Ciências da Saúde-volume 3, Pantanal Editora, 2020.

SOUZA, A. Q. L. D., SOUZA, A. D. L. D., ASTOLFI FILHO, S., PINHEIRO, M. L. B., SARQUIS, M. I. D. M., & PEREIRA, J. O. Atividade antimicrobiana de fungos endofíticos isolados de plantas tóxicas da Amazônia: *Palicourea longiflora* (aubl.) rich e Strychnos cogens bentham. Acta amazônica, v. 34, p. 185-195, 2004.

TORRES, F. L., BOGAS, A. C., DE ANDRADE, P. H. M., DE SOUSA, C. P., & LACAVA, P. T. Bioprospecção e potencial biotecnológico de fungos endofíticos associados a plantas do Cerrado. *Concilium*, *22*(2), 256–272, 2022.

VIEIRA, A. J. H.; SANTOS, J. I. Mecanismos de resistência de *Cândida albicans* aos antifúngicos anfotericina B, fluconazol e caspofungina. RBAC, v. 49, n. 3, p. 235-9, 2017.

WORLD HEALTH ORGANIZATION (OMS). Global priority list of antibioticresistant bacteria to guide research, discovery, and development of new antibiotics; 2017.