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Articles

Fungi from Amazonian soils with potential to control termites *Nasutitermes* sp. (Blattodea: Termitidae)

Fungos de solos amazônicos com potencial para o controle de cupins *Nasutitermes* sp. (Blattodea*:* Termitidae)

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ABSTRACT

Entomopathogenic fungi represent a promising low-impact alternative for pest control. This study aimed to isolate and select entomopathogenic fungi from Amazonian soils for the biological control of the termite *Nasutitermes* sp. (Blattodea: Termitidae). Ten soil samples were collected from different Amazonian sites, and the decimal dilution technique was employed for sample processing. Fungi were isolated by spreading the dilutions onto a selective medium containing 2% macerated termites as the sole carbon source. Termites of the genus *Nasutitermes* sp. were collected from natural mounds and used in pathogenicity assays with the three most frequently isolated fungal morphospecies, at concentrations of 10^5, 10^6, 10^7, and 10^8 conidia/mL, alongside a control group. The most frequently isolated fungus was identified through molecular techniques. Data were analyzed using analysis of variance (ANOVA), followed by Tukey's test. A total of 19 fungal isolates were obtained and grouped into six morphospecies. The most frequent were *Paecilomyces lilacinus* (42.11%), *Paecilomyces* sp. 2 (26.32%), and *Aspergillus* sp. 1 (10.53%). *Paecilomyces* sp. 2 (4.816) and *Aspergillus* sp. 1 (4.808) achieved 100% termite mortality at all tested concentrations from the sixth day onward. *Paecilomyces lilacinus* (4.807) also reached 100% mortality on the sixth day. The termite-based selective medium proved effective for isolating entomopathogenic fungi, as all tested strains showed potential for controlling *Nasutitermes* sp. This study presents the first report of virulence of *Paecilomyces lilacinus* against the termite *Nasutitermes* sp.

Keywords: Biological control; Entomopathogenic fungi; *Paecilomyces lilacinus*

RESUMO

Fungos entomopatogênicos representam uma alternativa promissora e de baixo impacto para o controle de pragas. Este estudo teve como objetivo isolar e selecionar fungos entomopatogênicos de solos amazônicos para o controle biológico do cupim *Nasutitermes* sp. (Blattodea: Termitidae). Dez amostras de solo foram coletadas em diferentes locais da Amazônia, e a técnica de diluição decimal foi empregada para o processamento das amostras. Os fungos foram isolados por meio da semeadura das diluições em meio seletivo contendo 2% de cupins macerados como única fonte de carbono. Cupins do gênero *Nasutitermes* sp. foram coletados de cupinzeiros naturais e utilizados em testes de patogenicidade com as três morfoespécies fúngicas mais frequentemente isoladas, nas concentrações de 10^5, 10^6, 10^7 e 10^8 conídios/mL, juntamente com um grupo controle. O fungo mais frequentemente isolado foi identificado por meio de técnicas moleculares. Os dados foram analisados por análise de variância (ANOVA), seguida do teste de Tukey. Um total de 19 isolados fúngicos foi obtido e agrupado em seis morfoespécies. As mais frequentes foram *Paecilomyces lilacinus* (42,11%), *Paecilomyces* sp. 2 (26,32%) e *Aspergillus* sp. 1 (10,53%). *Paecilomyces* sp. 2 (4.816) e *Aspergillus* sp. 1 (4.808) alcançaram 100% de mortalidade dos cupins em todas as concentrações testadas a partir do sexto dia. *Paecilomyces lilacinus* (4.807) também atingiu 100% de mortalidade no sexto dia. O meio seletivo à base de cupins mostrou-se eficaz para o isolamento de fungos entomopatogênicos, uma vez que todos os isolados testados apresentaram potencial para o controle de *Nasutitermes* sp. Este estudo apresenta o primeiro relato de virulência de *Paecilomyces lilacinus* contra o cupim *Nasutitermes* sp.

Palavras-chave: Controle biológico; Fungos entomopatogênicos; *Paecilomyces lilacinus*

1 INTRODUCTION

Termites belong to the order *Blattodea* and exhibit paurometabolous development (egg–nymph–adult). They are social, polymorphic insects that live in structured colonies (Albuquerque et al., 2008). From an ecological perspective, termites provide important environmental benefits: they improve the chemical and physical properties of the soil, contribute to the recycling of cellulose-based materials, and their underground structures (such as tunnels, galleries, and nests) can serve as shelters for other animals (Khan et al., 2022). However, in commercial plantations, termites can become pests by damaging the root systems of plants, with harmful effects occurring from transplanting to harvest. Reports of termite attacks have been documented in eucalyptus seedlings (Chaves et al., 2017), as well as in sugarcane and rice crops (Almeida; Alves, 2009).

In Brazil, agricultural pests are a major cause of significant economic losses (Adelino et al., 2021). A species is defined as a pest based on the economic damage it causes, as phytosanitary issues are critical limiting factors for both agricultural production and industrial performance. These limitations arise from the high costs associated with prevention, control, and damage mitigation (Ribeiro, 2020). Moreover, pest infestations can facilitate the invasion of other deteriorative agents, further reducing product quality and marketability (Srivastava; Mishra, 2021). Among the pests that cause economic harm to human activities are termites of the genus *Nasutitermes* sp. (Blattodea: Termitidae) (Govorushko, 2019).

Attacks by termites of the genus *Nasutitermes* are common in native tree species of the Amazon rainforest, in commercial plantations, and in cellulose-based materials (Acioli, 2018). These termites can also cause damage in urban areas by infesting furniture and other wooden objects, including those used in building structures (Da Silva Oliveira et al., 2017). Their impact is significant due to their high feeding plasticity, as they consume wood from a wide variety of species. Global losses attributed to termite activity are estimated to be around US$50 billion (Ribeiro, 2020).

The most commonly used method for termite control is chemical treatment, typically involving products based on fipronil or chlorpyrifos, available in both liquid and powder formulations (Brito; Silva, 2022). This method offers the advantage of delivering immediate results (Cooper; Dobson, 2007). However, it poses several risks due to its high toxicity to living organisms (Chaud et al., 2021) and the presence of xenobiotic compounds that degrade slowly in the environment, contributing to ecological damage. As a result, there is a growing body of research aimed at developing new, low-cost technologies for termite control that minimize environmental impact (Hanke et al., 2022).

Among the alternative methods for termite control is biological control (Saldanha et al., 2022), which involves regulating insect populations through natural enemies, such as parasitoids or pathogens, including fungi, bacteria, and viruses (Silva, 2014).

Fungi, in particular, can serve as biological control agents by acting through parasitism (Loeblein-Verdério et al., 2024), antibiosis (Rabuske et al., 2023), or by promoting positive interactions with plants (Monte et al., 2019).

The use of Hyphomycete fungi in pest control has been widely reported. The effectiveness of fungi such as *Metarhizium anisopliae* in insect management has led to the development of a commercially registered product in the United States, derived from *M. anisopliae*, which has been successfully employed in termite control (BUTT *et al*., 2001). Furthermore, entomopathogenic fungi from the genera *Metarhizium* and *Beauveria* have been identified as effective agents for termite control and the microbial management of urban pests. However, challenges remain regarding the stability and efficacy of these fungi under varying environmental conditions, as well as the optimization of application methods in urban settings (Milner; Pereira, 2007).

The primary advantages of biological control over chemical control include its less harmful impact on ecosystems (Braga-Sobrinho et al., 2022), as well as the absence of residual chemicals in the environment, thereby protecting biodiversity and preventing ecological imbalances (Van Leeuwen et al., 2020). However, biological control also has some drawbacks: it requires intensive planning and management, and its effects are often slower to manifest compared to conventional insecticides (Trevisan-Junior; Ghellee, 2022). In this sense, it is known that entomopathogenic fungi cause the death of insects because they infect and kill their hosts due to the production of mycotoxins (Padmaja; Aruna, 2019). In addition, they are quite efficient in controlling insect pests, due to their versatility and the fact that they have specialized infection mechanisms that cause physical damage due to the growth of mycelia, making difficult the stages of the host's life cycle (Vilcinskas, 2018).

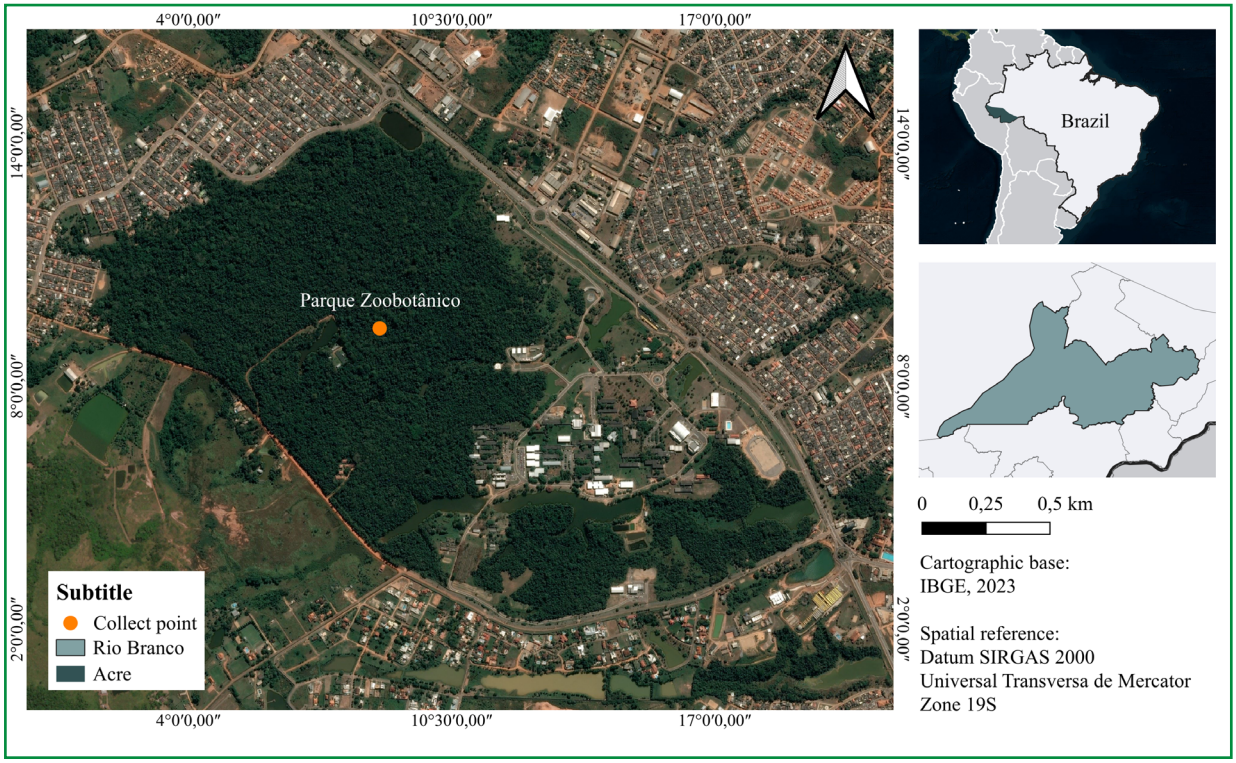
The use of entomopathogenic fungi for pest control has been increasingly studied (Angelone; Bidochka, 2018), although information on the potential of these fungi in the Amazon region remains limited. Therefore, the objective of this study was to isolate and select entomopathogenic fungi from Amazonian soils for the control of the termite *Nasutitermes* sp. (Blattodea: Termitidae).

2 MATERIALS AND METHODS

2.1 Soil collection

Ten soil samples were collected from the Parque Zoobotânico of the Universidade Federal do Acre (UFAC) in the Amazon region (Figure 1). Samples were taken randomly by first removing the surface litter layer, then collecting soil from a depth of 3 to 5 cm. The material was placed in labeled plastic bags and transported to the Microbiology Laboratory at UFAC for microorganism isolation.

Figure 1 – Location of collections of Amazonian soils at the Universidade Federal do Acre (UFAC)



Source: Authors (2025)

2.2 Isolation of entomopathogenic fungi

The soil samples were sieved, and 2 g of each sample was weighed and suspended in an Erlenmeyer flask containing 18 mL of sterilized 0.9% NaCl solution.

The suspension was stirred at 120 rpm for 1 hour. For sample preparation, the serial dilution technique was employed: 1 mL of the suspension was transferred to a tube containing 9 mL of 0.9% NaCl solution, generating dilutions of 10^{-1}, 10^{-2}, and 10^{-3} (Bills et al., 2004). Then, 200 µL aliquots of the 10^{-2} and 10^{-3} dilutions were inoculated onto a selective medium using the spread plate technique. The medium was prepared with 2% termite material and contained the following components: agar (15 g), chitosan (10 g), $NaNO_3$ (6 g), K_2CO_3 (5 g), KH_2PO_4 (1.5 g), $MgSO_4$ (0.5 g), trace amounts of $ZnSO_4$ and $FeSO_4$, and dried, macerated termite (7.6 g) per liter of distilled water. Plates were incubated at 28 ± 1ºC and observed daily for up to 30 days or until fungal colonies appeared.

2.3 Identification and storage of isolated fungi

After fungal growth on the termite-based selective medium, the fungi were purified using the exhaustion streak technique on Petri dishes containing Potato Dextrose Agar (PDA) medium (200 g potato infusion, 20 g dextrose, 15 g agar per liter). Isolated colonies were then transferred to test tubes containing PDA medium. The isolates were preserved using sterilized distilled water (Castellani, 1963) and mineral oil methods (Buell; Weston, 1947) in the Microbiology Laboratory Collection at UFAC.

After 14 days, the macroscopic characteristics of the fungi, including color, texture, and pigment production, were evaluated to group similar isolates into morphospecies. Microscopic identification was performed using the microculture technique, with fungi cultivated on PDA and oat agar media (30 g oat infusion and 15 g agar per liter) and incubated for 7 days at room temperature. Fungi producing reproductive structures were identified by comparison with descriptions in specialized literature (Barnett; Hunter, 1998).

2.4 Preparation of conidial suspensions for pathogenicity bioassay

The three most frequent morphospecies were cultivated on PDA medium at 28ºC for 14 days. On the day of the bioassay, conidia were gently scraped from the surface

of the plates and suspended in 10 mL of sterile distilled water containing 0.01% Tween 80. The conidial suspensions were quantified using a Neubauer chamber, and serial dilutions were prepared to obtain concentrations of 10^5, 10^6, 10^7, and 10^8 conidia/mL (Remadevi et al., 2010).

2.5 Virulence assay

Termites of the genus *Nasutitermes* sp. were manually collected from termite mounds located in the Parque Zoobotânico (UFAC). During collection, a channel was opened with the aid of a stick, or the entire termite nest was dismantled. The collected material was then placed in trays and stored in plastic bags.

For each fungal isolate, 45 Petri dishes were prepared, 20 for the first stage of the bioassay and 25 for the second stage, with filter paper placed inside each dish. In the first stage, termites were directly collected using brushes and placed on the plates in a ratio of 47 workers to 13 soldiers, reflecting the natural caste proportion found in the nests.

To evaluate fungal virulence, the experiment was conducted with five replicates per conidial concentration (10^5, 10^6, 10^7, and 10^8 conidia/mL) and a control group. For each replicate, 1 mL of conidial suspension was applied to a Petri dish and allowed to sit for 3 minutes to enable direct contact between the termites and the fungal inoculum. The termites were then transferred to a clean Petri dish. In the control group, 1 mL of 0.01% Tween 80 aqueous solution was used instead. Corrugated cardboard was subsequently added as both shelter and food source (Pires, 2010). Plates containing the termites were stored in plastic containers at 25°C and relative humidity ≥80%. Termite mortality was monitored every 24 hours for up to 10 days or until all individuals in the control group had died (Silva, 2014).

2.6 Reisolation of fungi obtained from termite corpses

The colonized termites were washed sequentially in 70% ethanol for 3 minutes, 2% sodium hypochlorite solution for 2 minutes, and then rinsed in sterilized distilled water for 3 minutes. After this sterilization process, the termites were dried on

sterilized filter paper and inoculated onto plates containing PDA medium. Following fungal growth, morphological characteristics were compared to confirm that the fungi isolated from the termites matched the original inoculum (Alves; Moraes, 1998).

2.7 Molecular Identification

A fungus from one of the most frequently isolated morphospecies, *Paecilomyces* sp. 1 (4.807), was inoculated on PDA medium and incubated at 28 ± 1°C for 14 days for DNA extraction.

DNA was extracted from the purified fungal isolates grown on PDA plates using the Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research), following the manufacturer's instructions. The rDNA ITS region was amplified in a 50 µL PCR reaction mixture containing 2 µL of DNA template (1–20 ng), 0.4 µM of each primer ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (WHITE *et al*., 1990), 1.5 mM $MgCl_2$, 0.2 µM dNTPs, 5 µL Taq buffer, and 1.25 U Taq DNA polymerase (Qiagen). PCR amplification was performed using a Bio-Rad thermal cycler with an initial denaturation at 95 °C for 2 minutes, followed by 35 cycles of 95 °C for 30 seconds, 55°C for 30 seconds, and 72 °C for 1 minute, and a final extension at 72 °C for 7 minutes.

PCR products, approximately 600 to 700 bp in size, were purified using the QIAquick PCR Purification Kit (Qiagen) and visualized on a 2% agarose gel. Forward and reverse sequencing reactions were performed on a 3730xl DNA Analyzer (Applied Biosystems). Forward and reverse reads for each isolate were aligned to generate consensus sequences. These consensus sequences were compared against the NCBI database using BLASTn, and the top hits were used for fungal identification.

2.8 Statistical analysis

The experiment was conducted in a completely randomized block design with five replications. Data were analyzed for normality of residuals and subjected to analysis of variance (ANOVA), followed by Tukey's test (Tukey,1945) for mean comparisons at a significance level of 0.05. For non-parametric data, the Kruskal-Wallis test was applied,

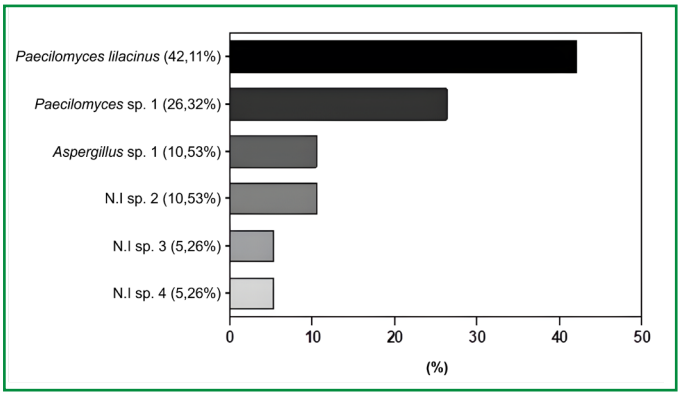
followed by the Student-Newman-Keuls (SNK) test for multiple comparisons of mean ranks, using the BioEstat software.

3 RESULTS

3.1 Isolation of fungi from soil

Nineteen fungal isolates from Amazonian soils were obtained using a selective medium with termites. These isolates were characterized based on macroscopic features and grouped into six morphotypes, with the most frequent being *Paecilomyces lilacinus* (42.11%) and *Paecilomyces* sp. 1 (26.32%). The remaining morphospecies were not identified, as they did not produce reproductive structures (Figure 2). Only the identified genera were used in the experimental assays.

Figure 2 – Fungi isolated from Amazonian soils in selective medium with termites

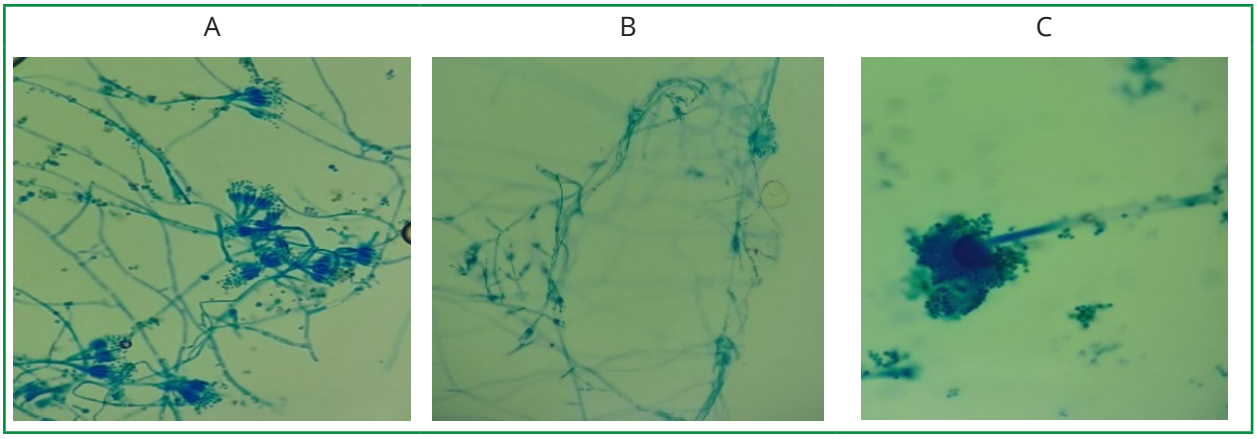


Source: Authors (2025)

3.2 Termite pathogenicity bioassay

Analysis of isolate pathogenicity over time after inoculation revealed that all isolates were highly virulent, with statistically significant differences among treatments during the initial days, indicating that the speed of action varied between species (Table 1). Termites in the control group showed no signs of fungal infection, and no statistically significant differences in mortality were observed between workers and soldiers.

Figure 3 – Microscopy of the isolated fungi soils in selective medium with termites. (A) *Paecilomyces lilacinus*, (B) *Paecilomyces* sp. 1 and (C) *Aspergillus* sp. 1



Source: Authors (2025)

Table 1 – Average mortality (%) of *Nasutitermes* sp. exposed to different conidial concentrations (conidia/mL) of *Paecilomyces lilacinus* (4.807), *Paecilomyces* sp. 1 (4.816), and *Aspergillus* sp. 1 (4.808) over the treatment period

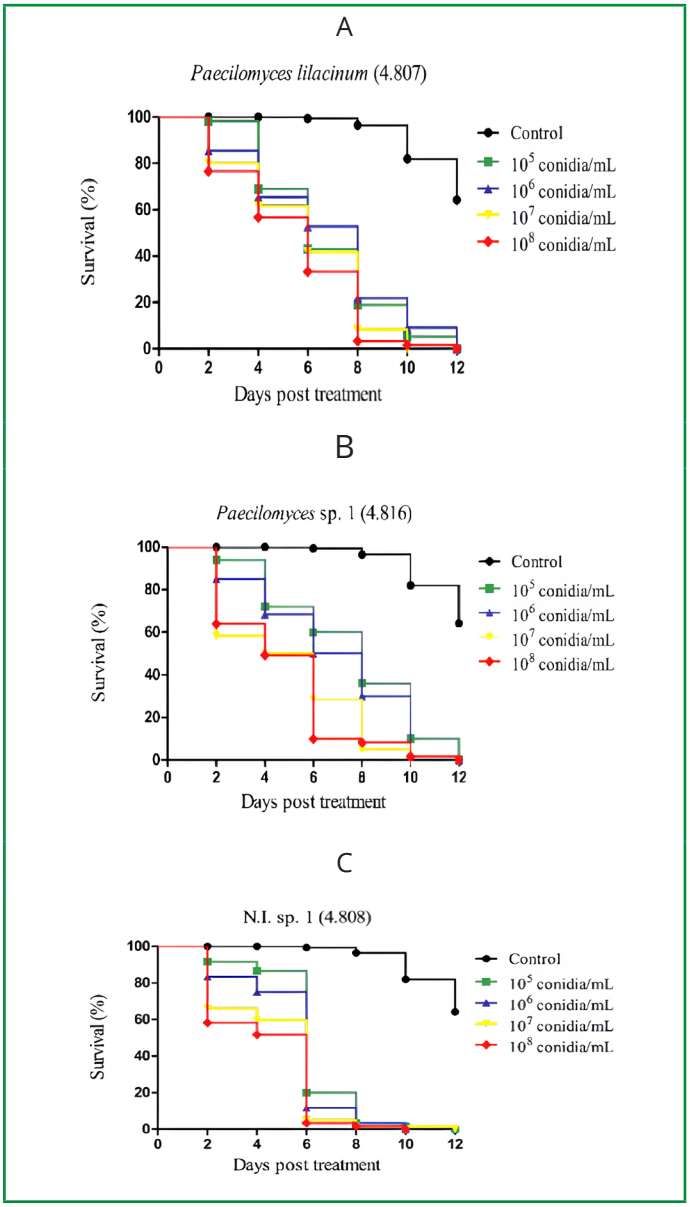
Day	Fungi	Treatment				
		Control	10^5	10^6	10^7	10^8
2º day	*Paecilomyces lilacinus* (4.807)	1,0 ±1,5c	2,3±2,8c	13±7,1b	20,3±5,1a	22,7±2,5a
	Paecilomyces sp. 1 (4.816)	1,0 ±1,5c	7,7±5,3b	12±2,2b	23,7±6,1a	30,7±2,2a
	Aspergillus sp. 1 (4.808)	1,0 ±1,5c	3,7± 3,2bc	7,9±1,8b	15,3±2,2a	9,3±4,1b
4º day	*Paecilomyces lilacinus* (4.807)	10,7±6,8bc	48,3±7,3a	43,3±14,9ab	59±9,5a	67,3±9,9a
	Paecilomyces sp. 1 (4.816)	10,7±6,8d	59,3±10,8c	78,7±10,2b	100±0a	100±0a
	Aspergillus sp. 1 (4.808)	10,7±6,7bc	18,0±11,1c	51,7±10,9b	100±0a	100±0a
6º day	*Paecilomyces lilacinus* (4.807)	26,0±4,8c	91,0±9,2ab	79,7±21,0a	42,9±15,4b	100±0a
	Paecilomyces sp. 1 (4.816)	26,0±4,8c	100±0a	100±0a	100±0a	100±0a
	Aspergillus sp. 1 (4.808)	26,0±4,8c	100±0a	100±0a	100±0a	100±0a
8º day	*Paecilomyces lilacinus* (4.807)	44±4,0b	100±0a	100±0a	100±0a	100±0a
	Paecilomyces sp. 1 (4.816)	44±4,0b	100±0a	100±0a	100±0a	100±0a
	Aspergillus sp. 1 (4.808)	44±4,0b	100±0a	100±0a	100±0a	100±0a

Source: Authors (2025)

In where: *Means followed by equal letters in the same row do not differ at the 5% significance level ($p \geq 0.05$).

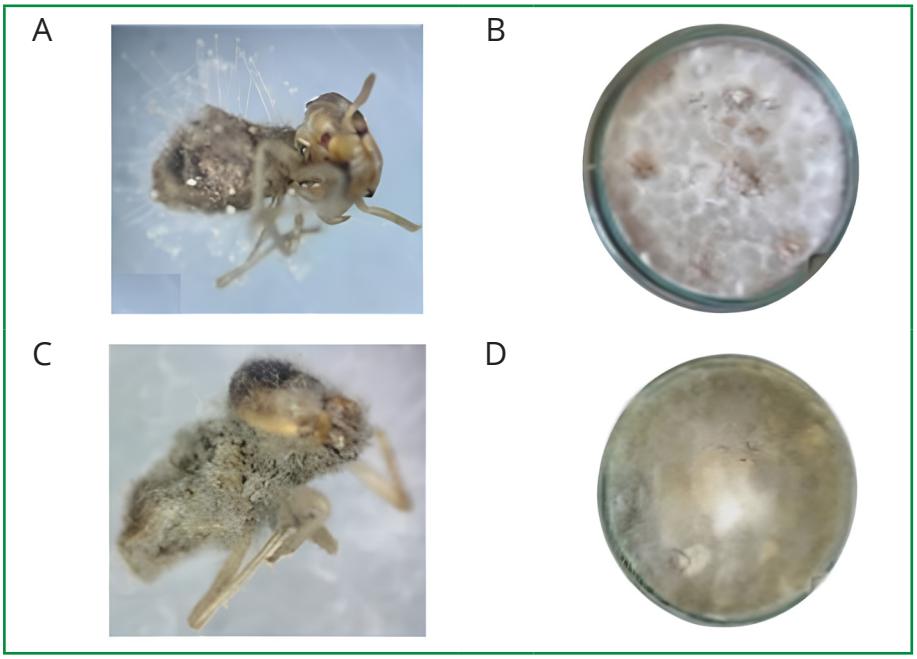
Paecilomyces sp. 1 (4.816) and *Aspergillus* sp. 1 (4.808) achieved 100% mortality at concentrations of 10^7 and 10^8 conidia/mL by the fourth day of testing. From the sixth day onward, both fungal isolates caused 100% mortality across all evaluated concentrations, indicating strong potential for biological termite control. *Paecilomyces lilacinus* (4.807) reached 100% mortality at the 10^8 conidia/mL concentration on the sixth day of treatment, and at all other concentrations by the seventh day (Figure 4).

Figure 4 – Mortality of *Nasutitermes* sp. termites by Amazonian fungi. A. *Paecilomyces lilacinus* (4.807); B. *Paecilomyces* sp. 1 (4.816); C. *Aspergillus* sp. 1 (4.808)



Source: Authors (2025)

Figure 5 – Cadaver of *Nasutitermes* sp. termite after exposure to conidia of Amazonian fungi A, C. and reisolation B, D of *Paecilomyces lilacinus* (4.807) and *Aspergillus* sp. 1 (4.808) respectively



Source: Authors (2025)

3.3 Molecular Identification

After obtaining the DNA sequences and comparing them with data available in GenBank, the fungus *Paecilomyces* (4.807) was identified as *Paecilomyces lilacinus* (Table 2).

Table 2 – Amazon soil fungi identified by DNA sequencing analysis

	Fungi no.	GenBank accession no.	Closest match in GenBank	Percentage identity (%)
C	4.807	OR447424	*Paecilomyces lilacinum* (MT453285)	99,63

Source: Authors (2025)

4 DISCUSSIONS

Entomopathogenic fungi occur in a wide range of environments (Lacey, 2016). Soil serves as an essential substrate, as it harbors numerous potential hosts such as insects and contains free carbon sources that can be utilized for energy (3, 2018). Studies using soil samples from the five Brazilian biomes (Amazon, Cerrado, Caatinga, Atlantic Forest, and Pampa) have reported the greatest diversity of entomopathogenic fungi in the Amazon biome (Zanardo, 2015). However, in the present study, the abundance of entomopathogenic fungi was relatively limited, likely due to the use of a selective isolation medium supplemented with 2% dried and macerated *Nasutitermes* sp. as the sole carbon source, which favored the growth of fungi capable of utilizing this substrate exclusively.

Regarding insect mortality, all termites exposed to the fungal strains exhibited mortality rates above 75% from the sixth day after inoculation, a pattern not observed in the control group. Since the germination rate of fungal conidia on the host cuticle is a key factor in determining potential virulence (Mascarin et al., 2019), and entomopathogenic fungi with mortality rates exceeding 40% are considered effective control agents (Alves, 2016), it can be inferred that the isolates *Paecilomyces lilacinus* (4.807), *Paecilomyces* sp. 1 (4.816), and *Aspergillus* sp. 1 (4.808) were effective in controlling *Nasutitermes* sp.

Analyzing the mortality rate over time for the different isolates reveals variations related to the infection mechanism of the fungi themselves. Processes such as infection, adhesion, germination, penetration, and colonization vary depending on the fungal isolate and environmental conditions, including temperature and humidity (Agostini et al., 2015).

Fungi of the genus *Paecilomyces* are widely used in biological control and can be isolated from most soils (Senthilkumar et al., 2020). *In vitro* studies highlight this genus as virulent against other insects, such as *Hedypathes betulinus* (Coleoptera:

Cerambycidae) (Leite et al., 2011) and *Oligonychus yothersi* (Acari: Tetranychidae) (Oliveira et al., 2004). This genus is considered promising for biological control due to its production of paecillotoxin, a compound commonly used for controlling a wide range of organisms (Khan et al., 2003).

Paecilomyces lilacinus occurs as a saprophyte in soil and decaying plant material and is described as a parasite of insects and nematodes (Ponte, 2017). In nematode control, its action is characterized by penetrating eggs and destroying the embryo, thereby exerting strong pressure on the reproductive capacity of colonized females (Senthilkumar et al., 2020). This fungus has also been reported in the biocontrol of leaf-cutting ants (*Atta* spp.), achieving mortality rates of around 80% (Loureiro; Monteiro, 2005). Furthermore, the application of fungal suspensions in termite nests has proven effective under field conditions, resulting in satisfactory colony mortality (Toscano et al., 2010). However, this is the first report of *Paecilomyces lilacinus* being used to control *Nasutitermes* sp.

5 CONCLUSIONS

The selective culture medium with termites proved effective for isolating entomopathogenic fungi, as all fungi tested in the bioassay exhibited high virulence against *Nasutitermes* sp., causing total insect mortality by the eighth day, a result not observed in the control group.

Amazonian entomopathogenic fungi represent a promising alternative to chemical insecticides for the control of *Nasutitermes* sp., particularly species of the genus *Paecilomyces*. This study constitutes the first report of *Paecilomyces lilacinus* as a biological control agent against termites.

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