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DOI: http://dx.doi.org/10.1590/1807-1929/agriambi.v28n7e279093 ORIGINAL ARTICLE

Entomopathogenic fungi: Control of *Aceria guerreronis* in commercial planting of *Cocos nucifera*[1]

Fungos entomopatogenicos: Controle de *Aceria guerreronis* em plantio comercial de *Cocos nucifera*

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HIGHLIGHTS:

The fungi Purpureocillium lilacinum *and* Beauveria bassiana *control mites in field conditions.*
Entomopathogenic fungi has an acaricide action.
The introduction of microorganisms is a more sustainable alternative for controlling A. guerreronis.

ABSTRACT: The coconut mite (*Aceria guerreronis* - Eriophyidae) attacks coconut fruits, inhabits the meristematic region, and causes losses in fruit production. Chemicals are the main control measures but successive applications can cause resistance in mites. In this sense, it is necessary to search for ecological alternatives that assist in sustainable management, as consumers seek products grown using more eco-friendly techniques. This study aimed to identify an entomopathogenic fungal isolate and evaluate its ability to control the mite *A. guerreronis*, which is present in commercial areas in the municipality of Santa Izabel do Pará, Brazil, in the Eastern Amazon. The efficiency of fungi on mites was tested using six treatments: water (control), chemical acaricide, and fungi of the genera *Purpureocillium*, *Metarhizium*, *Beauveria*, and *Trichoderma*; the treatments were applied to the bunches at a concentration of 10^8 conidia mL^{-1}. The results demonstrated a reduction in mites on fruits, with the *B. bassiana* and *P. lilacinum* treatments being the most successful. This study demonstrates that these fungi have acaricidal action and may present an economically viable and ecological alternative for controlling phytophagous mites in coconut cultivation in the Amazon.

Key words: biocontrol, coconut pests, Amazon biome, sustainability

RESUMO: O ácaro do coco (*Aceria guerreronis* - Eriophyidae) ataca os frutos do coqueiro, habita a região meristemática e causa perdas na produção de frutos. A principal medida de controle é por meio de produtos químicos. Sucessivas aplicações podem causar a resistencia dos ácaros, neste sentido, faz-se necessária a busca por alternativas ecológicas que auxiliem em um manejo sustentável, pois consumidores procuram produtos oriundos de técnicas mais saudáveis. O objetivo deste estudo foi identificar um isolado fúngico entomopatogênico e avaliar o controle sobre o ácaro *A. guerreronis*, presente em áreas comerciais no município de Santa Izabel do Pará, Brasil, Amazônia Oriental. A eficiência dos fungos sobre os ácaros, foi testada através de seis tratamentos: água (controle), acaricida químico e fungos dos gêneros *Purpureocillium*, *Metarhizium*, *Beauveria* e *Trichoderma*, na concentração de 10^8 conídios mL^{-1}, e aplicados sobre os cachos. Os resultados demonstraram que houve redução de ácaros nos frutos, sendo os tratamentos à base de *B. bassiana* e *P. lilacinum*, os mais eficientes, demonstrando que esses fungos possuem ação acaricida e podem ser uma alternativa economicamente viável e ecológica para o controle de ácaros fitófagos no cultivo de coqueiro na Amazônia.

Palavras-chave: biocontrole, pragas do coqueiro, bioma Amazônia, sustentabilidade

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Introduction

The Amazon forest is the largest biome in Brazil and is notable for its abundant biodiversity, especially of microorganisms such as fungi and bacteria. This microbial diversity has not been extensively explored, especially in relation to fungi (Cerqueira et al., 2018). Some species of fungi stand out as control agents for insect pests (Liu et al., 2022), and have the potential to control pest mites (Parveen et al., 2021). In a pathogenicity test on mites, the fungus *Beauveria bassiana* Vuillemin (Cordycipitaceae) showed potential for biological control applications (Pereira et al., 2019). In India, isolates of *B. bassiana* in pathogenicity testing caused mortality of up to 86.97% in the coconut mite (*Aceria guerreronis* Keifer - Eriophyidae) (Kalmath et al., 2012).

The *A. guerreronis* mite is one of the main pests of coconut trees. It inhabits the meristematic region of the fruit, causing production losses of 10 to 70% (Rezende et al., 2016). Continuous application of pesticide products can result in the insects becoming resistant (Ferreira et al., 2023). In Brazil, several pesticide products aimed at controlling *A. guerreronis* have been registered with the Ministry of Agriculture and Livestock (MAPA). The use of these products can cause serious risks to public health and the environment (Paiva-Guimarâes et al., 2019) and interfere with the biology of predators and the interaction between prey and predator (Barros et al., 2022). Ecological alternatives can help to reduce these problems and contribute to sustainable pest management. In this context, the objective of this study was to identify an entomopathogenic fungal isolate and evaluate its ability to control *A. guerreronis*, which is present in commercial areas in the municipality of Santa Izabel do Pará, Brazil, in the Eastern Amazon.

Material and Methods

The experiment was conducted in a commercial plantation of coconut trees intended for coconut water extraction, which is located in the municipality of Santa Izabel do Pará, state of Pará, Brazil, Eastern Amazon and forms part of the Reunidas SOCOCO farm (01º 13' 40.16" S; 48º 02' 54.35" W, and altitude of 24 m). The region is characterized by high annual rainfall of up to 3,000 mm and an average relative air humidity of 80%. According to Köppen-Geiger classification, the climate is type Af1, with a rainy period from January to May (Amazonian winter) and a less rainy period from June to December (Amazonian summer) (De Alfaia et al., 2023). The predominant soil in the area is Psamment (United States, 2014), with a green cover of the Pueraria type (*Pueraria phaseoloides* (Roxb.) Benth). The area receives cultural treatments every 60 days, involving manual vegetation reduction, as well as annual chemical crowning and fertilization.

The plantation was established in 2012 and features 2,974 plants distributed in 90 rows with 33 plants (plot K154), with a spacing of 7.4 × 7.5 × 7.5 m. From the first 40 lines, 54 plants featuring natural *A. guerreronis* infestation were selected. The border plants were disregarded. The plants did not receive chemical insecticides or acaricides in the year preceding the experiment. The experiment was conducted from January to October 2021, during which time the plants were nine years old and approximately 3 m tall.

DNA of the fungus *Purpureocillium* sp., belonging to the Micoteca of the Plant Protection Laboratory (LPP) of the Federal Rural University of the Amazon (UFRA), was extracted using the method described by Dissanayake et al. (2020). After DNA extraction, the sample was subjected to the PCR process, where the β-tubulin sequence was amplified with the help of primers T1-F (5'-AACATGCGTGAGATTGTAAGT-3') and βt2b-R (5'- ACCCTCAGTGTAGTGACCCTTGGC-3') which amplify approximately 600 bp (Glass & Donaldson, 1995). PCR reactions were performed with a final volume of 25 µL containing 1X 2X Master Mix (Promega) (0.05 U μL^{-1} Taq DNA polymerase, 4 Mm MgCl 2 reaction buffer, 0.4 Mm of each DNTP), 20 µM of each primer, and 100 ng of DNA. The reactions were performed in an Eppendorf thermocycler (Hamburg, Germany). Cycles for the ITS primer consisted of initial denaturation at 95 °C for 3 min, followed by 35 cycles at 95 °C for 30 s, 55 °C for 1 min, 72 °C for 90 s, and a final cycle of 72 °C for 10 min. The PCR products were analyzed on a 1.0% agarose gel, and electrophoresis was performed at 80 V for 40 min. To purify the PCR product, the ExonucleaseI, and Shrimp Alkaline Phosphatase (EXO/SAP) enzyme protocol (Promega) was used according to the manufacturer's recommendations. Sequencing was carried out at Actgene Ltda using ABI3730xl DNA Analyzer equipment (Applied Biosystems™). The sequences were deposited in GenBank under code OP957287.

Consensus sequencing was performed using the STADEN v.1.6 program package. Sequence analysis of the beta-tubulin (ß-tub) isolate was performed with the similarity index-based search system using the BLAST program that is available at NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Multiple sequence alignment was performed using MAFFT v. 7.110 (Katoh & Standley, 2013) and manually corrected when necessary. Phylogenetic inference in this study was based on Maximum Likelihood and Bayesian Inference. Maximum Likelihood analysis was performed using Mega v.7 (Kumar et al., 2016) based on the Tamura-Ney model (Tamura & Nei, 1993) with 1000 bootstraps. Bootstrap values were generated automatically by analysis program. The isolate *Drechmeria gunni* (accession number: DQ522488) was used as an outgroup. The Phylogenetic tree obtained via Bayesian analysis was performed using MrBayes v. 32.2 (Ronquist et al., 2012). The best replacement model was estimated using jModelTest 2.1.10 (Darriba et al., 2012) with the Akaike information criterion. The Bayesian analysis was based on the GTR+G model, where four Markov chains ran simultaneously for 10,000,000 generations and sampling was performed every 1,000 generations. The burn-in phase was performed to discard 18% of the initial trees, obtaining a standard deviation of less than 0.01; the remaining trees were used to construct a phylogram calculated using the Bayesian posterior probability.

Fungi were produced through mass multiplication in parboiled rice. From January to October 2021, the 54 selected plants were divided into nine randomized blocks and each of the six trees in each block were sprayed with

different treatments, namely, water (control), isolates of fungi *Trichoderma* sp., *Metarhizium anisopilae* (UFRA-MA-02), *B. bassiana* (UFRA-Bb05), and *Purpureocillium lilacinum* (UFRA01), and an abamectin-based acaricide. The selected plants were identified by treatment, and all bunches were sprayed. The treatments were applied using a manual knapsack sprayer equipped with a 40-pound pressure regulator and 110.2 fan nozzle, in a jet directed to the fruits of Clusters 12 to 17, using 2 L of syrup per plant. Monthly applications were carried out in the morning from 7:00 a.m. under favorable weather conditions (no rain and low wind speed).

At intervals of 15 days between applications, the fruits of cluster 14 were evaluated and quantified monthly for *A. guerreronis* injuries. The percentage of damaged fruits was calculated relative to the total number of fruits in bunch 14. For the collected fruits, a damage rating scale was used according to Souza et al. (2017). The percentage of damage in the perianth region around the bract was determined based on the maximum and minimum levels of damage observed.

One fruit from Cluster 14 was randomly collected for each treatment, placed in separate marked plastic bags, and transported to the Laboratory of Entomology at UFRA to evaluate the presence of dead and live mites; this procedure was adapted from Fernando et al. (2007). The bracts and fruits were examined under a stereomicroscope and dead mites were collected with a brush, placed on microscopic slides containing a drop of blue cotton dye, and observed under an optical microscope to confirm the presence of hyphae or fungal spores. Live mites were transferred with a brush to a vial containing 1 mL of 70% alcohol and a drop of Tween 80 to break the surface tension.

Data relating to the average number of mites, percentage of damage to the fruit, and percentage of fruits with damage to the bunch were analyzed per treatment, using analysis of variance (ANOVA). The significant differences between the means were calculated using the Tukey test ($p \leq 0.05$). The averaged mite data were transformed using the Box Cox method and the number of live mites between treatments. The analyses were conducted using the statistical software R (version 4.2.1, R Core Team, 2021).

Results and Discussion

With the primers ß-tubulin-F (5 ACGCTGCTCATCTCCAAGAT 3') and ß-tubulin-R (5' TCAATGCAGAAGGTCTCGTC 3'), it was possible to amplify the sequence of the isolated *Purpureocillium* sp. containing 643 bp. The BLAST program revealed a high degree of similarity (100%) between the isolate's ß-tubulin sequence and the type isolate of *P. lilacinum* (CBS 284.36). A total of 16 sequences were used in this study (Table 1). The phylogenetic trees obtained using Maximum Likelihood (Figure 1) and Bayesian analysis (Figure 2) had identical topologies and were not significantly different. In both trees, the isolate belonged to the species *P. lilacinum* (Thom.) Samson, part of the Ophiocordycipitaceae family with bootstrap support of 99% and posterior probability of 96%.

Fruits from bunch 14 on each of the 54 plants were evaluated, totaling 552 fruits, from which 436 were collected; of these, 49.09% (214 fruits) were without injuries, 48.17% (210 fruits) exhibited injuries caused by *A. guerreronis*, 2.52% (11 fruits) exhibited injuries caused by *S. furcatus*, and 0.23% (one fruit) exhibited injuries caused by both *A. guerreronis* and *S. furcatus*.

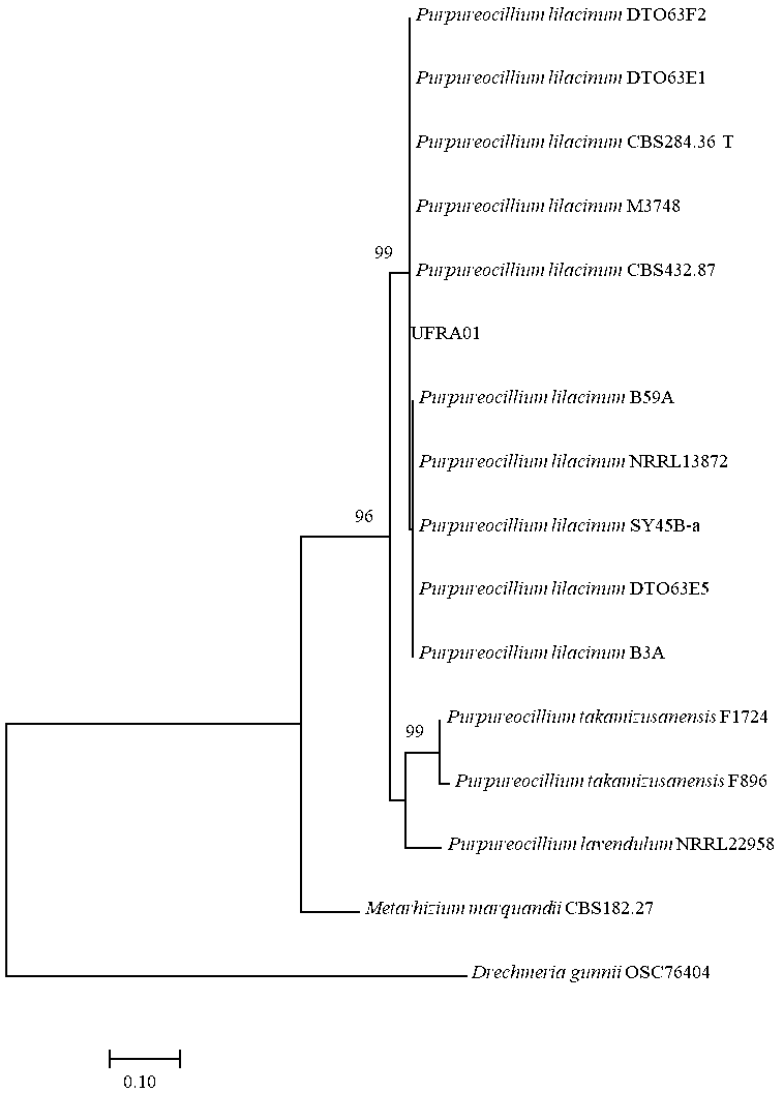
No dead mites were found under the bracts in any of the treatment groups. However, outside the bract, dead mites with spores of *P. lilacinum* and *B. bassiana* were found in two fruits (Figure 3). The Kruskal-Wallis test showed that the number of live *A. guerreronis* was significantly influenced by the treatments, with collection carried out every 15 days after application ($x^2 = 70.04$; $df = 5$; $p \leq 0.05$). The highest number of *A. guerreronis* were obtained with the control treatment (water), followed by the *Trichoderma* sp., Abamectin, *M. anisopliae*, and *B. bassiana* treatments. The least *A. guerreronis* were found in the *P. lilacinum* treatment group (Figure 4).

During the rainy period from January to May (Amazonian winter), fruits treated with *P. lilacinum* and *B. bassiana* exhibited a lower average population of *A. guerreronis* (Table 2). However, during the less rainy period of June to October (Amazonian summer), fruits treated with *P. lilacinum* still exhibited a lower average *A. guerreronis* population, in contrast to all other treatments (Table 3). Comparing the two periods,

Table 1. Sequences of fungal isolates used in the phylogenetic analysis to identify the species *Purpureocillium lilacinum*

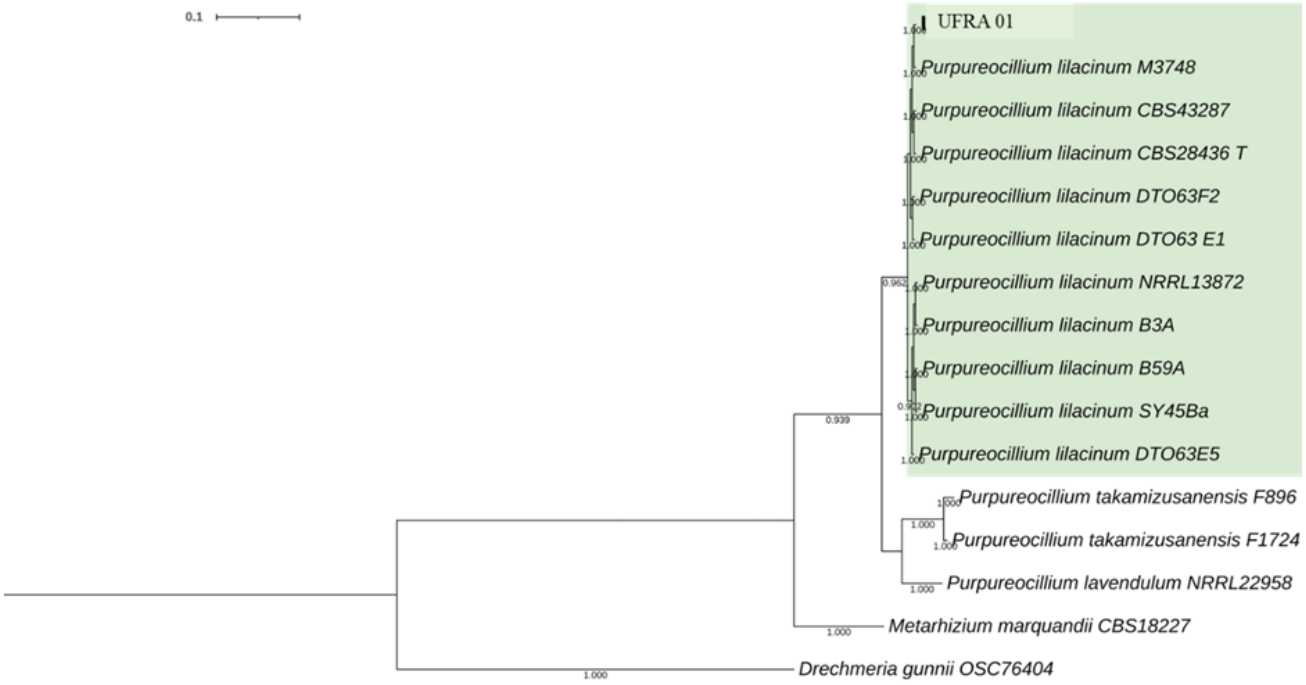
Species	Isolated	GenBank accession number	Reference
		ß-tubulin	
P. lilacinum	CBS 284.36^T	AY624227	Luangsa-Ard et al. (2005)
P. lilacinum	NRRL13872	GU979997	Unpublished
P. lilacinum	DTO 63E5	GU968702	Houbraken et al. (2010)
P. lilacinum	CBS 432.87	AY624228	Luangsa-Ard et al. (2005)
P. lilacinum	M3748	KC157849	Unpublished
P. lilacinum	B3A	HM242265	Johny et al. (2012)
P. lilacinum	B59A	HM242266	Johny et al. (2012)
P. lilacinum	SY45B-a	HM242267	Johny et al. (2012)
P. lilacinum	DTO 63F2	GU968703	Houbraken et al. (2010)
P. lilacinum	DTO 63E1	GU968701	Houbraken et al. (2010)
P. lilacinum	UFRA01	OP957287	In this study
P. lavendulum	NRRL 22958	GU980007	Unpublished
P. takamizusanensis	F1724	GU980010	Unpublished
P. takamizusanensis	F896	GU980011	Unpublished
Metarhizium marquandii	CBS 182.27^T	AY624229	Luangsa-Ard et al. (2005)
Drechmeria gunnii (Out-group)	OSC 76404	DQ522488	Spatafora et al. (2007)

T - Type isolate



Statistical support values greater than 70% are shown at the nodes using bootstrapping. *Drechmeria gunnii* isolate was designated as an out-group. T - Type isolate

Figure 1. Phylogenetic tree obtained via Maximum Likelihood analysis of the ß-tubulin sequences of the UFRA 01 isolate used in this study



Drechmeria gunnii isolate was designated as an out-group. T - Type isolate

Figure 2. Phylogenetic tree obtained via Bayesian analysis of ß-tubulin sequences from the UFRA 01 isolate used in this study

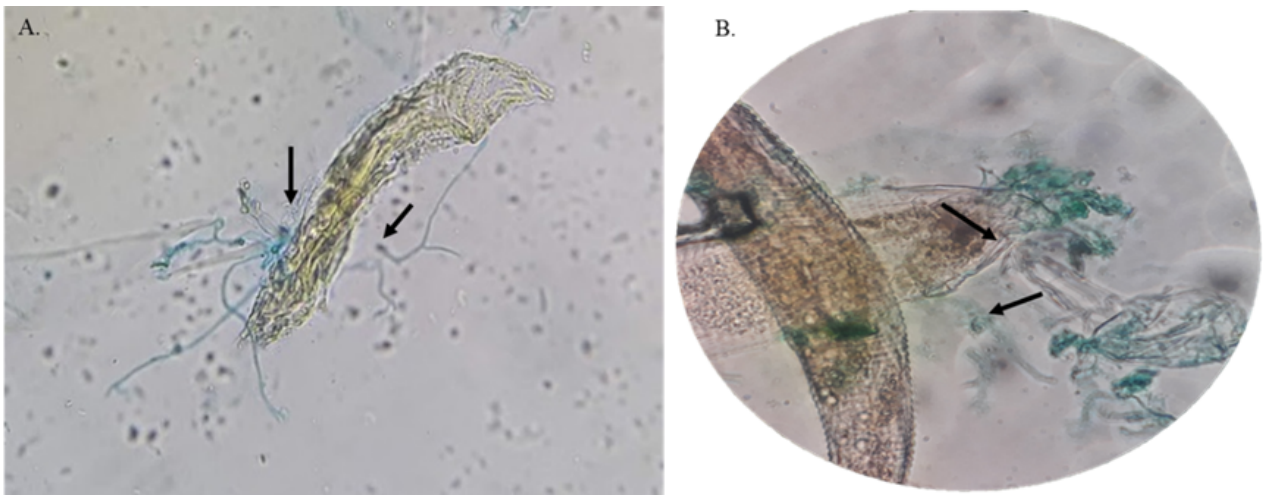
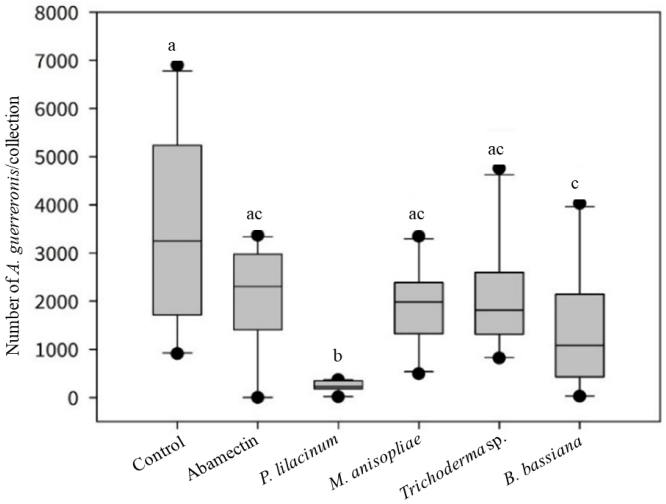


Figure 3. Dead mites found outside the bract in fruits treated with (A) *P. lilacinum* and (B) *B. bassiana* in a commercial plantation of *Cocos nucifera*, municipality of Santa Izabel do Pará, PA, Eastern Amazon, Brazil (arrows indicate spore germination)



Averages followed by the same letter do not differ statistically according to the Kruskal-Wallis test ($p \leq 0.05$)

Figure 4. Number of *A. guerreronis* collected from fruits of *Cocus nucifera* following different treatments

revealed that from June to October there was a significant increase of 22% in the average population of *A. guerreronis* in the control treatment (water). This period featured reduced rainfall, increased temperature, and reduced relative air humidity (Figure 5), leading to a consequent increase in the mite population.

Isolate UFRA01 belongs to the species *Purpureocillum lilacinum*, which according to Yamamoto et al. (2020) is sometimes misidentified as *Isaria* spp., as the anamorphs of both groups are similar. Luangsa-ard et al. (2011), in an in-depth morphological and phylogenetic study, proposed the creation of the genus *Purpureocillium* to accommodate the species *Paecilomyces lilacinus*, modifying it to *P. lilacinum*, which is why morphological identification should be accompanied by molecular analyses. In Brazil, *P. lilacinum* is used to control parasitic nematodes on plants. In addition to its role as a bionematicicide, this fungus also has insecticidal (Liu et al., 2022) and acaricidal properties (Silva et al., 2022).

The absence of dead mites under the bracts after treatment application suggests that in the field and on the fruits, the fungi may be able to control mites under the bracts through enzymes or the production of toxic metabolites, produced by the fungi in contact with mites. Mites under the floral bracts that cover the perianth of the fruit would be protected from the treatment spray (De Alfaia et al., 2023); however, the fungi might dislodge them (expel them), since no mummified mites (dead mite covered in fungus) were found, in fruit treated with *B. bassiana* and *P. lilacinum*. These fungal treatments differed from the standard (acaricide) and control (water) treatments, in that a significant number of fungi treated fruits were damaged but had a low number of mites under the bracts. In addition, *P. lilacinum* treatment resulted in a greater number of fruits that were not infested by *A. guerreronis*.

Two dead mites containing spores of *P. lilacinum* and *B. bassiana* were found outside the bracts. The action of fungi may also be related to the moment of mite dispersion when they leave the perianth to disperse and walk on the fruit

Table 2. Percentage of damaged fruits in bunch 14, % of damage to the fruit, average *A. guerreronis* population in fruits of *Cocos nucifera*, 15 days after treatment application during the Amazon rainy season (January to May/2021) in the municipality of Santa Izabel do Pará, PA, Eastern Amazon, Brazil

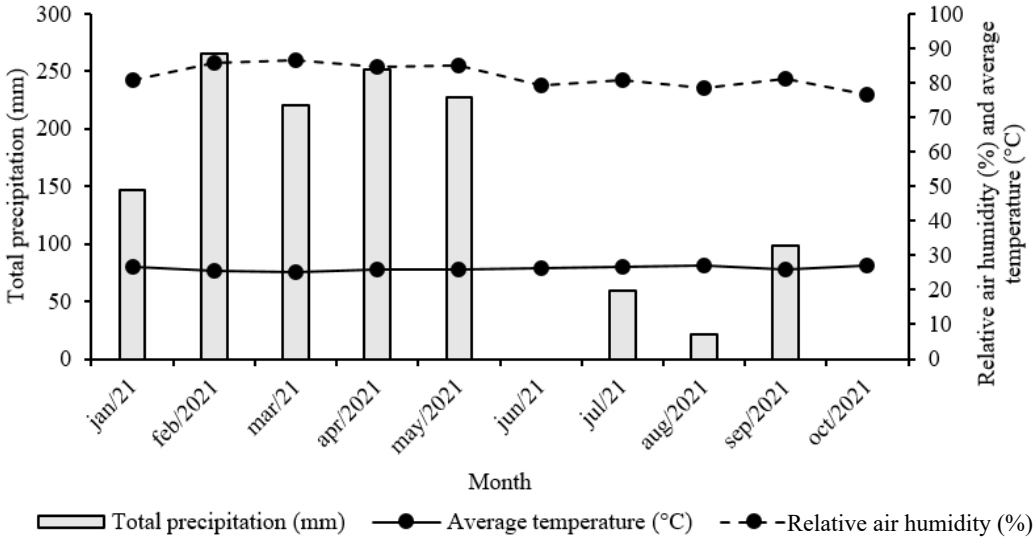
Treatment	% of damaged fruits in bunch 14	% damage to the fruit	Average of *A. guerreronis*	% of control
Water	10.29 ± 2.07 a	0.09 ± 0.05 a	2400.82 ± 376.36 a	-
Abamectin	17.02 ± 5.67 a	0.22 ± 0.04 a	1930.04 ± 352.90 a	20
Trichoderma sp.	15.08 ± 1.47 a	0.16 ± 0.03 a	2496.14 ± 254.60 a	-4
M. anisopliae	17.93 ± 4.86 a	0.20 ± 0.04 a	1967.35 ± 177.72 a	18
B. bassiana	12.03 ± 3.88 a	0.14 ± 0.04 a	749.65 ± 228.15 b	69
P. lilacinum	13.14 ± 2.70 a	0.18 ± 0.02 a	207.36 ± 20.93 b	91
CV (%)	60.81	51.01	14.30	-

Means with the same letters in the column do not differ from each other by the Tukey test at $p \leq 0.05$

Table 3. Percentage of damaged fruits in bunch 14, % of damage in the fruit, average *A. guerreronis* population in fruits of *Cocos nucifera*, 15 days after treatment application during the Amazon dry season (June to October/2021) in the municipality of Santa Izabel do Pará, PA, Eastern Amazon, Brazil

Treatment	% of damage fruits per *A. guerreronis*	% fruit damage	Average *A. guerreronis*	% of control
Water	8.52 ± 1.48 a	0.14 ± 0.02 a	3084.64 ± 323.87 a	-
Abamectin	11.66 ± 1.04 a	0.15 ± 0.02 a	2001.24 ± 464.69 a	35
Trichoderma sp.	15.19 ± 3.36 a	0.06 ± 0.02 a	1924.12 ± 64.19 a	38
M. anisopliae	10.74 ± 1.92 a	0.16 ± 0.03 a	1834.54 ± 97.23 a	41
B. bassiana	10.47 ± 1.91 a	0.13 ± 0.03 a	1983.63 ± 390.99 a	36
P. lilacinum	13.36 ± 1.53 a	0.13 ± 0.01 a	244.52 ± 33.10 b	92
CV (%)	39.80	39.97	3.86	-

Means with the same letters in the column do not differ from each other by the Tukey test at $p \leq 0.05$



Source: Climate data from the SOCOCO Company

Figure 5. Climatic conditions during the application period (January to October/2021), in the municipality of Santa Izabel do Pará, PA, Eastern Amazon, Brazil

epicarp (Silva et al., 2022). Barreto et al. (2004) evaluated the effects of different isolates of *B. bassiana* and *M. anisopliae* on *Mononychellus tanajoa* Bondar (Tetranychidae) in the laboratory, and concluded that the isolates of *B. bassiana* were more efficient. In India, *Beauveria* isolates caused mortality equivalent to that of the fungi *Hirsutella tompsonni* Fischer in a pathogenicity test on the mite *A. guerreronis* (Kalmath et al., 2012). In a pathogenicity test of two isolates of *B. bassiana* and one of *M. anisopliae* on the mite *Phyllocoptes gracilis* Nalepa (Eriophyidae), the isolate of *Beauveria* cause greater mite mortality (Minguely et al., 2021).

Fruits treated with *P. lilacinum* presented the lowest average population of *A. guerreronis*, corroborating the results of Fiedler &Sosnowska (2007) who tested *Paecilomyces lilacinus* (Thom.) Samson on the two-spotted spider mite *Tetranychus urticae* (Tetranychidae) on bean plants under laboratory and greenhouse conditions. The mortality rate was 78% in the laboratory and 60% in the greenhouse; it is worth noting that until 2011, the fungus *P. lilacinum* was known as *Paecilomyces lilacinus*. According to Shin et al. (2017), *P. lilacinum* tolerates temperatures of up to 38 °C, the average temperature in the municipality of Santa Izabel during the studied period was close to 30 °C (Figure 5). Temperatures were higher during the dry period (Figure 5).

In terms of the percentage of fruits damaged by *A. guerreronis*, treatment with the fungus *P. lilacinum* did significantly alter outcomes, compared to the standard treatment with abamectin. According to Calvet et al. (2018), *A. guerreronis* can modify its behavior to increase its fitness, and the presence of products in the fruits may have contributed to the dispersion of the mites. According to Azevedo et al. (2022), mite dispersion can occur through the action of wind or arthropods that transport specimens from one plant to another.

Conclusions

1. Through molecular characterization it is possible to identify fungi of the species *P. lilacinum*.

2. The natural populations of *A. guerreronis* in *Cocos nucifera* fruits were reduced after the application of the entomopathogenic fungi *P. lilacinum*, and to a lesser extent *B. Bassiana*.

3. Fungi of the species *B. bassiana* and *P. lilacinum* isolated from Amazonian soils could be used to develop bioacaricides to control *A. guerreronis*.

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