Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Native fungi from Amazon with potential for control of *Aedes aegypti* L. (Diptera: Culicidae)

Fungos nativos da Amazônia com potencial de controle de *Aedes aegypti* L. (Diptera: Culicidae)

G. R. Q. Mendonça[a]*, L. P. Peters[b], L. M. Lopes[c], A. H. Sousa[c] and C. M. Carvalho[a,c]

[a]Universidade Federal do Acre, Programa de Pós-graduação em Ciência, Inovação e Tecnologia para Amazônia, Rio Branco, AC, Brasil
[b]Universidade Federal do Acre, Centro de Ciências da Saúde e do Desporto, Rio Branco, AC, Brasil
[c]Universidade Federal do Acre, Centro de Ciências Biológicas e da Natureza, Rio Branco, AC, Brasil

Abstract

Aedes aegypti L. (Diptera: Culicidae) is the main transmitter of pathogens that cause human diseases, including dengue, chikungunya, zika and yellow fever. Faced with this problem, this study aims to select fungi with entomopathogenic potential against *Ae. aegypti* and develop formulations that optimize the control action of entomopathogenic fungi in the semi-field condition. 23 fungal strains native from Amazon were inoculated in Potato-Dextrose-Agar (PDA) culture medium for 14 days and then transferred by scraping to tubes containing 0.9% NaCl solution. To obtain the larvae, eggs were collected using traps in peridomestic environments for 7 days. 20 larvae of *Ae. aegypti* in 125 mL erlenmeyers containing 20 mL of conidial suspension at a concentration of $1x10^6$ conidia/mL for initial selection and $1×10^4$, $1×10^5$, $1×10^6$ and $1×10^7$ conidia/mL for determination of LC_{50}. Mortality was checked every 24 h for 5 days. The three fungi with the best virulence rates were identified using molecular techniques. The compatibility between fungi at a concentration of $1×10^6$ conidia/mL and oily adjuvants, mineral oil and vegetable oil (andiroba, chestnut and copaiba) at concentrations of 0.1, 0.5 and 1% was evaluated. The germination capacity of 100 conidia per treatment was evaluated after incubation at 28 ºC for 24 h. To evaluate the entomopathogenic potential of the fungal formulations, conidial suspensions ($1×10^6$ conidia/mL) were added with 0.1% mineral and vegetable oil. The treatments were submitted to laboratory and semi-field conditions and mortality was verified every 24 h for 5 days. *Beauveria* sp. (4,458) (LC_{50} = 8.66× 10^3), *Metarhizium anisopliae* (4,420) (LC_{50} = 5.48×10^4) and *M. anisopliae* (4,910) (LC_{50} = 1.13×10^5) were significantly more effective in the larval control of *Ae. aegypti*, in relation to the other fungal morphospecies evaluated. Mineral oil was better compatible in all treatments evaluated. *Beauveria* sp. (4,458) was considerably less virulent under semi-field conditions. *M. anisopliae* (4,910) formulated with mineral oil increased larval mortality to 100% on the 4th day in the laboratory and on the 5th day in the semi-field. Fungal formulations developed from native Amazonian isolates represent a promising tool for the development of strategies to control *Ae. aegypti*.

Keywords: biological control, arboviruses, *Beauveria*, *Metarhizium*.

Resumo

Aedes aegypti L. (Diptera: Culicidae) é o principal transmissor de patógenos que causam doenças humanas, incluindo dengue, chikungunya, zika e febre amarela. Diante desse problema, este estudo tem como objetivo selecionar fungos com potencial entomopatogênico frente ao *Ae. aegypti* e desenvolver formulações que otimizem a ação de controle de fungos entomopatogênicos na condição de semi-campo. 23 cepas fúngicas nativas da Amazônia foram inoculadas em meio de cultura Potato-Dextrose-Agar (PDA) por 14 dias e depois transferidas por raspagem para tubos contendo solução de NaCl 0,9%. Para obtenção das larvas, os ovos foram coletados por meio de armadilhas em ambientes peridomiciliares por 7 dias. 20 larvas de *Ae. aegypti* em erlenmeyers de 125 mL contendo 20 mL de suspensão de conídios na concentração de $1×10^6$ conídios/mL para seleção inicial e $1×10^4$, $1×10^5$, $1×10^6$ e $1×10^7$ conídios/mL para determinação de CL_{50}. A mortalidade foi verificada a cada 24 horas durante 5 dias. Os três fungos com as melhores taxas de virulência foram identificados por meio de técnicas moleculares. Foi avaliada a compatibilidade entre fungos na concentração de $1×10^6$ conídios/mL e adjuvantes oleosos, óleo mineral e óleo vegetal (andiroba, castanha e copaíba) nas concentrações de 0,1, 0,5 e 1%. A capacidade germinativa de 100 conídios por tratamento foi avaliada após incubação a 28 ºC por 24 h. Para avaliar o potencial entomopatogênico das formulações fúngicas, suspensões de conídios ($1×10^6$ conídios/mL) foram adicionadas de óleo mineral e vegetal a 0,1%. Os tratamentos foram submetidos a condições de laboratório e semi-campo e a mortalidade foi verificada a cada 24 horas durante 5 dias. *Beauveria* sp. (4.458) (LC_{50} = 8,66×10^3), *Metarhizium anisopliae* (4.420) (LC_{50} = 5,48×10^4) e *M. anisopliae* (4.910) (LC_{50} = 1,13×10^5) foram significativamente mais eficazes no controle larval de *Ae. aegypti*, em relação às demais morfoespécies fúngicas avaliadas. O óleo mineral foi melhor compatível em todos os tratamentos avaliados. *Beauveria* sp. (4.458) foi consideravelmente menos virulento em condições de semi-campo. *M. anisopliae* (4.910) formulado com óleo mineral aumentou a mortalidade larval para 100% no 4º dia no laboratório e no 5º dia no semi-campo. Formulações fúngicas desenvolvidas a partir de isolados nativos da Amazônia representam uma ferramenta promissora para o desenvolvimento de estratégias de controle de *Ae. aegypti*.

Palavras-chave: controle biológico, arboviroses, *Beauveria*, *Metarhizium*.

*e-mail: gleisonrafael13@gmail.com
Received: May 19, 2023 – Accepted: August 14, 2023

1. Introduction

Among the hundreds of arboviruses already known, approximately thirty are capable of causing disease in humans (Nicoletti, 2020). Of this diversity, only dengue, chikungunya, zika and yellow fever are responsible for almost all cases, and the vector is the *Aedes aegypti* L. mosquito (Diptera: Culicidae) (Caragata et al., 2019).

Some characteristics are essential for the effectiveness of *Ae. aegypti* as a vector, such as its easy adaptation to urban environments, common occurrence in domestic spaces and obtaining nutrition for reproduction from human blood (Wang et al., 2018).

Between January and September 2020, approximately 20,902 notifications of probable cases of dengue, chikungunya or zika were registered in the Brazilian Amazon Region (Brasil, 2020), thus showing the importance of this vector.

In the Amazon, populations of *Ae. aegypti* persist throughout the year, and in the rainy seasons the population number of vectors is drastically high, which results in epidemic outbreaks of viral diseases (Rodrigues et al., 2019).

The most effective way is to control the vector, and options of biological insecticides formulated from entomopathogenic fungi to control *Ae. aegypti* has shown promise, with the aim of replacing or integrating with the usual chemicals (Lacey et al., 2015).

The application of fungal entomopathogens in the environment encounters challenging and complex scenarios of biotic and abiotic stress (Ortiz-Urquiza and Keyhani, 2015). Sunlight, temperature and humidity are among the most critical factors capable of affecting the persistence of these microorganisms (Páramo et al., 2015).

Thus, the proposition of formulations from microbial agents to control human disease vectors is interesting (Fernandes et al., 2015). Adjuvant components improve the effect of the entomopathogenic fungus on the target insect, providing greater radiation protection, nutrients for fungal growth, storage conditions, greater practicality in application and greater adaptation to the natural environment, enabling the use of the pathogen in the field (Butt et al., 2016).

The Brazilian Amazon Region stands out for its ecosystem complexity that has a great diversity of entomopathogenic microorganisms with biotechnological potential, until then, little investigated for the control of disease vectors.

Thus, this study aims to select Amazonian fungi with entomopathogenic potential against *Ae. aegypti* and test formulations that optimize the control action of entomopathogenic fungi under semi-field conditions.

2. Material and Methods

2.1. Fungi maintenance and preparation of conidia suspensions

Twenty-three fungal morphospecies with good conidia production capacity were reactivated, stored in the collection of entomopathogenic fungi at Microbiology Laboratory from Universidade Federal do Acre (UFAC). The reactivation of fungal samples stored in mineral oil and distilled water was carried out by inoculating the fungi in Potato-Dextrose-Agar-BDA medium (200g potato, 20g dextrose and 15g agar in 1 L of distilled water) and incubation at room temperature for 14 days. After mycelial growth, the fungi were transferred to tubes with PDA medium and stored at room temperature (Ayala-Zermeño et al., 2017).

To assess virulence against *Ae. aegypti*, the fungi were cultivated in PDA medium at 28 ºC for 14 days and subsequently submitted to removal of the colonies surface by scraping and transferred to tubes containing 0.9% NaCl solution, with vigorous agitation in electric shaker and suspension standardization for the concentration of 10^6 conidia/mL in a Neubauer chamber (Remadevi et al., 2010).

2.2. Virulence assay against Aedes aegypti

To obtain the larvae of *Ae. aegypti*, eggs were collected from traps installed in properties, randomly chosen, in urban areas of the city of Rio Branco, Acre. The ovitraps were installed in extra-domestic areas, in shaded places and close to other mosquito breeding sites for 7 days. The pallets were collected and placed in plastic containers with tap water and fish food to facilitate the hatching of the larvae (Carvalho et al., 2004).

20 larvae of *Ae. aegypti* in 125 mL Erlenmeyer flask, containing 20 mL of the conidial suspension in distilled water at a concentration of 10^6 conidia/mL, constituting the group of exposed larvae, and 20 mL of the sterile distilled water solution, corresponding to the control group, with 3 repetitions. Mortality was verified every 24 h for 5 days (Carvalho et al., 2004).

The three fungi that showed the best virulence rates were submitted to new biological assays following the same methodological steps described in this topic, however, at different conidial concentrations, 1×10^4, 1×10^5, 1×10^6 and 1×10^7 conidia/mL in order to obtain a mortality variability considerable to calculate the lethal dose (LC50) (Carvalho et al., 2017).

2.3. Molecular characterization of fungi entomopathogenic to Aedes aegypti

The total genomic DNA of fungi with entomopathogenic potential were extracted using the cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1987). The fungus was grown in PDA culture medium for 14 days at 25 °C. 50 mg of pathogen mycelium was collected and subsequently macerated with liquid nitrogen in a crucible until a fine powder was formed.

3% CTAB extraction buffer was prepared using NaCl, 0.5 M EDTA pH 8.0 and 1.0 M TRIS-HCl pH 8.0. Subsequently, the sample was homogenized and placed in a water bath at 64 °C for one hour, homogenizing it every 15 minutes. For each sample, 0.5 mL of CIA (24:1; Chloroform: Isoamyl Alcohol) was added, being manually shaken for one minute, followed by centrifugation at 8000 rpm for 10 min.

The supernatant was transferred to a new microtube, again adding 0.5 mL of CIA, homogenizing and centrifuging at 8000 rpm for 10 min. Then, the supernatant was taken to another microtube and 0.35 ml of ice-cold (-20 °C) isopropanol was added. The sample was homogenized again and kept at -20 °C for 1 h.

After this period, the samples were centrifuged at 8000 rpm for 10 min. The pellet was washed with 70%

ethanol (70 mL of 100% ethanol and 30 mL of milli-Q water), centrifuged at 7500 rpm for 5 min and then dried at room temperature. Finally, the pellet was resuspended in 50 µL of sterile milli-Q water, adding 2 µL of RNAse (37 °C) to the sample.

To evaluate the DNA extraction, it was run on a 2% agarose gel, melted in 50 mL of TBE (Tris/Borate/EDTA Buffer). The buffer solution was prepared using 10.8 g TRIS, 5.5 g boric acid (H_3BO_3) and 0.74 g/4 mL 0.5 M EDTA pH 8.0. The procedure was performed in an electrophoresis vat, using the DNA 1kb Plus Ladder 100 bp molecular marker (Invitrogen). The DNA run was at 120 V for 45 min. The visualization was done in a photodocumentator.

The universal primers ITS1 (5′-TCCGTAGGT GAACCTGCGG-3′) and ITS4 (5′TCCTCCGCTTATTGATATGC-3′) (White et al., 1990) were used to amplify the ITS (Internal Transcribed Spacer) region. The PCR mix reaction consisted of around 40 ng DNA; 0.5 µM each primer; 0.2 µM dNTP; 1.25 U Taq DNA polymerase (Ludwig) and 2.5 µL buffer. The PCR reactions were cycled as follows: denaturation at 94 °C for 3 min, followed by 30 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min and a final extension at 72°C for 7 min. The amplification products were submitted to 1.0% agarose gel electrophoresis for 1 h. 1 Kb DNA Ladder (Invitrogen Life Technologies) was used as a base pair molecular marker.

Purification of the amplified product via PCR of the rDNA region composed of ITS1-5,8S-ITS2 was done with the Easy Pure PCR Purification Kit according to the manufacturer's instructions. Sequencing was done with the ITS1 and ITS4, using the MegaBACE 1000, a 96 capillary DNA analysis system with GE Healthcare technology. Sequencing reactions were done according to the protocol for the MegaBACE 1000, using the DYEnamic ET Dye Terminator Kit (with Thermo Sequenase™ II DNA Polymerase) and the sequences were analyzed by the Sequence Analyzer software using the Base Caller Cimarron 3.12. Sequencing reactions generally reach between 500 and 600 bases with 98.5% analysis fidelity.

The sequences were verified and edited using Bioedit 7.0.9.1 (Hall, 1999). Only regions of the sequences considered to be of higher quality were used, being processed to generate new FASTA files. The sequences were compared with other sequences present in the GeneBank and UNITE databases. After identifying species close and related to the isolates of interest, sequences of type organisms were used for phylogenetic analysis. The phylogenetic tree was constructed using the Neighbor Joining method (Jukes and Cantor, 1969), using the MEGA 11.0 program.

2.4. Evaluation of the compatibility between fungal conidia and vegetable and mineral oils

Formulations were prepared with entomopathogenic fungus at a concentration of 1×10^6 conidia/mL plus mineral oil and vegetable oils from Amazonian species andiroba (*Carapa guianensis*), Brazil nut (*Bertholletia excelsa*) and copaíba (*Copaifera multijuga*) at concentrations of 0.1, 0.5 and 1%. Vegetable oils were obtained from RESEX Alto Tarauacá, located in the municipality of Jordão, state of Acre.

To determine conidial germination indices, 10 µL of each treatment were placed at three equidistant points in Petri dishes containing PDA medium. These plates were incubated at 28 ºC for 24 h, and then, a drop of lactophenol blue was placed for observation of conidial viability under an optical microscope (Francisco et al., 2006). The germination capacity of 100 fungal conidia per treatment was evaluated.

2.5. Evaluation of the virulence of fungal formulations against Aedes aegypti in laboratory and semi-field

The three fungi with the highest lethality rates were prepared with formulations composed of conidial suspension at a concentration of 1×10^6 conidia/mL plus mineral oil or vegetable oil from the Amazonian species andiroba, chestnut and copaiba in the proportion of 0.1% (Camargo et al., 2012).

To evaluate the virulence of the fungal formulations under laboratory and semi-field conditions, 20 *Ae. aegypti* were deposited in 125 mL erlenmeyers. The treatments consisted of 20 mL of the fungal formulation: (a) aqueous conidial suspension, (b) conidial suspension + 0.1% mineral oil, (c) conidial suspension + 0.1% vegetable oil, (d) mineral oil 0.1%, (e) 0.1% vegetable oil and (f) 0.9% NaCl solution control. Three replications were made for each treatment at each site. Survival was assessed every 24 h for 5 days. The *in vitro* assays were kept in the Microbiology Laboratory as previously described and for the semi-field assays, the containers were kept in a shady peridomicile environment, similar to the typical mosquito collection sites.

2.6. Statistical analysis

Biological assay data were statistically analyzed according to normality. Analysis of variance (ANOVA) followed by Tukey's test for parametric data was used to compare the multivariate means. For the evaluation of non-parametric data, the Kruskal Wallis test was used, followed by the Student-Newman-Keuls (SNK) test to compare the mean rankings. Larval mortality data were submitted to Kaplan-Meier analysis using the GraphPad Prism 5.0 software. The determination of the lethal concentrations (LC) were estimated using the SAS 9.4 software.

3. Results

Virulence from 23 fungal morphospecies from the collection of fungi with entomopathogenic potential from the Microbiology Laboratory from UFAC at a concentration of $1x10^6$ conidia/mL was evaluated against *Ae. aegypti.*

Ae. aegypti was more susceptible to *Beauveria* sp. (4,458), which increased the larval mortality rate to 100% on the 5th day of the experiment (Table 1). *Metarhizium* sp. 1 (4,420) and *Metarhizium* sp. 2 (4,910) caused significantly higher mortality than the other isolates of the genus *Metarhizium*, with 80% and 95%, respectively, on the 5th day of the experiment.

The eight fungal morphospecies with mortality rates above 50% belong to the genera *Beauveria* and *Metarhizium*.

Table 1. Larval mortality rate of *Ae. aegypti* subjected to fungal conidia.

Fungi	Mortality rate (%) ± SD				
	1º day	2º day	3º day	4º day	5º day
Beauveria sp. 1 (4.458)	0 ± 0^{b}	15 ± 5^{b}	41.6 ± 7.6^{b}	70 ± 5^{b}	100 ± 0^{a}
Beauveria sp. 2 (4.394)	0 ± 0^{b}	7 ± 2.9^{f}	22 ± 2.9^{g}	32 ± 5^{i}	50 ± 5^{f}
Beauveria sp. 3 (4.438)	0 ± 0^{b}	15 ± 5^{b}	30 ± 0^{e}	43 ± 7.6^{e}	55 ± 5^{e}
Beauveria sp. 4 (4.798)	0 ± 0^{b}	3 ± 2.9^{h}	17 ± 2.9^{i}	32 ± 2.9^{i}	40 ± 0^{h}
Metarhizium sp. 1 (4.420)	0 ± 0^{b}	13 ± 2.9^{c}	35 ± 0^{c}	65 ± 0^{c}	80 ± 5^{c}
Metarhizium sp. 2 (4.910)	3 ± 2.9^{a}	27 ± 2.9^{a}	53 ± 5.8^{a}	80 ± 8.7^{a}	95 ± 5^{b}
Metarhizium sp. 3 (4.843)	0 ± 0^{b}	13 ± 7.6^{c}	32 ± 11.5^{d}	47 ± 5.8^{d}	55 ± 10^{e}
Metarhizium sp. 4 (4.903)	0 ± 0^{b}	8 ± 10.4^{e}	23 ± 15.2^{f}	43 ± 7.6^{q}	55 ± 15^{e}
Metarhizium sp. 5 (4.959)	0 ± 0^{b}	5 ± 8.7^{g}	30 ± 5^{e}	37 ± 5.8^{f}	60 ± 0^{d}
Metarhizium sp. 6 (4.887)	0 ± 0^{b}	0 ± 0^{j}	2 ± 2.9^{n}	8 ± 7.6^{i}	20 ± 5^{l}
Metarhizium sp. 7 (4.539)	0 ± 0^{b}	12 ± 2.9^{d}	23 ± 2.9^{f}	38 ± 2.9^{r}	45 ± 10^{g}
Metarhizium sp. 8 (4.892)	0 ± 0^{b}	3 ± 2.9^{h}	15 ± 5^{j}	32 ± 7.6^{i}	40 ± 10^{h}
Metarhizium sp. 9 (4.901)	0 ± 0^{b}	0 ± 0^{j}	2 ± 2.9^{n}	7 ± 2.9^{r}	10 ± 5^{o}
Paecilomyces sp. 1 (4.748)	0 ± 0^{b}	3 ± 2.9^{h}	13 ± 5.8^{k}	32 ± 2.9^{i}	40 ± 0^{h}
Paecilomyces sp. 2 (4.451)	0 ± 0^{b}	5 ± 0^{g}	23 ± 7.6^{f}	33 ± 2.9^{h}	40 ± 10^{h}
Paecilomyces sp. 3 (4.493)	0 ± 0^{b}	2 ± 2.9^{i}	12 ± 5.8^{l}	17 ± 5.8^{o}	25 ± 5k
Paecilomyces sp. 4 (4.428)	0 ± 0^{b}	0 ± 0^{j}	12 ± 5.8^{l}	18 ± 7.6^{n}	30 ± 0^{j}
Paecilomyces sp. 5 (4.449)	0 ± 0^{b}	8 ± 2.9^{e}	18 ± 7.6^{h}	25 ± 5^{k}	30 ± 10^{j}
Paecilomyces sp. 6 (4.455)	0 ± 0^{b}	13 ± 2.9^{c}	22 ± 2.9^{g}	33 ± 2.9^{h}	45 ± 5^{g}
Paecilomyces sp. 7 (4.432)	0 ± 0^{b}	5 ± 0^{g}	15 ± 0^{j}	30 ± 0^{j}	35 ± 5^{i}
Paecilomyces sp. 8 (4.490)	0 ± 0^{b}	5 ± 0^{g}	15 ± 5^{j}	22 ± 5.8^{l}	35 ± 0^{i}
Paecilomyces sp. 9 (4.407)	0 ± 0^{b}	3 ± 2.9^{h}	12 ± 2.9^{l}	20 ± 8.7^{m}	30 ± 0^{j}
Paecilomyces sp. 10 (4.471)	0 ± 0^{b}	7 ± 2.9^{f}	8 ± 2.9^{m}	12 ± 2.9^{p}	15 ± 0^{m}
Control	$\mathbf{0 \pm 0^{b}}$	$\mathbf{0 \pm 0^{j}}$	$\mathbf{2 \pm 2.9^{n}}$	$\mathbf{5 \pm 0^{s}}$	$\mathbf{12 \pm 5^{n}}$

The virulence data of these fungi against *Ae. aegypti* at a concentration of 1×10^{6} conidia/mL were analyzed by Kaplan Meier (Figure 1). The survival curves of larvae treated with fungal conidia of the three *Beauveria* morphospecies differed significantly from the control treatment ($\chi 2$ = 64.58; df = 4; $p < 0.0001$). The same was observed for the morphospecies of the genus *Metarhizium* ($\chi 2 = 64.58$; df = 4; $p < 0.0001$).

The three fungal morphospecies that increased mortality at a rate equal to or greater than 80% were selected for the following stages of the study, the fungi *Beauveria* sp. 1 (4,458), *Metarhizium* sp. 1 (4,420) and *Metarhizium* sp. 2 (4,910). These three fungi were were identified using molecular methods (Table 2).

The results of the molecular analysis of the sequences obtained from fungi 4.420 and 4.910, morphologically identified as *Metarhizium*, allowed us to confirm that they belonged to the species *M. anisopliae* with an identity rate of 99% (Table 2 and Figure 2b). On the other hand, with the results of the molecular characterization of the fungus 4,458 it was possible to reach only the genus level (*Beauveria* sp.) with 92% identity. However, phylogenetic

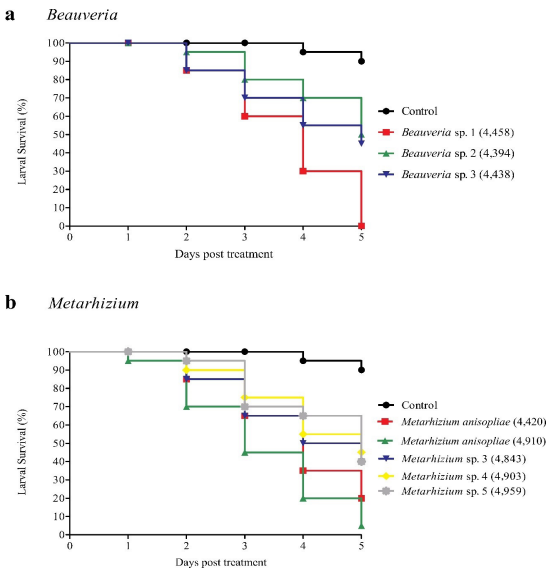

Figure 1. Larval mortality rates of *Aedes aegypti* against fungi native from amazon of the genera (a) *Beauveria*; and (b) *Metarhizium*.

analysis shows that this fungus is closer to the species *B. bassiana* (Figure 2a).

The three fungi with the highest mortality rates were evaluated for *in vitro* virulence against *Ae. aegypti* at concentrations 1×10^4, 1×10^5, 1×10^6 and 1×10^7 conidia/mL. The survival curves of the larvae treated with the fungal conidia of the three evaluated fungi differed significantly from the control treatments, with (A) *Beauveria* sp. (4,458) ($\chi2 = 2.05$; df = 4; $p < 0.0001$), (B) *Metarhizium anisopliae* (4,420) ($\chi2 = 3.55$; df = 4; $p < 0.0001$) and (C) *Metarhizium anisopliae* (4,910) ($\chi2 = 3.68$; df = 4; $p < 0.0001$) (Figure 3).

The fungal isolates were effective in increasing larval mortality rates at all conidial concentrations evaluated. The larvae of *Ae. aegypti* were more susceptible to the isolate *Beauveria* sp. (4,458) ($LC_{50} = 8.66\times10^3$) in relation to *Metarhizium anisopliae* (4,420) ($LC_{50} = 5.48\times10^4$) and *Metarhizium anisopliae* (4,910) ($LC_{50} = 1.13\times10^5$) (Table 3).

The conidial compatibility of *Beauveria* sp. 1 (4,458), *Metarhizium anisopliae* (4,420) and *Metarhizium anisopliae* (4,910) with mineral oil and vegetable oils of andiroba, chestnut and copaiba at concentrations of 0.1, 0.5 and 1%.

Significant differences in compatibility were observed between the types of oils used (andiroba, chestnut, copaiba

Table 2. Maximum nucleotide identity match of native Amazonian fungi with entomopathogenic potential against *Aedes aegypti* larvae based on ITS sequences using BLASTn analysis.

Fungi	GenBank Accession No.	Species	Identity
Beauveria sp. 1 (4,458)	ON794468	*Beauveria* sp. (UDB0780969)	92%
Metarhizium sp. 1 (4,420)	ON794467	*Metarhizium anisopliae* (MH104861)	99%
Metarhizium sp. 2 (4,910)	ON794469	*Metarhizium anisopliae* (KX809519)	99%

Table 3. Concentration-response of *Beauveria* sp. (4,458), *Metarhizium anisopliae* (4,420) and *Metarhizium anisopliae* (4,910) against *Aedes aegypti* larvae.

Isolate	Inclination ± M. S. E.	LC_{50} (IF 95%)	TR (IC 95%) LC_{50}	LC_{95} (IF 95%)	TR (IC 95%) LC_{95}	χ^2	*P*
Beauveria sp. (4,458)	0.653±0.082	8.661×10^3 ($3.22 \times 10^{-3} - 1.71 \times 10^{-4}$)	-	2.850×10^6 ($1.232 \times 10^{-6} - 1.027 \times 10^{-7}$)	-	2.05	0.35
Metarhizium anisopliae (4,420)	0.646±0.069	5.488×10^4 ($2.97 \times 10^{-4} - 9.14 \times 10^{-4}$)	6.33 (2.46-16.31)	1.917×10^7 ($7.528 \times 10^{-6} - 7.535 \times 10^{-7}$)	6.33 (2.02 – 19.79)	2.81	0.24
Metarhizium anisopliae (4,910)	0.866±0.197	1.137×10^5 ($1.06 \times 10^{-5} - 1.84 \times 10^{-7}$)	13.13 (4.20-41.04)	8.976×10^6 ($6.747 \times 10^{-5} - 5.627 \times 10^{-7}$)	13.13 (3.56 – 48.38)	13.55	0.001

M. S. E. = Mean Standard Error; LC = Lethal Concentration; TR = Toxicity Rate; IF = Inhibitory Factor; IC = Concentration Interval.

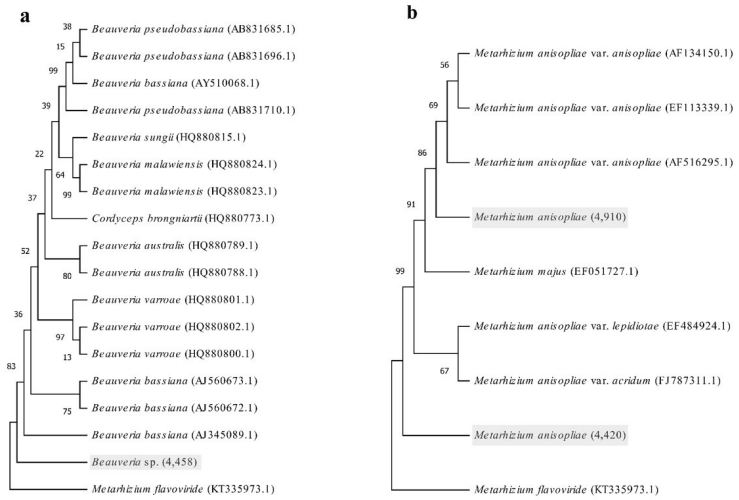

Figure 2. Phylogenetic tree based on sequences from the ITS region of rDNA, which were aligned by the ClustalW program using the MEGA v 11 program. (a) *Beauveria* sp. (4,458); (b) *Metarhizium anisopliae* (4,420) and *Metarhizium anisopliae* (4,910).

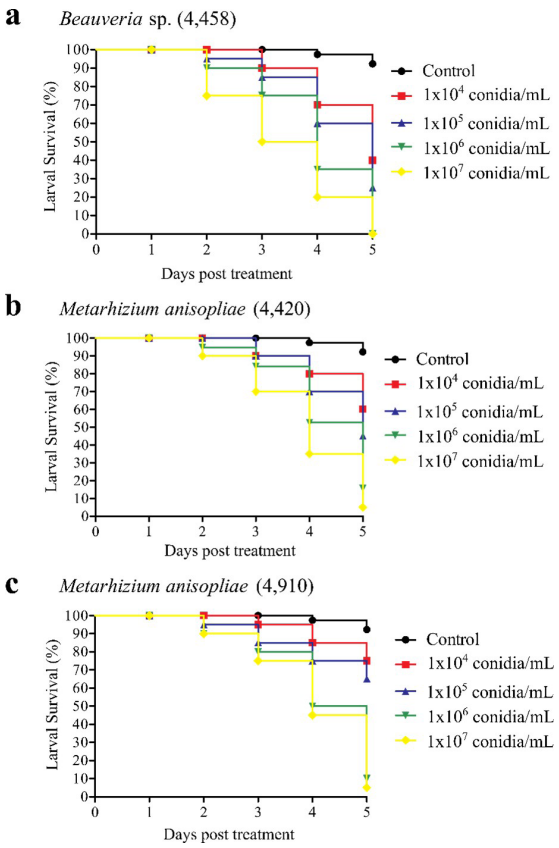

Figure 3. Survival curves of entomopathogenic fungi against *Aedes aegypti* at concentrations 1×10^4, 1×10^5, 1×10^6 and 1×10^7 conidia/mL. (a) *Beauveria* sp. (4,458); (b) *Metarhizium anisopliae* (4,420); (c) *Metarhizium* anisopliae (4,910).

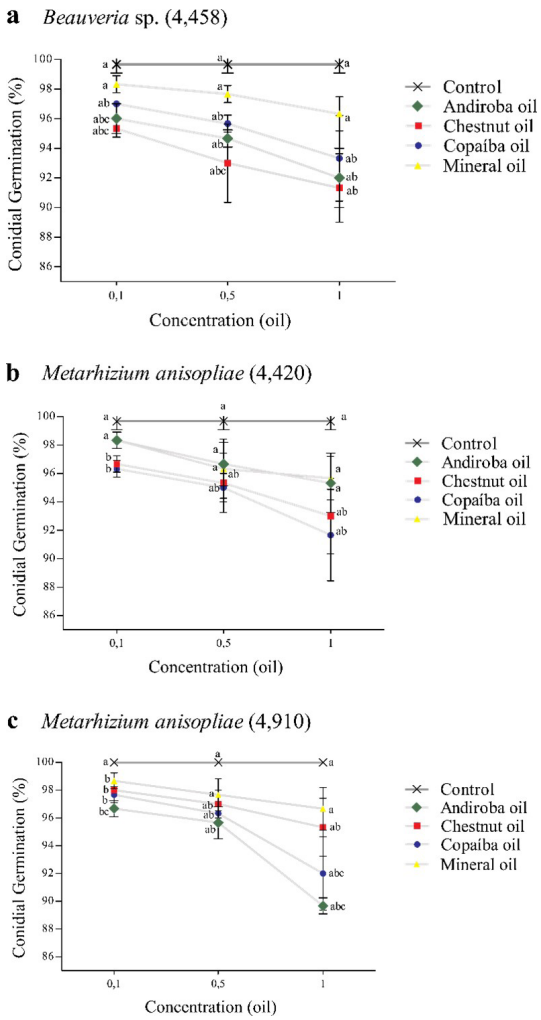

Figure 4. Evaluation of the compatibility of fungal conidia with vegetable oils of the Amazonian species *Carapa guianensis* (andiroba), *Copaifera multijulga* (copaíba), *Bertholletia excelsa* (chestnut) and mineral oil at concentrations of 0.1%, 0.5% and 1%. (a) *Beauveria* sp. (4,458); (b) *Metarhizium anisopliae* (4,420), c. *Metarhizium anisopliae* (4,910).

or mineral) and the conidia of *Beauveria* sp. (4,458) ($P < 0.0001$; F4.10 = 25.89), *Metarhizium anisopliae* (4,420) ($P = 0.0017$; F4.10 = 9.85) and *Metarhizum anisopliae* (4,910) ($P < 0, 0001$; F4.10 = 24.13) (Figure 4).

Mineral oil was the most compatible with the conidia of the three fungi evaluated. With *Beauveria* sp. 1 (4,458), the concentrations of 0.1, 0.5 and 1% of mineral oil obtained germination rates of 98% (±0.5), 97% (±0.5) and 96% (±1.15), respectively. *M. anisopliae* (4,420) obtained germination rates of 98.3% (±0.5), 96.3% (±2.08) and 95.6% (±1.52) when combined with mineral oil at concentrations 0 .1, 0.5 and 1%, respectively. *M. anisopliae* (4,910) was compatible with mineral oil at concentrations of 0.1, 0.5 and 1%, with germination rates of 98% (±0.5), 97% (±1.15) and 96% (± 1.5), respectively.

When comparing the compatibility between fungi and vegetable oils, *Beauveria* sp. (4,458) was the most compatible with copaiba, with a germination rate of 97% (± 0) at 0.1% concentration, 95% (± 0.5) at 0.5% concentration and 93% (± 2.9) at 1% concentration. *M. anisopliae* (4,420) was compatible with andiroba obtaining germination rates of 98% (± 0.5) to 0.1%, 96% (± 1.5) to 0.5% and 95% (± 2.08) to 1%. *M. anisopliae* (4,910) was most compatible with Brazil nuts with germination rates of 98% (±0) to 0.1%, 97% (±1.0) to 0.5% and 95% (±2.08) to 1%.

The concentrations of the oils used (0.1%, 0.5% and 1%) also showed significant differences in the treatments of *Beauveria* sp. (4,458) ($P < 0.0001$; F2.20 = 15.72), *Metarhizium anisopliae* (4,420) ($P < 0.0001$; F2.20 = 15.70) and *Metarhizium anisopliae* (4,910) ($P < 0, 0001$; F2.20 = 52.94). It was observed that 0.1% of mineral and vegetable oil caused less influence on conidia germination rates, which decreased as the concentration increased to 0.5% and 1%.

The entomopathogenicity of aqueous and oily formulations of three native Amazonian fungi exposed to laboratory and semi-field conditions was evaluated (Figure 5). The formulations of *Beauveria* sp. 1 (4,458) were susceptible to semi-field conditions, with mortality rates that differed significantly from laboratory-maintained formulations ($P < 0.0001$; F1.12 = 69.82). The aqueous suspension of *Beauveria* sp. (4,458) was less effective when exposed to semi-field conditions, obtaining mortality rates

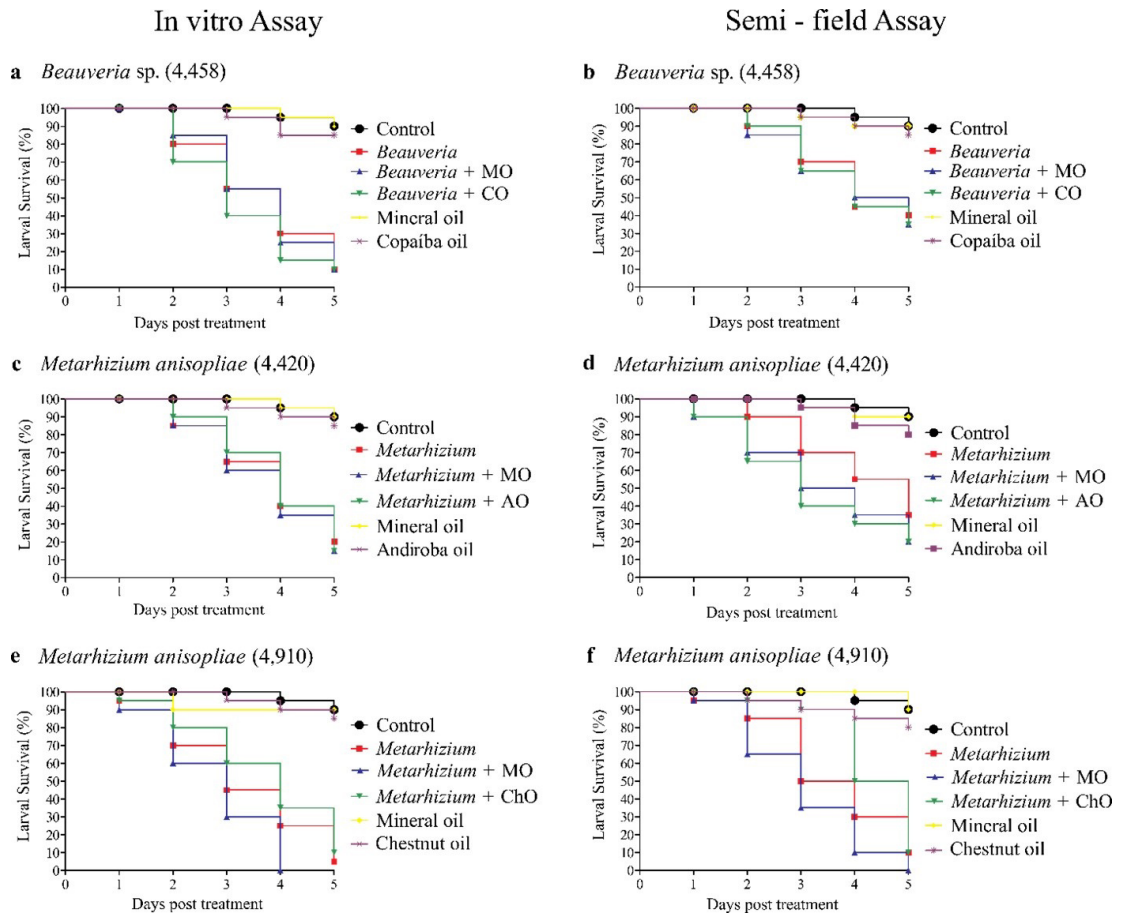

Figure 5. Evaluation of the entomopathogenicity of fungal formulations against *Ae. aegypti* in laboratory and semi-field conditions. A. *Beauveria* sp. (4,458) in the laboratory; B. *Beauveria* sp. (4,458) in semi-field; C. *Metarhizium anisopliae* (4,420) in the laboratory; d. *Metarhizium anisopliae* (4,420) in semi-field; e. *Metarhizium anisopliae* (4,910) in the laboratory; f. *Metarhizium anisopliae* (4,910) in semi-field. MO (Mineral oil); CO (Copaiba oil); AO (Andiroba oil); ChO (Chestnut oil).

of 60%, while in the laboratory it increased larval mortality by up to 90%. There were also significant differences between the formulations of this fungus and the control treatments ($P < 0.0001$; $F5.12 = 159.23$).

The formulations of *M. anisopliae* (4,420) ($P = 0.1152$; $F1.12 = 2.88$) and *M. anisopliae* (4,910) ($P = 0.6818$; $F1.12 = 0.18$) showed no difference significant in the larval mortality rates when related to the exposure conditions of the treatments. However, there were significant differences when related to the formulations of *M. anisopliae* (4,420) ($P = < 0.0001$; $F5.12 = 104.86$) and *M. anisopliae* (4,910) ($P = < 0.0001$; $F5, 12 = 1184.28$) and control treatments.

Metarhizium anisopliae (4,420), when formulated with oily adjuvants, was able to maintain virulence against *Ae. aegypti* in semi-field condition, with mortality rates of 80%. The larvae of *Ae. aegypti* were more sensitive to the formulations of the fungus *Metarhizium anisopliae* (4,910), which increased the mortality rate to 100% in treatments with mineral oil on the 4th day in the laboratory and on the 5th day in the semi-field. The aqueous and nut oil formulations were also able to maintain the mortality rate above 90%, in both exposure conditions.

4. Discussion

This study confirms the entomopathogenicity of fungal strains native to the Brazilian Amazon against *Ae. aegypti*, where *Beauveria* sp. (4,458), *Metarhizium anisopliae* (4,420) and *Metarhizium anisopliae* (4,910) were able to significantly increase the *in vitro* larval mortality rate.

The genus *Beauveria* is extensively studied for the biological control of disease vectors (Bitencourt et al., 2018). Aqueous suspensions of *Beauveria* sp. (4,458) had the highest virulence rates against *Ae. aegypti*. Fungi of the genus *Beauveria* are not toxic to mammals, and their use is not expected to have harmful effects on human health or the environment (Ragavendran et al., 2017).

B. bassiana conidia are effective in killing mosquito larvae and adults when applied to breeding sites (Daniel et al., 2017). The spores come into contact with the insect's exoskeleton, develop hyphae that secrete enzymes, and dissolve the cuticle (Lacey, 2017). These fungal hyphae feed on body tissue, produce toxins and reproduce, and when under favorable humidity conditions, they multiply and release more spores into the environment to repeat the cycle (Mascarin et al., 2019).

B. bassiana produces several mycotoxins, such as beauvericins, bassianolides, beauveriolides, tenelins, bassianins, pyridoverolidicins, oosporeins and bassiacridins (Daniel et al., 2017). Beauvericin is an ionophoric cyclodepsipeptide detected in several fungi, mainly in *Beauveria* and *Paecilomyces* (Isaka et al., 2005). This mycotoxin forms complexes with cations, which results in increased permeability to natural and artificial membranes (Toman et al., 2011), in addition to inducing programmed cell death similar to apoptosis (Wätjen et al., 2014).

Fungi of the genus *Metarhizium* are widely evaluated for their potential to control mosquitoes that carry human diseases. These fungal entomopathogens infect their hosts through the cuticle and develop in the hemolymph and internal organs. The dynamics of invasion is determined by the specific virulence of the fungus, a high initial inoculum of conidia and high humidity that favors the extracuticular development of the entomopathogen (Vivekanandhan et al., 2020).

Metarhizium has a matrix of insecticides and other bioactive secondary metabolites, especially destruxins (Shoukat et al., 2020). These metabolites play an important role in weakening the host's immune defense, damaging the muscular system and Malpighian tubules, leading to feeding and mobility difficulties (Amerasan et al., 2016).

Several biosynthetic pathways have been discovered by sequences of the *Metarhizium* genome (Donzelli and Krasnoff, 2016), and include pathways responsible for chemicals known as cytochalasins and ovalicin, pathways without products yet known in *Metarhizium* but present in other fungi, such as Ergot alkaloids, diketopiperazine and resorcylic acid lactones, in addition to pathways that are so unique that their produced molecules are not yet characterized (Wang et al., 2018). These pathways signal that *Metarhizium*'s ability to produce secondary metabolites is much greater than its known chemistry. Thus, *Metarhizium* strains native to the Amazon are possibly a source of new molecules still unknown.

Several other studies have emphasized the search for new fungal strains that are candidates for the development of new bioproducts against *Aedes aegypti*. When isolating the fungal strains *Fusarium equiseti* (MK371718) and *Fusarium proliferatum* (MK371715) from forest soils in the Pakistan region, considerable larvicidal activity against *Aedes aegypti* was observed in laboratory conditions, where LC_{50} of 3.8×10^{8}, 2.9×10^{7}, 2.0×10^{7} and 7.1×10^{6} conidia/mL after 24h, 48h, 72h and 96h, respectively, for the *F. equiseti* isolate, while for the *F. proliferatum* isolate, LC_{50} of 1.21×10^{8}, 9.6×10^{7}, 4.2×10^{7} and 2.6×10^{7} conidia/mL after 24h, 48h, 72h and 96h, respectively. Thus, *F. equiseti* is also noteworthy as a promising biotechnological candidate for the control of *Ae. aegypti* (Abrar et al., 2021). To evaluate the entomopathogenicity of *Bacillus thuringiensis* against *Aedes aegypti*, strains of this entomopathogenic bacterium were isolated from soils in the state of Maranhão – Brazil, and it was observed that the BtMA-750 strain stood out with lethal concentrations 50 and 90 of 0.003 mg/mL and 0.012 mg/mL, respectively, which is a great alternative with possible application in the biological control of this vector (Vieira-Neta et al., 2021).

The selection of virulent strains is fundamental in the process of developing a biopesticide. However, screening different types of formulations is also essential to optimize fungus performance and minimize the negative effects caused by abiotic factors (Bitencourt et al., 2018).

It was observed that the conidial germination of the evaluated fungi was higher the lower the concentration of vegetable/mineral oil, and the formulation with 0.1% vegetable/mineral oil had a better germination rate in relation to the formulations with 0.5 and 1% vegetable/mineral oil. Some studies have already evaluated the mortality rates of *Ae. aegypti* when subjected to fungal formulations with vegetable and mineral oil (Paula et al., 2008; Mnyone et al., 2011; Carolino et al., 2014; Lobo et al., 2016).

Beauveria sp. (4,458) was sensitive to the semi-field condition, causing the mortality of 65% of *Ae. aegypti* when formulated with mineral and vegetable oil. This strain showed the best virulence rates in the laboratory when compared to *M. anisopliae* (4,420) and *M. anisopliae* (4,910). However, when subjected to semi-field condition, it was considerably less effective. Several biological attributes must be evaluated and combined to develop a bioproduct, thus, conclusions regarding the efficiency of a strain cannot be based solely on virulence under controlled conditions (Michereff-Filho et al., 2021).

High temperatures and solar UV radiation are the main factors that limit entomopathogenic fungi in environments exposed to sunlight in the control of *Ae. aegypti* (Darbro et al., 2012). When evaluating the persistence of *B. bassiana* conidia in non-target arthropods after application in pasture and alfalfa agroecosystems, it was observed that more than 90% of the deposited conidia were unviable after two days of application (Goettel et al., 2021).

Oil adjuvants were able to maintain the virulence of *M. anisopliae* (4,420) and *M. anisopliae* (4,910) against *Ae. aegypti* in semi-field condition. Considering that vegetable oils are biodegradable and ecological, the application of mineral oil in a fungal formulation would probably increase the post-application residual effect of a bioinsecticide, due to a slower disintegration by microbial activity, compared to vegetable oil (Rodrigues et al., 2019). Thus, the application of mineral oil in low concentrations and in a focal region, suggests a less harmful environmental impact, as the bioinsecticide application container can be discarded under controlled conditions (Lobo et al., 2016).

Fungi of the genus *Metarhizium* produce large amounts of new conidia in the environment after effective control, regardless of conidial concentrations or tested formulations (Choi et al., 2020). This characteristic makes this strain highly interesting for applications in new breeding sites in regions that are difficult to access by health agents, a common case in the Amazon (Rodrigues et al., 2019).

5. Conclusions

Fungal strains native from Amazon *Beauveria* sp. (4,458), *Metarhizium anisopliae* (4,420) and *M. anisopliae* (4,910) significantly increased the larval mortality of *Ae. aegypti in vitro* and were more compatible with mineral oil than vegetable oils.

The formulation of the fungus *M. anisopliae* (4,910) with 0.1% mineral oil represents a promising tool for the development of strategies to control *Ae. aegypti*.

Acknowledgements

The Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the granting of the first author's master's scholarship and to Santander for the granting of a scholarship through the Bolsas Ibero Americanas 2020 program

References

ABRAR, A., SARWAR, S., ABBAS, M., CHAUDHRY, H., GHANI, N., FATIMA, A. and TAHIR, A., 2021. Identification of locally isolated entomopathogenic *Fusarium* species from the soil of Changa Manga Forest, Pakistan and evaluation of their larvicidal efficacy against *Aedes aegypti*. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 83, pp. e246230. http://dx.doi.org/10.1590/1519-6984.246230. PMid:34495158.

AMERASAN, D., NATARAJ, T., MURUGAN, K., PANNEERSELVAM, C., MADHIYAZHAGAN, P., NICOLETTI, M. and BENELLI, G., 2016. Myco-synthesis of silver nanoparticles using *Metarhizium anisopliae* against the rural malaria vector *Anopheles culicifacies* Giles (Diptera: culicidae). *Journal of Pest Science*, vol. 89, no. 1, pp. 249-256. http://dx.doi.org/10.1007/s10340-015-0675-x. PMid:28217039.

AYALA-ZERMEÑO, M.A., GALLOU, A., BERLANGA-PADILLA, A.M., ANDRADE-MICHEL, G.Y., RODRÍGUEZ-RODRÍGUEZ, J.C., ARREDONDO-BERNAL, H.C. and MONTESINOS-MATÍAS, R., 2017. Viability, purity, and genetic stability of entomopathogenic fungi species using different preservation methods. *Fungal Biology*, vol. 121, no. 11, pp. 920-928. http://dx.doi.org/10.1016/j.funbio.2017.07.007. PMid:29029699.

BITENCOURT, R.O.B., FARIAS, F.S., FREITAS, M.C., BALDUINO, C.J.R., MESQUITA, E.S., CORVAL, A.R.C. and ANGELO, I.C., 2018. In vitro Control of *Aedes aegypti* larvae using *Beauveria bassiana*. *International Journal of Bioengineering and Life Sciences*, vol. 12, no. 10, pp. 400-404.

BRASIL. Ministério da Saúde, Secretaria de Vigilância em Saúde, 2020. Monitoramento dos casos de arboviroses urbanas transmitidas pelo *Aedes Aegypti* (Dengue, Chikungunya e Zika), Semanas Epidemiológicas 1 a 15. *Boletim Epidemiológico*, vol. 51, no. 16, pp. 1-45.

BUTT, T.M., COATES, C.J., DUBOVSKIY, I.M. and RATCLIFFE, N.A. 2016. Entomopathogenic fungi: new insights into host–pathogen interactions. In: L. Brian and J.S.L. Raymond, eds. *Advances in genetics*. College Park, USA: Academic Press, vol. 94, pp. 307-364. https://doi.org/10.1016/bs.adgen.2016.01.006.

CAMARGO, M.G., GOLO, P.S., ANGELO, I.C., PERINOTTO, W.M., SÁ, F.A., QUINELATO, S. and BITTENCOURT, V.R., 2012. Effect of oil-based formulations of acaripathogenic fungi to control *Rhipicephalus microplus* ticks under laboratory conditions. *Veterinary Parasitology*, vol. 188, no. 1-2, pp. 140-147. http://dx.doi.org/10.1016/j.vetpar.2012.03.012. PMid:22480883.

CARAGATA, E.P., TIKHE, C.V. and DIMOPOULOS, G., 2019. Curious entanglements: interactions between mosquitoes, their microbiota, and arboviruses. *Current Opinion in Virology*, vol. 37, no. 1, pp. 26-36. http://dx.doi.org/10.1016/j.coviro.2019.05.005. PMid:31176069.

CAROLINO, A.T., PAULA, A.R., SILVA, C.P., BUTT, T.M. and SAMUELS, R.I., 2014. Monitoring persistence of the entomopathogenic fungus *Metarhizium anisopliae* under simulated field conditions with the aim of controlling adult *Aedes aegypti* (Diptera: culicidae). *Parasites & Vectors*, vol. 7, no. 1, pp. 198-205. http://dx.doi.org/10.1186/1756-3305-7-198. PMid:24766705.

CARVALHO, J.R., PRATISSOLI, D., VIANNA, U.R. and HOLTZ, A.M., 2017. *Análise de probit aplicada a bioensaios com insetos*. Colatina: IFES, 102 p.

CARVALHO, M.S.L., CALDAS, E.D., DEGALLIER, N., VILARINHOS, P.T., SOUZA, L.C., YOSHIZAWA, M.A., KNOX, M.B. and OLIVEIRA, C., 2004. Susceptibility of *Aedes aegypti* larvae to the insecticide temephos in the Federal District, Brazil. *Revista de Saúde Pública*, vol. 38, no. 5, pp. 623-629. http://dx.doi.org/10.1590/S0034-89102004000500002. PMid:15499431.

CHOI, C.J., LEE, J.Y., WOO, R.M., SHIN, T.Y., GWAK, W.S. and WOO, S.D., 2020. An effective entomopathogenic fungus *Metarhizium anisopliae* for the simultaneous control of *Aedes albopictus* and *Culex pipiens* mosquito adults. *Journal of Asia-Pacific Entomology*, vol. 23, no. 2, pp. 585-590. http://dx.doi.org/10.1016/j.aspen.2020.04.007.

DANIEL, J.F., SILVA, A.A., NAKAGAWA, D.H., MEDEIROS, L.S.D., CARVALHO, M.G., TAVARES, L.J. and RODRIGUES-FILHO, E., 2017. Larvicidal activity of *Beauveria bassiana* extracts against *Aedes aegypti* and identification of Beauvericins. *Journal of the Brazilian Chemical Society*, vol. 28, no. 6, pp. 1003-1013.

DARBRO, J.M., JOHNSON, P.H., THOMAS, M.B., RITCHIE, S.A., KAY, B.H. and RYAN, P.A., 2012. Effects of *Beauveria bassiana* on survival, blood-feeding success, and fecundity of *Aedes aegypti* in laboratory and semi-field conditions. *The American Journal of Tropical Medicine and Hygiene*, vol. 86, no. 4, pp. 656-664. http://dx.doi.org/10.4269/ajtmh.2012.11-0455. PMid:22492151.

DONZELLI, B.G.G. and KRASNOFF, S.B., 2016. Molecular genetics of secondary chemistry in *Metarhizium* fungi. *Advances in Genetics*, vol. 94, no. 1, pp. 365-436. http://dx.doi.org/10.1016/bs.adgen.2016.01.005. PMid:27131330.

DOYLE, J.J. and DOYLE, J.L., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin*, vol. 19, no. 1, pp. 11-15.

FERNANDES, É.K., RANGEL, D.E., BRAGA, G.U. and ROBERTS, D.W., 2015. Tolerance of entomopathogenic fungi to ultraviolet radiation: a review on screening of strains and their formulation. *Current Genetics*, vol. 61, no. 3, pp. 427-440. http://dx.doi.org/10.1007/s00294-015-0492-z. PMid:25986971.

FRANCISCO, E.A., MOCHI, D.A., CORREIA, A.D.C.B. and MONTEIRO, A.C., 2006. Influence of culture media in viability test of conidia of entomopathogenic fungi. *Ciência Rural*, vol. 36, no. 4, pp. 1309-1312. http://dx.doi.org/10.1590/S0103-84782006000400043.

GOETTEL, M.S., DOUGLAS INGLIS, G., DUKE, G.M., LORD, J.C. and JARONSKI, S.T., 2021. Measurement of internal *Beauveria bassiana* to ascertain non-target impacts on arthropods *in field environments*. *Biocontrol Science and Technology*, vol. 31, no. 8, pp. 1-16. http://dx.doi.org/10.1080/09583157.2021.1895072.

HALL, T., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, vol. 41, no. 41, pp. 95-98.

ISAKA, M., KITTAKOOP, P., KIRTIKARA, K., HYWEL-JONES, N.L. and THEBTARANONTH, Y., 2005. Bioactive substances from insect pathogenic fungi. *Accounts of Chemical Research*, vol. 38, no. 10, pp. 813-823. http://dx.doi.org/10.1021/ar040247r. PMid:16231877.

JUKES, T.H. and CANTOR, A., 1969. Evolution of protein molecules. *Mammalian Protein Metabolism*, vol. 3, no. 1, pp. 21-132. http://dx.doi.org/10.1016/B978-1-4832-3211-9.50009-7.

LACEY, L.A., 2017. Microbial control of medically important mosquitoes in tropical climates. In: L.A. LACEY, ed. *Microbial control of insect and mite pests*. London: Academic Press, pp. 409-430. http://dx.doi.org/10.1016/B978-0-12-803527-6.00028-7

LACEY, L.A., GRZYWACZ, D., SHAPIRO-ILAN, D.I., FRUTOS, R., BROWNBRIDGE, M. and GOETTEL, M.S., 2015. Insect pathogens as biological control agents: back to the future. *Journal of Invertebrate Pathology*, vol. 132, no. 1, pp. 1-41. http://dx.doi.org/10.1016/j.jip.2015.07.009. PMid:26225455.

LOBO, L.S., RODRIGUES, J. and LUZ, C., 2016. Effectiveness of *Metarhizium anisopliae* formulations against dengue vectors under laboratory and field conditions. *Biocontrol Science and Technology*, vol. 26, no. 3, pp. 386-401. http://dx.doi.org/10.1080/09583157.2015.1123220.

MASCARIN, G.M., LOPES, R.B., DELALIBERA JÚNIOR, Í., FERNANDES, É.K.K., LUZ, C. and FARIA, M., 2019. Current status and perspectives of fungal entomopathogens used for microbial control of arthropod pests in Brazil. *Journal of Invertebrate Pathology*, vol. 165, no. 1, pp. 46-53. PMid:29339191.

MICHEREFF FILHO, M., FARIA, M., WRAIGHT, S.P. and SILVA, K.F.A.S., 2021. Micoinseticidas e micoacaricidas no Brasil: como estamos após quatro décadas? *Arquivos do Instituto Biológico*, vol. 76, no. 1, pp. 769-779.

MNYONE, L.L., KIRBY, M.J., MPINGWA, M.W., LWETOIJERA, D.W., KNOLS, B.G., TAKKEN, W. and RUSSELL, T.L., 2011. Infection of *Anopheles gambiae* mosquitoes with entomopathogenic fungi: effect of host age and blood-feeding status. *Parasitology Research*, vol. 108, no. 2, pp. 317-322. http://dx.doi.org/10.1007/s00436-010-2064-y. PMid:20872014.

NICOLETTI, M. 2020. Three scenarios in insect-borne diseases. In: M. NICOLETTI, ed. *Insect-borne diseases in the 21st century*. London: CRC Press, pp. 1-99.

ORTIZ-URQUIZA, A. and KEYHANI, N.O., 2015. Stress response signaling and virulence: insights from entomopathogenic fungi. *Current Genetics*, vol. 61, no. 3, pp. 239-249. http://dx.doi.org/10.1007/s00294-014-0439-9. PMid:25113413.

PÁRAMO, M.E.R., LASTRA, C.C.L., GARCÍA, J.J., FERNANDES, É.K., MARRETO, R.N. and LUZ, C., 2015. Effect of ultraviolet-A radiation on the production of *Leptolegnia chapmanii* (Saprolegniales: Saprolegniaceae) zoospores on dead *Aedes aegypti* (Diptera: Culicidae) larvae and their larvicidal activity. *Journal of Invertebrate Pathology*, vol. 130, no. 1, pp. 133-135. http://dx.doi.org/10.1016/j.jip.2015.08.002. PMid:26259676.

PAULA, A.R., BRITO, E.S., PEREIRA, C.R., CARRERA, M.P. and SAMUELS, R.I., 2008. Susceptibility of adult *Aedes aegypti* (Diptera: Culicidae) to infection by *Metarhizium anisopliae* and *Beauveria bassiana*: prospects for Dengue vector control. *Biocontrol Science and Technology*, vol. 18, no. 10, pp. 1017-1025. http://dx.doi.org/10.1080/09583150802509199.

RAGAVENDRAN, C., DUBEY, N.K. and NATARAJAN, D., 2017. *Beauveria bassiana* (Clavicipitaceae): a potent fungal agent for controlling mosquito vectors of *Anopheles stephensi, Culex quinquefasciatus* and *Aedes aegypti* (Diptera: Culicidae). *RSC Advances*, vol. 7, no. 7, pp. 3838-3851. http://dx.doi.org/10.1039/C6RA25859J.

REMADEVI, O.K., SASIDHARAN, T.O., BALACHANDER, M. and BAI, N.S., 2010. *Metarhizium* based mycoinsecticides for forest pest management. *Journal of Biopesticides*, vol. 3, no. 2, pp. 470-473.

RODRIGUES, J., BORGES, P.R., FERNANDES, É.K.K. and LUZ, C., 2019. Activity of additives and their effect in formulations of *Metarhizium anisopliae* sl IP 46 against *Aedes aegypti* adults and on post mortem conidiogenesis. *Acta Tropica*, vol. 193, no. 1, pp. 192-198. http://dx.doi.org/10.1016/j.actatropica.2019.03.002. PMid:30836061.

SHOUKAT, R.F., HASSAN, B., SHAKEEL, M., ZAFAR, J., LI, S., FREED, S. and JIN, F., 2020. Pathogenicity and Transgenerational Effects of *Metarhizium anisopliae* on the Demographic Parameters of *Aedes albopictus* (Culicidae: diptera). *Journal of Medical Entomology*, vol. 57, no. 3, pp. 677-685. http://dx.doi.org/10.1093/jme/tjz236. PMid:31819965.

TOMAN, P., MAKRLÍK, E. and VAŇURA, P., 2011. On the complexation of the sodium cation with beauvericin: experimental and theoretical study. *Monatshefte für Chemie-Chemical Monthly*, vol. 142, no. 8, pp. 779-782. http://dx.doi.org/10.1007/s00706-011-0499-1.

VIEIRA-NETA, M.R.A., SOARES-DA-SILVA, J., VIANA, J.L., SILVA, M.C., TADEI, W.P. and PINHEIRO, V.C.S., 2021. Strain of *Bacillus thuringiensis* from Restinga, toxic to *Aedes* (Stegomyia) *aegypti* (Linnaeus) (Diptera, Culicidae). *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 81, no. 4, pp. 872-880. http://dx.doi.org/10.1590/1519-6984.228790. PMid:33053121.

VIVEKANANDHAN, P., SWATHY, K., KALAIMURUGAN, D., RAMACHANDRAN, M., YUVARAJ, A., KUMAR, A.N., MANIKANDAN, A.T., POOVARASAN, N., SHIVAKUMAR, M.S. and KWEKA, E.J., 2020. Larvicidal toxicity of *Metarhizium anisopliae* metabolites against three mosquito species and non-targeting organisms. *PLoS One*, vol. 15, no. 5, pp. e0232172. http://dx.doi.org/10.1371/journal.pone.0232172. PMid:32365106.

WANG, Y.H., CHANG, M.M., WANG, X.L., ZHENG, A.H. and ZOU, Z., 2018. The immune strategies of mosquito *Aedes aegypti* against microbial infection. *Developmental and Comparative Immunology*, vol. 83, no. 1, pp. 12-21. http://dx.doi.org/10.1016/j.dci.2017.12.001. PMid:29217264.

WÄTJEN, W., DEBBAB, A., HOHLFELD, A., CHOVOLOU, Y. and PROKSCH, P., 2014. The mycotoxin beauvericin induces apoptotic cell death in H4IIE hepatoma cells accompanied by an inhibition of NF-κB-activity and modulation of MAP-kinases. *Toxicology Letters*, vol. 231, no. 1, pp. 9-16. http://dx.doi.org/10.1016/j.toxlet.2014.08.021. PMid:25178661.

WHITE, T.J., BRUNS, T., LEE, S.J.W.T. and TAYLOR, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protocols: a guide to methods and applications*, vol. 18, no. 1, pp. 315-322. http://dx.doi.org/10.1016/B978-0-12-372180-8.50042-1.