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Entomopathogenic fungi from Brazil for control of Aedes aegypti L. (Diptera: Culicidae): A systematic review

Fungos entomopatogênicos do Brasil no controle de Aedes aegypti L. (Diptera: Culicidae): Uma revisão sistemática

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RESUMO

Foi realizado levantamento bibliográfico para revisão sistemática dos estudos realizados entre 2000 e 2020 com fungos entomopatogênicos isolados no Brasil para controle do mosquito vetor *Aedes aegypti*. As buscas foram realizadas nas bases de dados Google Scholar, Science Direct, Pubmed e Scielo. Foram utilizados os seguintes descritores: fungos entomopatogênicos; *Aedes aegypti*; Brasil. Os critérios de inclusão foram estudos sobre a atividade de fungos isolados no Brasil contra *Ae. aegypti* entre 2000 e 2020; estudos que não utilizaram fungos isolados no Brasil ou que não especificaram a origem dos fungos foram excluídos. Foram recuperados 2.361 artigos; após a aplicação dos critérios de inclusão e exclusão, foram selecionados 30 artigos. *Metarhizium anisopliae* é o fungo mais estudado contra *Ae. aegypti* no Brasil. Foi citada em 36,6% dos estudos, seguida por *Beauveria bassiana* (12,2%). A idade adulta é o estágio de desenvolvimento mais frequentemente avaliado para *Ae. aegypti*, sendo os óleos minerais e vegetais os adjuvantes mais citados nas formulações. Em associação com *Metarhizium anisopliae*, o único agente químico avaliado foi o imidaclopride, que é eficaz no controle de espécimes adultos de *Ae. aegypti*. Este estudo traz um cenário de estudos utilizando fungos nativos do Brasil para o controle de *Ae. aegypti* e expõe as lacunas que precisam ser preenchidas para o desenvolvimento de novos bioprodutos.

Palavras-chave: Arbovírus. *Metarhizium anisopliae*. *Beauveria bassiana*.

ABSTRACT

A bibliographic survey was carried out for a systematic review of studies conducted between 2000 and 2020 with entomopathogenic fungi isolated in Brazil to control the mosquito vector *Aedes aegypti*. The searches were performed in the Google Scholar, Science Direct, Pubmed and Scielo databases. The following descriptors were used: entomopathogenic fungi; *Aedes aegypti*; Brazil. The inclusion criteria were studies on the activity of fungi isolated in Brazil against *Ae. aegypti* between 2000 and 2020; studies that did not use fungi isolated in Brazil or that did not specify the origin of the fungi were excluded. A total of 2,361 articles were retrieved; after application of the inclusion and exclusion criteria, 30 articles were selected. *Metarhizium anisopliae* is the most studied fungus against *Ae. aegypti* in Brazil. It was cited in 36.6% of the studies, followed by *Beauveria bassiana* (12.2%). Adulthood is the most frequently assessed stage of development for *Ae. aegypti*, and mineral and vegetable oils are the most frequently cited adjuvants in formulations. In association with *Metarhizium anisopliae*, the only chemical agent assessed was Imidacloprid, which is effective at controlling adult specimens of *Ae. aegypti*. This study brings a scenario of studies using fungi native to Brazil for the control of *Ae. aegypti* and exposes the gaps that need to be filled for the development of new bioproducts.

Keywords: Arboviruses. *Metarhizium anisopliae*. *Beauveria bassiana*.

1. INTRODUCTION

Vector mosquitoes, which is the case of *Aedes aegypti*, are capable of transmitting several disease-causing pathogens to humans, including the ones that cause dengue (DENV), chikungunya (CHIKV), zika (ZIKV) and yellow fever (YFV), creating serious public health problems in Brazil (Caragata et al. 2019; Brasil 2020).

Technologies aimed at developing vaccines to control the transmission of these arboviruses are still incipient. Therefore, the more common form of combat is to control the vector mosquito *Ae. aegypti* (Tyagi and Dhanasekaran, 2018). Conventionally, breeding sites are eliminated and chemical and/or biological insecticides are applied in places of proliferation of *Ae. aegypti* (Rodríguez-Pérez and Reyes Villanueva, 2018).

Chemical insecticides, such as the organophosphate adulticide Malathion and the larvicide Piriproxifen, are the main current control measures for the vector mosquito in Brazil (Brasil, 2020). However, the use of these chemical agents has already been found to be ineffective in several regions of the country, owing to increased resistance of larvae and mosquitoes against the active ingredient of various chemicals, such as organophosphates and pyrethroids (Gomes et al., 2015). In addition, these products can damage the environment and harm humans, as emphasized in several studies (Vivekanandhan 2018).

The use of entomopathogenic fungi to control *Ae. aegypti* in Brazil is a technology that can help reduce the spread of these arboviruses, including strains of generalist fungal entomopathogens isolated in Brazil, such as *Metarhizium anisopliae* and *Beauveria bassiana*, which are widely studied (Mascarin et al., 2019). The search for a fungal strain to control *Ae. aegypti* involves several steps, which are developed into studies, with different approaches, from the evaluation of the resistance of these fungi (when under conditions of abiotic stress) to the analysis of effective control when these strains are associated with additives, in the case of formulations, or even with chemicals currently used to control *Ae. aegypti* (Rodrigues et al., 2019).

Although a wide range of entomopathogenic fungi has already reported in Brazil, there is little information on promising strains isolated in Brazil that can be developed as a biological product for control of *Ae. aegypti*. Thus, the objective of this study is to gather and update information available on the potential use of entomopathogenic fungi isolated in Brazil to control the vector mosquito *Aedes aegypti*.

2. METHOD

This study is a systematic review of studies carried out with fungi isolated in Brazil for the control of the mosquito vector *Aedes aegypti*. The theoretical framework was obtained from the following data platforms: Google Scholar, Science Direct, Pubmed and Scielo. For selection of articles, the following descriptors were used: Entomopathogenic fungi; *Aedes aegypti*; Brazil. The decisive inclusion criteria for studies of the activity of fungi isolated from Brazil against *Ae. aegypti* between the years 2000 and 2020, a period that included the last twenty years during the study. The exclusion criteria were duplicate articles and studies that did not specify the origin of the fungus or that did not use fungi isolated in Brazil.

3. RESULTS

A total of 2.361 articles were obtained from the descriptors Entomopathogenic fungi; *Aedes aegypti*; Brazil. There were 2.240 articles in Google Scholar, 49 articles in Scielo, 38 in Science Direct and 34 articles in Pubmed (Figure 1).

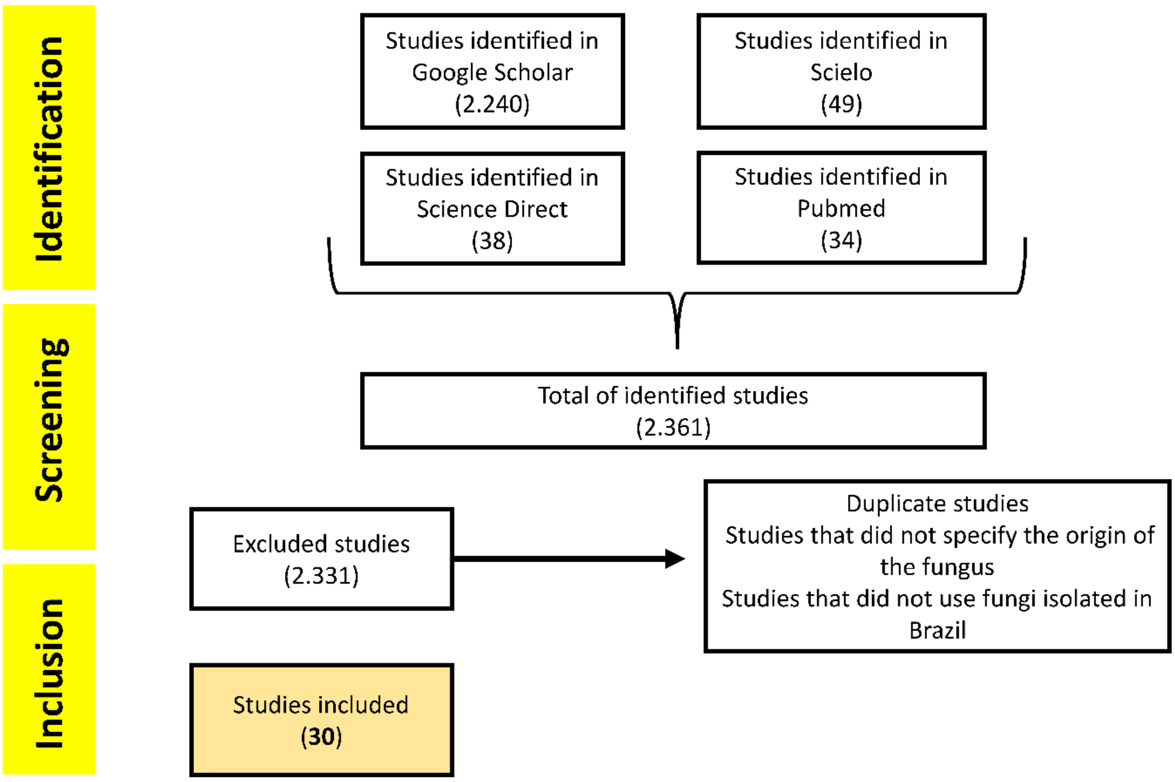

Figure 1. Study selection process.

By analyzing the title, abstract and material and methods of the articles, 30 articles were selected for meeting the inclusion criteria (Table 1).

Table 1. List of articles selected from the pre-defined inclusion criteria, organized according to the study objective, author, year of publication and database from which it was obtained.

Objective	Author	Year of publication	Data base
in vitro entomopathogenicity (25,8%)	Silva et al.	2004	Google Scholar
	De Paula et al.	2008	Google Scholar
	Pereira et al.	2009a	Google Scholar
	Leles et al.	2010	Pubmed
	Montalva et al.	2016	Science Direct
	Carolino et al.	2019	Google Scholar
	Montalva et al.	2019	Science Direct
fungal formulations (22,6%)	Leles et al.	2012	Springer
	Sousa et al.	2013	Pubmed
	Gomes et al.	2015	Pubmed
	Lobo et al.	2016	Google Scholar
	Bitencourt et al.	2018	Google Scholar
	Paula et al.	2019	Pubmed
	Rodrigues et al.	2019	Science Direct
fungal resistance to abiotic factors (12,9%)	Luz et al.	2008	Google Scholar
	Santos et al.	2009	Science Direct
	Falvo et al.	2016	Pubmed
	Falvo et al.	2018	Google Scholar
fungal epizootic in *Ae. aegypti* (9,7%)	Pereira et al.	2005	Google Scholar
	Pereira et al.	2009b	Google Scholar
	Rocha et al.	2009	Google Scholar
integration between fungus and some usual chemical product (9,7%)	Paula et al.	2011a	Pubmed
	Paula et al.	2013a	Pubmed
	Paula et al.	2013b	Pubmed
fungi under field conditions (6,4%)	Carolino et al.	2014	Pubmed
	Paula et al.	2018	Pubmed
Ae. aegypti subjected to different nutritional conditions (6,4%)	Paula et al.	2011b	Pubmed
	Cabral et al.	2020	Pubmed
insecticidal activity of fungal extracts (6,4%)	Bucker et al.	2013	Google Scholar
	Daniel et al.	2017	Google Scholar

From the works analyzed, 41 fungi isolated in Brazil have entomopathogenic activity against *Ae. aegypti* evaluated, among the main ones, are representatives of the species *Metarhizium anisopliae* (36.6%) and *Beauveria bassiana* (12.2%) (Figure 2).

Table 2. Fungal strains isolated in Brazil evaluated for entomopathogenic activity against different stages of development of *Ae. aegypti* in studies published between 2000 and 2020.

Fungal strain	Life stage	Mortality	Author
Metarhizium anisopliae IP 46	Egg	98,7%	Luz et al. 2008
	Egg	LC 50 2.8x10^2 conidia	Santos et al. 2009
	Adulthood	100%	Leles et al. 2010
	Egg	47,8% - 74,7%	Leles et al. 2012
	Adulthood	43,8% - 70,8%	Falvo et al. 2016
	Larvae	60% - 90%	Falvo et al. 2018
	Adulthood	LT 50 11,7 days	Rodrigues et al. 2019
Metarhizium anisopliae ESALQ 818	Adulthood	78,67%	De Paula et al. 2008
	Larvae	88%	Pereira et al. 2009
	Adulthood	55 - 57,8%	Paula et al. 2011
	Adulthood	56%	Paula et al. 2013
	Adulthood	30 - 71,2%	Carolino et al. 2014
	Larvae	26,7 - 75,6%	Gomes et al. 2015
	Adulthood	52 - 68%	Paula et al. 2018
Metarhizium anisopliae IP23	Larvae	100 %	Silva et al. 2004
Metarhizium anisopliae IP 84	Larvae	100 %	Silva et al. 2004
Metarhizium anisopliae IP 117	Larvae	100 %	Silva et al. 2004
Metarhizium anisopliae IP 123	Egg	88,7%	Luz et al. 2013
Metarhizium anisopliae CG 423	Egg	18,7%	Luz et al. 2013
Metarhizium anisopliae CG144	Adulthood	40%	De Paula et al. 2008
	Larvae	90%	Pereira et al. 2009
Metarhizium anisopliae LPP133	Adulthood	89,33%	De Paula et al. 2008
	Adulthood	8%	Pereira et al. 2009
	Larvae	48,9 - 76,7%	Paula et al. 2011
Metarhizium anisopliae LPP45	Adulthood	88,67%	De Paula et al. 2008
	Larvae	14%	Pereira et al. 2009
Metarhizium anisopliae LPP 96	Larvae	22%	Pereira et al. 2009
Metarhizium anisopliae LPP 137	Larvae	20%	Pereira et al. 2009
Metarhizium anisopliae LPP 87	Larvae	20%	Pereira et al. 2009
Metarhizium anisopliae LPP 128	Larvae	14%	Pereira et al. 2009
Metarhizium anisopliae LEF 2000	Pupa	Conidia 42,5% Blastospores 100%	Carolino et al. 2019
Metarhizium flavoviridae ARSEF 2948	Adulthood	40 %	Leles et al. 2010
Beauveria bassiana CG 24	Adulthood	30%	De Paula et al. 2008
	Adulthood	82,7%	Pereira et al. 2009
	Larvae	48% – 64%	Paula et al. 2018
Beauveria bassiana CG 494	Adulthood	70,67%	De Paula et al. 2008
	Larvae	6%	Pereira et al. 2009
Beauveria bassiana CG 479	Larvae	Blastospores 42% Conidia 57,44%	Bitencourt et al. 2018
Beauveria bassiana IP3A	Egg	71,2 %	Luz et al. 2013
Beauveria bassiana LPP27	Adulthood	26%	De Paula et al. 2008
Beauveria brongniartii CG619	Adulthood	80 %	Leles et al. 2010
	Egg	18,7%	Luz et al. 2013
Paecilomyces carneus CG525	Adulthood	100 %	Leles et al. 2010
	Egg	97,5%	Luz et al. 2013
Paecilomyces lilacinus CG362	Adulthood	100 %	Leles et al. 2010
	Egg	78,7	Luz et al. 2013
Paecilomyces marquandii CG 190	Adulthood	64 %	Leles et al. 2010
	Egg	94,9%	Luz et al. 2013
Paecilomyces variotti CG503	Egg	12,5 %	Luz et al. 2013

Isaria farinosa CG195	Adulthood Egg	92,5 % 98,7%	Leles et al. 2010 Luz et al. 2013
Isaria amoenorosea CG75	Egg	98,7%	Luz et al. 2013
Cladosporium cladosporioides CG635	Egg	18,7%	Luz et al. 2013
Nomuraea rileyi CG 381	Egg	13,7%	Luz et al. 2013
Sporothrix insectorum CG826	Egg	12,5%	Luz et al. 2013
Pochonia chlamydosporia IP315	Adulthood	85 %	Leles et al. 2010
Lecanicillium psalliotae IP301	Adulthood	100 %	Leles et al. 2010
Tolypocladium cylindrosporum IP 419	Larvae and Adulthood	Larvae 75 - 100% Adult 27,5 - 95%	Montalva et al. 2019
Tolypocladium cylindrosporum IP 425	Larvae and Adulthood	Larvae 22,5 - 82,5 % Adult 17,5 - 87,5%	Montalva et al. 2019
Conidiobolus macrosporus ARSEF12830	Adulthood	100%	Montalva et al. 2016
Gliocladium sp. IP290	Adulthood	70 %	Leles et al. 2010
Eulachovaea sp. IP218	Egg	27,5%	Luz et al. 2013
Fusarium sp. IP2	Egg	11,2%	Luz et al. 2013
Fusarium sp. IP4	Egg	1,2%	Luz et al. 2013
Penicillium sp. IP 182	Egg	80%	Luz et al. 2013

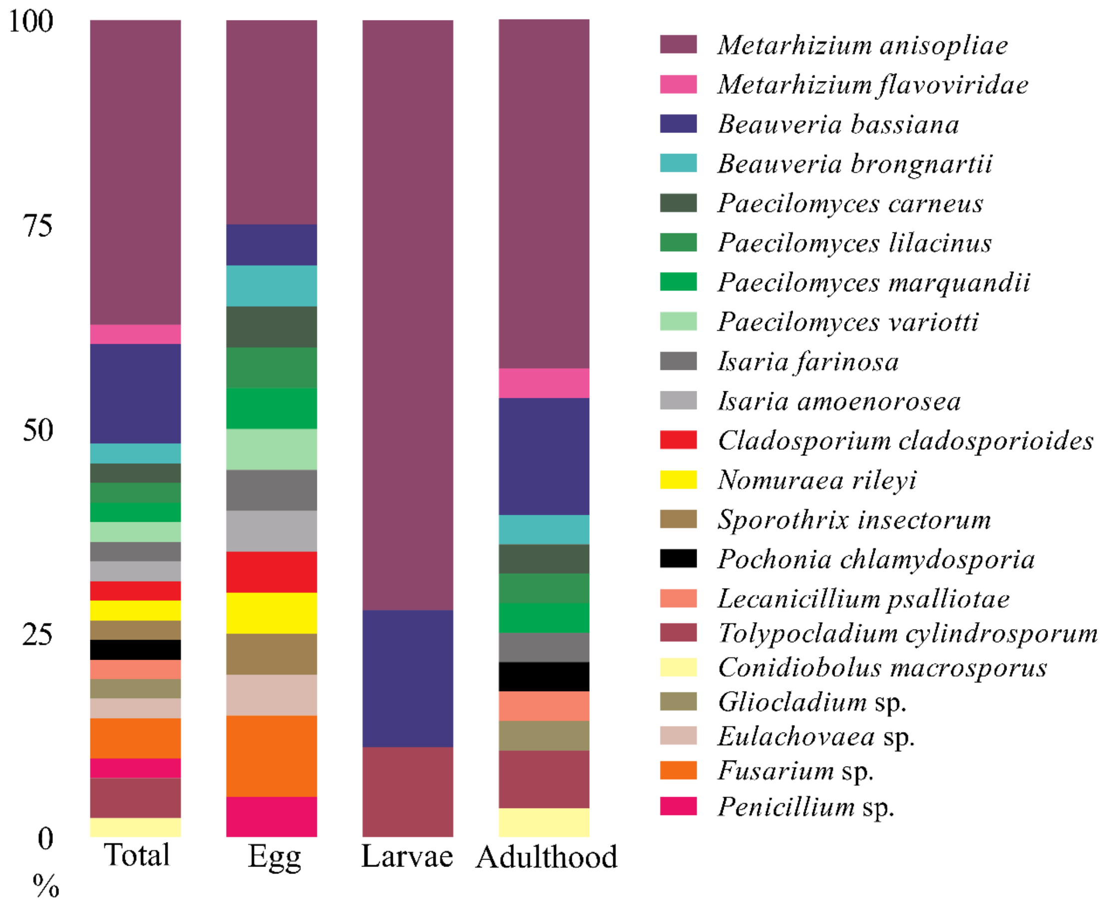

Figure 2. Frequency of entomopathogenic fungi isolated in Brazil against *Ae. aegypti* according to their stage of development (egg, larvae and adulthood) in studies published between 2000 and 2020.

Among the studies evaluated, only six studies (20%) evaluated the entomopathogenic activity of strains of *Metarhizium anisopliae* and *Beauveria bassiana* with adjuvants in eggs, larvae and adulthoods of *Ae. aegypti* (Table 3).

Table 3. Formulations of fungal strains isolated in Brazil that were evaluated for potential entomopathogenic activity against different stages of development of *Ae. aegypti* in studies published between 2000 and 2020.

Fungal strain	Life stage	Adjuvant	Conidia concentration	Mortality	Author
Metarhizium anisopliae IP46	Egg	Mineral oil	3,3 x $10^3/10^4/10^5$	CL50 $4{,}8\times10^3$ conidia	Sousa et al. 2013
	Egg	Vegetable oil (graxol - 1 µL)	1×10^6	64 - 100%	Lobo et al. 2016
	Adulthood	Mineral oil 0.25 µL Vegetable oil 0.25 µL Diatomaceous earth 0.6 mg	3.3×10^6	LT50 10,2 days	Rodrigues et al. 2019
Metarhizium anisopliae ESALQ 818	Larvae	Neem oil (0,001%)	1×10^7 and 1×10^8	63,4 - 87,8 %	Gomes et al. 2015
	Larvae	Neem oil (0,01%)	1×10^9	83 - 87%	Paula et al. 2019
Beauveria bassiana CG 479	Larvae	Conidia/Blastospores + Mineral oil (1, 0,5 and 0,1%)	1×10^7	83,78 - 91,44%	Bitencourt et al.. 2018

Of the studies with fungi isolated in Brazil, only three (10%) evaluated the entomopathogenic efficacy against *Ae. aegypti* in integration with chemical additives, being tested two strains of *Metarhizium anisopliae* associated with Imidacloprid against the adulthood mosquito (Table 4).

Table 4. Fungal strains isolated in Brazil that were evaluated for potential entomopathogenic activity associated with chemicals against the adulthood mosquito of *Ae. aegypti* in studies published between 2000 and 2020.

Fungal strain	Chemical additive	Conidia concentration	Mortality	Author
Metarhizium anisopliae LPP133	Imidacloprid 0,1, 1, 10, 50 and 100 ppm	1×10^9	55,6% - 92,3%	Paula et al. 2011
Metarhizium anisopliae ESALQ 818	Imidacloprid 0,1 ppm	1×10^9	56,7% - 61,7%	Paula et al. 2013a
	Imidacloprid 0,1 ppm	1×10^9	69% - 88%	Paula et al. 2013b

4. DISCUSSION

Of the isolated natives of Brazil, 41 fungal strains were evaluated for entomopathogenic potential against the vector mosquito *Aedes aegypti*. The species *Metarhizium anisopliae* stands out among the others, and its entomopathogenic action has been analyzed at different stages of development of *Ae. aegypti*. Only with the strain *M. anisopliae* IP 46, seven studies reported promising activity against eggs, larvae and adults of *Ae. aegypti* (Luz et al. 2008; Santos et al. 2009; Leles et al. 2010; Leles et al. 2012; Falvo et al. 2016; Falvo et al. 2018; Rodrigues et al. 2019).

M. anisopliae produces a group of cyclic depsipeptides known as destruxins (DTXs), a class of hexadepsipeptides produced by different soil fungi, and most of the 35+ characterized as toxic to insect pests have been described from this species (Ravindran et al. 2016). DTXs are the only toxic compounds ever reported to be present in insect cadavers in sufficient quantities to cause host death (Nowak et al. 2020).

Beauveria bassiana isolates also integrate much of the evidence of entomopathogenicity against eggs, larvae and adults of *Ae. aegypti* (De Paula et al. 2008; Pereira et al. 2009; Luz et al. 2013; Bitencourt et al. 2018; Paula et al. 2018). *B. bassiana* fungi have a set of genes that enable them to act by oral toxicity. At least thirteen heat-labile bacteria-like enterotoxins, eight Cry-like delta enterotoxins and three bacteria-like zeta toxin proteins have been reported for the species, while other entomopathogenic fungi have one or none. This finding suggests that *B. bassiana* could have greater oral toxicity than other entomopathogenic fungi (Mannino et al. 2019).

Entomopathogenic fungi have been more frequently used to control the adult stage of *Ae. aegypti*, compared to the other life stages of the vector. This approach usually involves the use of surfaces impregnated with fungi to which the mosquitoes are attracted, and after a brief contact with the fungal inoculum, they become infected and die (Falvo et al. 2020). Black cloths impregnated with *M. anisopliae* have been shown to significantly reduce the survival rates of *Ae. aegypti* under simulated field conditions (Paula et al. 2019).

When compared to the entomopathogenicity of two strains of *Tolypocladium cylindrosporum* against larvae and adults of *Ae. aegypti*, it was found that larvae are more susceptible, in comparison to adults, to the effect of the application of this entomopathogen. *T. cylindrosporum* has been often reported as a pathogen against the larval stage of aquatic mosquitoes (Rocha et al. 2015). A probable cause could be the capacity of conidial dissemination in breeding sites, resulting in the infection of other larvae and females (Montalva et al. 2019).

Six studies evaluated the synergistic effect between isolates of *Metarhizium anisopliae* and *Beauveria bassiana* with different mineral and/or vegetable oils against eggs, larvae and adults of *Ae. aegypti* (Sousa et al. 2013; Gomes et al. 2015; Lobo et al. 2016; Bitencourt et al. 2018; Paula et al. 2019; Rodrigues et al. 2019).

The selection of virulent isolates is crucial for development of bioproducts (Tyagi and Dhanasekaran 2018). However, the screening of different types of formulations is also necessary to optimize fungal performance, in addition to minimizing the negative effects caused by abiotic factors (Bitencourt et al. 2018).

The use of oils associated with entomopathogenic fungi are often applicable for control of vector mosquitoes (Lobo et al. 2016; Bitencourt et al. 2018). Conidia of *M.* anisopliae formulated in pure mineral oil or associated with water causes conidial adhesion in greater numbers and more homogeneously than in oil-free formulations (Rodrigues et al. 2019). In addition, oil-formulated propagules develop faster in the cuticle than unformulated conidia (Mannino et al. 2019). Oils also protect the propagules after application or after adhesion to the cuticle against harmful abiotic stresses (Paula et al. 2019).

By analyzing the entomopathogenicity of the fungus *M. anisopliae* IP 46 against adults of *Ae. aegypti*, a shorter lethal time to kill 50% of mosquitoes (10.2 days) was found in treatments formulated with mineral oil and vegetable oil, compared to treatment with oil-free formulations (11.7 days) (Rodrigues et al. 2019).

Conidia of *M. anisopliae* float in water because they have a hydrophobic surface (Vivekanandhan et al. 2020). Thus, when the larvae open their perispiracular valves for air intake, the conidia reach the insect's surface and attach to the tip of the siphon, so the hyphae grow in the trachea and can kill the insect by asphyxia (Mannino et al. 2019). If the same conidia are offered with a non-ionic detergent that deposits the spores on the bottom of the container, the larvae can eat them (Butt et al. 2013). Conidia can also be found in the intestine and kill the insect by secreting toxins, but they do not invade the rest of the host (Lacey et al. 2017).

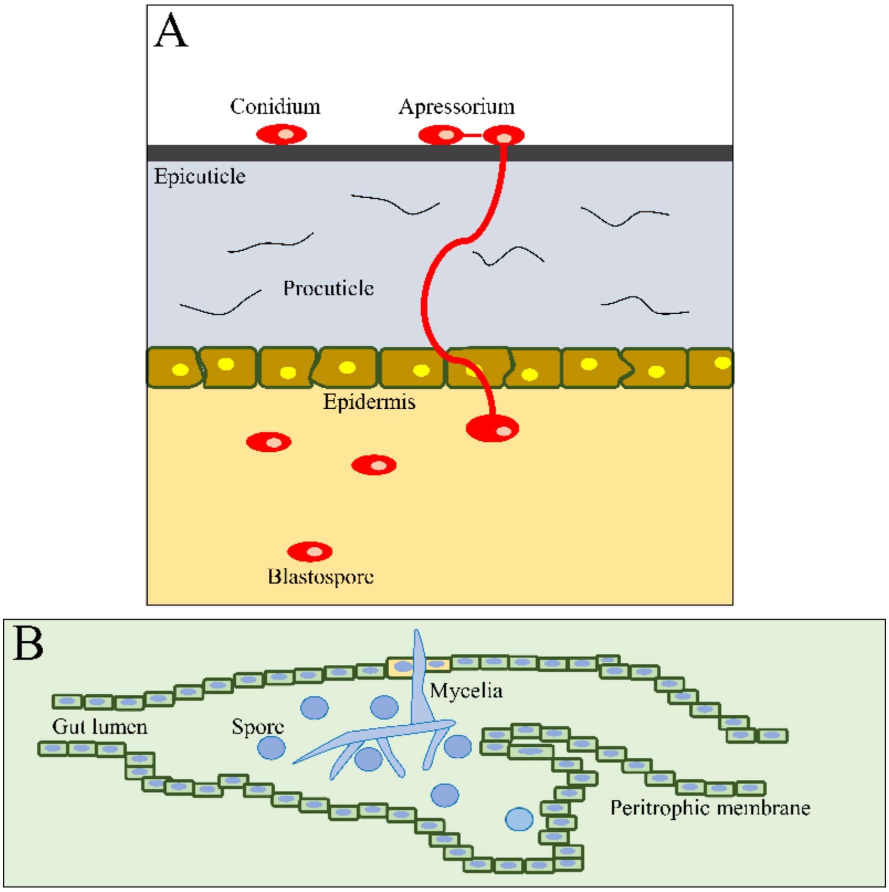

Figure 3. Infection mechanisms developed by entomopathogenic fungi in *Aedes aegypti*. **A**. Entomopathogen conidia attach to the cuticle of the larva or adulthood mosquito, penetrate the exoskeleton, and cross the integument to reach the hemocele. **B**. Mycelia and spores of entomopathogens ingested by larvae reach the intestine, causing mosquito mortality. Conidia germinate and penetrate through the midgut epithelium to restart the infective cycle.

Formulations with diatomaceous earth, which is known to cause abrasive damage to cuticular surfaces, have also been evaluated against *Ae. aegypti* associated with fungi isolated in Brazil (Rodrigues et al. 2019). Pioneering studies have reported the efficacy of controlling entomopathogenic fungi associated with diatomaceous earth against triatomine vectors (Luz et al. 2012).

Two studies (6.6%) compared entomopathogenic efficacy of the strains according to the infective structure being used (Bitencourt et al. 2018; Carolino et al. 2019). Blastopores have morphological characteristics that differentiate them from aerial conidia and make them more effective in water, which is an environment where *Ae. aegypti* is present during most of its life cycle (Alkhaibari et al. 2016). This propagule is a hydrophilic structure with a thin cell wall, which makes them less hydrophobic but not necessarily less resistant to adverse environmental conditions in comparison to conidia (Bitencourt et al. 2018).

The application of entomopathogenic fungi together with chemical insecticides covers different modes of action; applied in combination or at different times, it has become a necessary method (Litwin et al. 2020). For control of *Ae. aegypti*, findings were reported only from studies that had checked the effectiveness of controlling adults using the fungal species *M. anisopliae* associated with Imidacloprid (Paula et al. 2011; Paula et al. 2013).

The association of the fungus *M. anisopliae* LPP133 with Imidacloprid increased adult mortality of *Ae. aegypti* at rates that ranged between 55.6 and 92.3%, compared to treatment without the chemical, which ranged between 48.9 and 76.7% (Paula et al. 2011). This practice allows the reduction of the application concentrations of chemical insecticides as well as the time of action of the entomopathogen, thus attenuating factors that may limit the acceptance of these control methods.

5. CONCLUSIONS

Of the entomopathogenic fungi isolated in Brazil, *Metarhizium anisopliae* is the most studied against *Ae. aegypti*. The adult stage of *Ae. aegypti* is the most evaluated stage of development regarding the entomopathogenicity of fungi isolated in Brazil. The most frequently cited adjuvants for formulations with entomopathogenic fungi isolated in Brazil against *Ae. aegypti* are mineral and vegetable oils. Imidacloprid is the only chemical agent ever evaluated in association with *M. anisopliae* to control the adult of *Ae. aegypti*. The evaluation of the entomopathogenicity of new fungi native to Brazil against eggs, larvae and adults, as well as the effective association between fungi and vegetable oils of native species, in addition to integrating different species of fungi and other chemical products not yet investigated, are gaps to be considered completed to obtain new bioproducts effective in the control of *Ae. aegypti*.

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