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Virulence of entomopathogenic fungi isolated from wild mosquitoes against *Aedes aegypti*

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Abstract

The entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* are highly virulent control tools for insect pests and have been under evaluation for the control of globally important mosquito vectors such as *Aedes aegypti*. Here, we identified and isolated other virulent entomopathogenic fungi against *Ae. aegypti*. We collected 7 species of mosquitoes by human landing catch in 5 municipalities in Central and Northern Mexico and isolated 28 species of fungi. We harvested fungal conidia from six and assessed virulence against *Ae. aegypti* females. We observed variation in virulence of fungi in *Ae. aegypti* with the most virulent being *Aspergillus tamarii*, with a LT_{50} of 6.4 (±0.65) days and the least virulent was *Trichoderma euskadiense* with a LT_{50} of 16.3 (±1.5) days. Additional assays evaluated the impact of the fungi on *Ae. aegypti* fecundity and fertility and *A. tamarii* had the highest for both, resulting in 60% and 37% decrease, respectively. These results provide support for the potential utility of *A. tamarii* as an entomopathogenic control tool for the dengue vector, *Ae. aegypti*, pending further evaluations of environmental and nontarget safety.

Key words: *A. aegypti*, *Aspergillus tamarii*, biological control, entomopathogenic fungi

Introduction

Aedes and *Culex* mosquitoes are disease vectors of multiple arboviruses such as dengue, Zika, chikungunya, and West Nile virus. As no effective human vaccines are available, reducing the abundance of mosquito vectors are the primary control tool to interrupt arbovirus transmission (Suesdek 2019). However, the indiscriminate use of chemical insecticides has environmental and human health consequences (Rezende-Teixeira *et al*. 2022), can impact non-target organisms (Hoang & Rand 2015), and often contribute to the development of insecticide-resistant mosquito populations (Smith *et al*. 2016; Vontas *et al*. 2012). Alternative control tools are needed to augment integrated mosquito management of *Ae. aegypti* (Achee *et al*. 2019). The paucity of natural enemies of *Ae. aegypti* (Hembree 1979; Laird 1985) has led to renewed research into biocontrol with entomopathogenic fungus.

Entomopathogenic fungi for mosquito control is promising given the low or no toxicity to non-target organisms and because of their high specificity against insect pests (Islam *et al*. 2021). Fungi can infect different mosquito life stages by penetrating the integument during ingestion of spores or metabolites (Tawidian *et al*. 2019), by direct contamination using cotton sheets, mud panels, polyester nets, or clay pots impregnated with conidia (Farenhorst *et al*. 2008; Mnyone *et al*. 2010), or by auto-dissemination *via* copula (García-Munguía *et al*. 2011; Garza-Hernández *et al*. 2015; Reyes-Villanueva *et al*. 2011; Reyes-Villanueva *et al*. 2021).

Metarhizium anisopliae and *Beauveria bassiana* are two of the most efficient active ingredients for commercial formulations of bioinsecticides (Faria & Wraight 2007). Although there is no evidence of mosquitoes developing resistance to fungal bioinsecticides, this resistance is predicted more slowly than chemical insecticides (Wasinpiyamongkol

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& Kanchanaphum 2019). Thus, it is important to expand the repertoire of fungal bioinsecticides to alternate the use of available fungal bioinsecticides (Bitencourt *et al*. 2021).

Here, fungus-infected wild mosquitoes were collected in Mexico from which *Aspergillus tamari* was isolated. Using laboratory bioassays, we provide evidence that this natural enemy of *Ae. aegypti* is a potential candidate for the biocontrol of the dengue vector, with similar virulence as *B. bassiana*.

Materials and methods

Mosquito collection

Mosquito collections were conducted in five sites from Mexico: The municipality of Santiago Tianguistenco (19°10′56.4″N; 99°27′49.5″W; 2600 masl) was sampled in August, 2017 and Gómez Farías (23°03′22.3″N; 99°11′47.9″W; 900 masl) in July, 2018. The other municipalities: Chihuahua (28°59′18.1″N; 106°36′25.4″W; 2300 masl), Ciudad Juárez (31°41′09.2″N; 106°25′33.8″W; 1100 masl) and Reynosa (26°01′04.0″N; 98°16′29.3″W; 50 masl) were sampled in October, 2018. The human landing collection technique was used, and adult specimens were killed by exposing them for 5 s. to chloroform vapors (Nautiyal *et al*. 2015). The mosquitoes were identified using the Carpenter and LaCasse key (Carpenter & La Casse 1974) and processed for fungus isolation.

Fungus isolation

On the same day of collection, individual mosquitoes were placed on Petri dishes containing Potato Dextrose Agar (PDA, DIBICO® 1059, México) supplemented with 50 mg/L streptomycin sulfate under aseptic conditions. Plates were kept in the dark at 25°C using a styrofoam cooler and transported to a local lab. Individual mosquitoes were incubated for 5 days at 25°C, 60% RH in the dark, and those showing fungal germinations were transferred to a new Petri plate for identification.

Fungus identification

Morphological characters of fungus and molecular biology techniques were used to identify the fungus (Watanabe 2010). Fungus isolates were further grown on PDA for 4–7 d. at 25°C, 60% RH in the dark to obtain fresh mycelium for DNA extraction. DNA extraction was performed as reported elsewhere (Moslem *et al*. 2010). Nuclear ribosomal internal transcribed spacer (ITS) of rDNA were amplified by PCR using universal primers: ITS1 (5 - TCC GTA GGT GAA CCT GCG G - 3′) and ITS4 (5′ - TCC TCC GCT TAT TGA TAT GC - 3′) (White *et al*. 1990). PCR cycling conditions were as follows: 1 cycle of 5 min. at 94°C followed by 35 cycles of 1 min. at 94°C and final cycles of 1 min. at 55°C, 1 min. at 72°C, and 7 min. at 72°C. PCR products (550–800 bp) were purified using the QIAquick PCR Purification Kit Print (Qiagen, USA) following manufacturer's instructions. Sequencing was conducted using forward primer of ITS1: 5′-TCC GTA GGT GAA CCT GCG G-3′ on an ABI 3730xl sequencer (Eurofins Genomics, USA). The sequences were then blasted (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The identified fungus was cultured for the production of conidia.

Conidia and *A. aegypti* mosquitoes

The identified fungus was incubated on PDA at 25°C, 60% RH in the dark for 20 day to allow sporulation. Conidia harvesting was carried out as reported elsewhere (Reyes-Villanueva *et al*. 2011). Conidia suspensions were used to prepare a concentration of 6×10^8 conidia mL^{-1} per isolate using a Fisher hemocytometer. Afterward, 7 mL of each conidia suspension was poured on an area 56.75 cm^2 of a sterile Whatman filter paper in a Petri dish and left to dry out at 25°C, 60% RH for 24 h. The final concentration of conidia on the filter paper was approximately 4×10^7 conidia cm^2 for each fungus. The impregnated filter paper was placed into an exposure chamber constructed by two half Petri dish halves taped together with a 4-cm hole covered with a net in the top to allow the introduction and removal of mosquitoes using a mouth aspirator. The exposure chamber (8.5-cm diameter × 1.6-cm high) containing conidia was used to test *Ae. Aegypti* for virulence.

A. aegypti adult mosquitoes were obtained using egg strips from F8 and F10 generations of field-caught specimens collected in Chiapas, Mexico (14°53′01.6″N; 92°15′53.6″W; 141 masl, 14°54′58.7″N; 92°15′34.9″W; 193 masl). The insect room and rearing conditions were as reported elsewhere (García-Munguía *et al*. 2011). Freshly hatched females (4–6-day-old) were used for the bioassays.

Bioassays

A bioassay was carried out by direct contamination of conidia to *Ae. aegypti* females for 48 h. to estimate the lethal time (LT_{50}) of each isolate. Saline solution without fungus was also exposed to mosquitoes which served as control. Three replicates of 20 females each were placed into an exposure chamber containing the filter paper impregnated with conidia of each isolate. Females had access to 10% sucrose solution on a cotton pad placed on the surface of the chamber. After contamination, each group of female mosquitoes was transferred to a 1-L plastic pot with a cotton pad soaked in 10% sucrose solution on the top. Pots and exposure chambers

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were maintained at insectary conditions, namely, 27.5 (±1) °C, 80 (±10) % RH in a 12:12 h L:D photoperiod. The mortality of mosquitoes was monitored daily, and the carcasses of mosquitoes were washed with 1% sodium hypochlorite for 20 s and three times with sterile deionized water for 20 s. Individual dead mosquitoes were placed on humid sterile filter paper in a Petri dish sealed off with parafilm® and maintained at 25°C to allow sporulation.

In order to estimate the effects of isolates on fecundity and fertility rates, *i.e.*, the total of laid eggs per female and successfully hatched eggs, respectively, two additional bioassays were performed. The fertility rate was calculated as the total number of larvae/total number of eggs ×100. Two replicates of 20 females each were exposed to conidia for 48 h. using the aforementioned conditions. After fungal contamination, females were blood-fed on the forearm of a volunteer (JAAD) for 30 min. Individual engorged females were transferred to a 1-L plastic pot with a cotton pad containing 10% sucrose solution. The pot contained a 1-oz. plastic cup half-filled with deionized water and lined with filter paper to allow oviposition. After 7 day, the eggs on strips were counted through a stereomicroscope, transferred to a plastic container (not airtight) for embryonation, and kept at insectary conditions for 72 h. Following the drying period, each egg strip was transferred to a 1-L pot half-filled with deionized water for egg hatching. After 7 day, first instar larvae were recorded.

Statistical analysis

The LT_{50} was calculated through survival curve analysis using the Kaplan–Meier model considering the 60 females per treatment in the bioassays. Each survival curve was computed by pooling the three replicates per treatment and the three replicates per control. A Log-rank test was performed to compare survival curves.

Data from fecundity and fertility rates were tested for normality (Shapiro–Wilk test) and homogeneity of variances (Bartlett's test). Analysis of fecundity was performed by a Kruskal-Wallis test, followed by a Wilcoxon rank sum test to compare the eggs laid for treatment and control groups. Fertility data were arcsine-transformed prior to analysis. Then a student's *t*-test was performed to compare the hatching rate of eggs in the treatment and control groups. All statistical analyses were performed using SAS OnDemand for Academics (SAS OnDemand for Academics 2021).

Results

Mosquito collection and fungal isolation

A total of 83 adult mosquitoes of seven different taxa were collected in Mexico. These mosquitoes were plated on PDA for fungal isolation. Of these, 86.74% (72/83) had fungal growth, and in some mosquitoes more than one fungal species were isolated. The morphological and molecular identification of fungal species resulted in a total of 79 fungal isolates belonging to 28 species and 16 genera (Table S1). The most abundant fungal taxa were *Aspergillus* spp. (31.64%) followed by *Alternaria* spp. (16.45%) and *Cladosporium* spp. (16.45%) (Table S2).

Mosquito bioassays

Aspergillus tamarii, *Aspergillus sclerotiorum*, *Beauveria bassiana*, *Cladosporium cladosporioides*, *Byssochlamys spectabilis,* and *Trichoderma euskadiense* were selected for bioassays. The results (Table 1) indicate that the virulence of these fungi varied significantly from control, except for *T. euskadiense* ($\chi^2 = 1.59$, $df = 1$, $P = 0.206$). Decreasing daily survival rates appear to be similar for *Ae. aegypti* using either *A. tamarii*, *B. bassiana*, or *A. sclerotiorum* (Fig. 1). Although,

Table 1 Lethal Time (LT_{50}) for *Ae. aegypti* females after contamination with each fungal isolate and percent of sporulation on mosquito cadavers.

	LT_{50} ± SE (days)	LT_{50} ± SE (days)		
Fungus isolate	Treatment	Control	Sporulation rate on cadavers (% ± SE)†	Statistical metrics
A. tamarii	6.4 ± 0.65	20.26 ± 1.45	98.33 ± 1.66	$\chi^2 = 64.24$, $df = 1$ $P < 0.0001$
B. bassiana	7.18 ± 0.72	19.55 ± 1.10	88 ± 4.40	$\chi^2 = 66.87$, $df = 1$ $P < 0.0001$
B. spectabilis	13.46 ± 1.27	20.26 ± 1.29	65 ± 5.77	$\chi^2 = 12.34$, $df = 1$ $P = 0.0004$
A. sclerotiorum	8.6 ± 0.70	20.96 ± 1.18	86.66 ± 4.40	$\chi^2 = 65.92$, $df = 1$ $P < 0.0001$
C. cladosporioides	9.2 ± 0.74	20.70 ± 1.30	56.66 ± 17.34	$\chi^2 = 50.37$, $df = 1$ $P < 0.0001$
T. euskadiense	16.3 ± 1.5	19.3 ± 1.41	65 ± 2.88	$\chi^2 = 1.59$, $df = 1$ $P = 0.206$

Abbreviation: SE, standard error of the mean.
†Number of cadavers showing fungus sporulation (% ± SE) after 7 days incubation in wet chambers.

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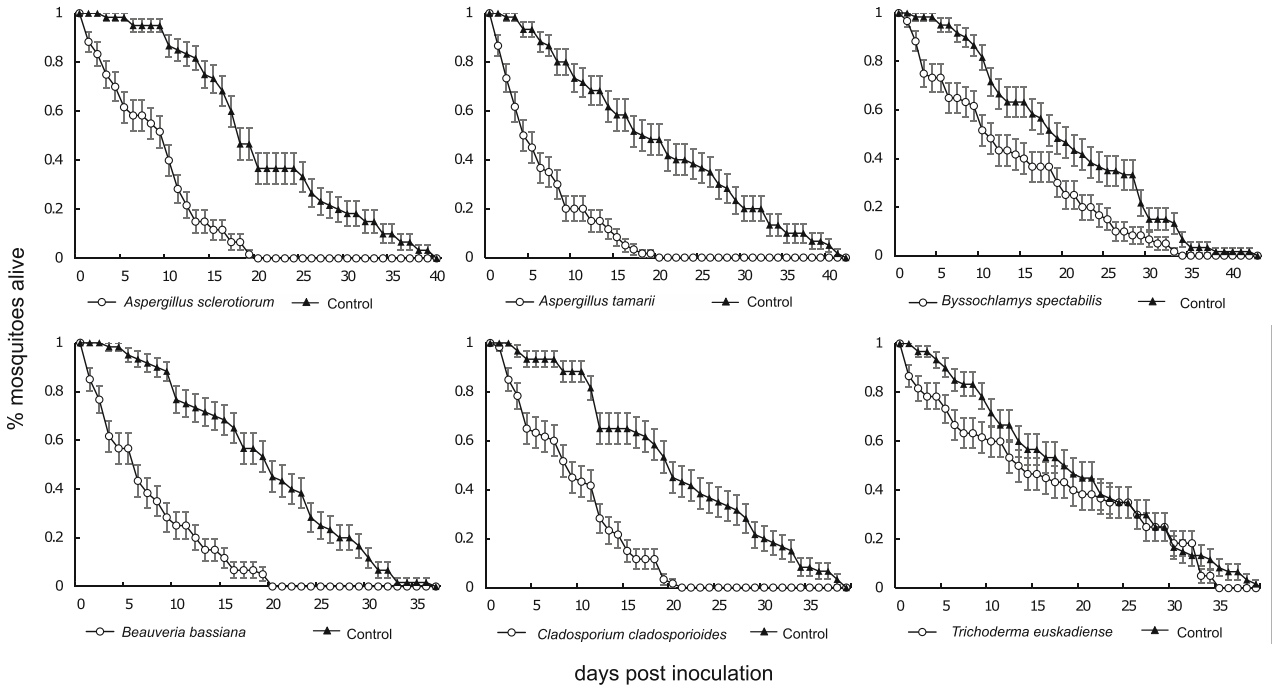

FIGURE 1 Survival curves (±standard error, SE) calculated by the Kaplan–Meier model of *Ae. aegypti* females exposed to 6×10^8 conidia mL^{-1} of each fungus isolate (white circle) and control (black triangle). In each experiment, 60 *Ae. aegypti* females were exposed to each fungus and saline solution without fungus served as control for 48 h. *A. tamarii* killed *Ae. aegypti* faster than other fungus isolates. Mortality by each fungus was demonstrated by sporulation in cadavers (Table 1).

Table 2 Effect of fungal infection on the fecundity rate of *Ae. aegypti* (mean ± SE).

Fungus isolate	Treatment	Control	Statistical metrics
A. tamarii	12.87 ± 2.12	37.36 ± 4.41	$Z = 4.4285$, $P < 0.0001$
B. bassiana	18.5 ± 3.9	40.42 ± 3.4	$Z = 4.2554$, $P < 0.0001$
B. spectabilis	25.5 ± 3.11	37.02 ± 3.14	$Z = 2.6317$, $P = 0.0085$
A. sclerotiorum	19.37 ± 3.43	27.12 ± 3.23	$Z = -2.2054$, $P = 0.0274$
C. cladosporioides	26.97 ± 3.41	41.62 ± 3.87	$Z = -2.8071$, $P = 0.0050$
T. euskadiense	21.52 ± 3.3	27.7 ± 2.84	$Z = -2.0507$, $P = 0.0403$

Abbreviation: SE, standard error of the mean.

the fungus with the highest virulence was *A. tamarii* with a LT_{50} of 6.4 (±0.65) d. after contamination, compared to that of the control of 20.26 (±1.45) d. ($\chi^2 = 64.24$, $df = 1$ $P < 0.0001$). In addition, out of 60 exposed mosquitoes, 59 showed *A. tamarii* sporulation after 7 day. in wet chambers (Figure S1), indicating that 98% had succumbed to fungal infection. The LT_{50} of the other evaluated fungi varied from 7.18 (±0.72) d. (*B. bassiana*) to 16.3 (±1.5) day (*T. euskadiense*) in comparison to those observed in controls of 19.3 (±1.41) day and 20.96 (±1.18) day, respectively.

There was a significant effect on the fecundity of females of *Ae. aegypti* exposed to the six fungi evaluated (Kruskal-Wallis test, $\chi^2 = 80.74$, $df = 11$ $P < 0.0001$) (Table 2). *A. tamarii* had the most significant impact on fecundity, decreasing by 60% the fecundity *versus* the control group (Wilcoxon rank sum test, Z = 4.4285, $P < 0.0001$). Similarly, the fecundity of females exposed to *B. bassiana* was highly affected, diminishing by 50% the number of eggs laid (Wilcoxon rank sum test, Z = 4.2554, $P < 0.0001$). Females exposed to *C. cladosporioides*, *A. sclerotiorum*, *B. spectabilis*, and *T. euskadiense* decreased by 36%, 27%, 25%, and 22%, respectively, compared to those of controls.

Furthermore, the fungal infection also affected the fertility rate of *Ae. aegypti* females. Figure 2 depicts a significant decrease in the fertility rate when using four out of the six fungi assessed. The mean of hatched eggs per female exposed

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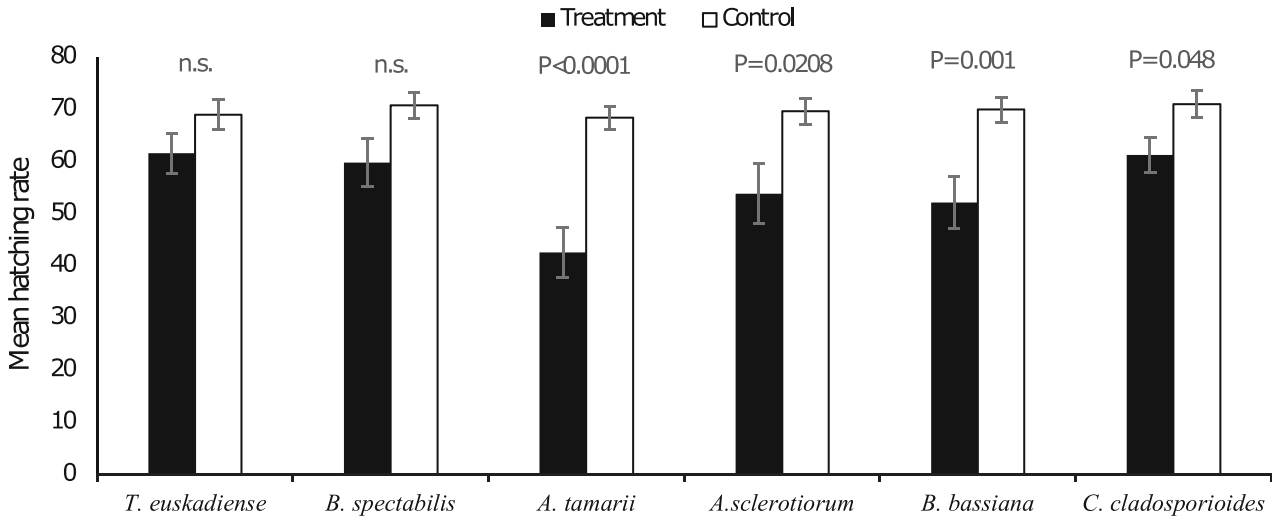

Figure 2 Mean of the eggs hatched (±SE) per *Ae. aegypti* female exposed to fungus or with saline solution without fungus as control.

to *A. tamarii* was of 42.56% (±3.74) in comparison to that of 68.37% (±3.33) in the control (37% decrease) ($t_{(45)} = 4.38$, $P < 0.0001$). A reduction of 25.4% in the fertility rate was observed for *B. bassiana* ($t_{(35)} = 3.12$, $P = 0.0036$). However, when *Ae. aegypti* females were exposed to *A. sclerotiorum* and *C. cladosporioides*; the fertility rate only decreased by 22% and 13%, respectively. No significant effect was observed for *B. spectabilis* ($t_{(55)} = 1.32$, $P = 0.19$) and *T. euskadiense* ($t_{(65)} = 1.51$, $P = 0.13$) in comparison to those in controls.

Discussion

Here, the most abundant fungi were *Aspergillus*, *Cladosporium*, and *Alternaria*, which have already been reported as saprobic, airborne contaminant, or opportunistic entomopathogens capable of infecting and killing mosquitoes (Jaber *et al*. 2016). Although we isolated these fungi from mosquitoes, they could have resulted from environmental exposure after the collection event.

Field data in our entomologic surveys coincided with those reported in Brazil where an association between *Acremonium* sp., *Aspergillus*, *Fusarium* sp., *Gliocladium* sp., *Paecilomyces* sp., and *Penicillium* spp. and Culicidae larvae collected from temporary, semipermanent, and permanent containers was documented (Pereira *et al*. 2009). Da Costa and de Oliveira (1998) reported a high incidence of *Penicillium* spp. infecting both adults and larvae of *Anopheles*, *Aedes*, *Culex*, and *Mansonia* collected from natural and artificial habitat in three states of Brazil. While Pereira *et al*. (2005) found an 18% incidence of 4th-instar larvae of Culicidae (*Limatus* spp., *Cx. urichii*, *Culex* sp., *Ae. aegypti,* and *Ochlerotatus argyrothorax*) infected with *Zancudomyces culisetae*. Also, in Tanzania, *Akanthomyces muscarius* was isolated from a dead culicid showing signs of fungal infection from resting traps (Luz *et al*. 2010).

Although many species of fungi have been found infecting immature and adult stages of mosquitoes in nature (Scholte *et al*. 2004), *M. anisopliae* and *B. bassiana* have been the main species used against medically important disease vectors such as *Ae. aegypti*, *Ae. albopictus*, *Cx. tarsalis*, *Cx. pipiens*, *Cx. quinquefasciatus*, *Anopheles albimanus* and *An. stephensi* (Clark *et al*. 1968; de Paula *et al*. 2008; Greenfield *et al*. 2015; Ragavendran *et al*. 2017; Scholte *et al*. 2007). This is because their conidia are easy to produce and harvest (Rodriguez-Perez & Reyes-Villanueva 2018).

Here, we demonstrate that five out of six fungi successfully reduced the survival adult *Ae. aegypti* in comparison to those of control groups. The finding of a LT_{50} of 6.4 day for the Reynosa strain of *A. tamarii* (UAMH 12497) and 16.3 day for *T. euskadiense* is concordant with that report of Leles *et al*. (Leles *et al*. 2010).

Aspergillus nomius isolated from dead Buprestidae (Coleoptera) was as pathogenic as *B. bassiana* to *Ae. albopictus* (Jaber *et al*. 2016), while *Akanthomyces muscarius* was pathogenic to *Ae. aegypti*, *An. arabiensis* and *Cx. quinquefasciatus* (Luz *et al*. 2010), indicating the existence of highly virulent fungi against medically important mosquitoes. Thus, the fungi (*e.g.,* Reynosa strain *A. tamarii*) assessed here have the potential to kill and affect (lethal or sublethally) *Ae. aegypti*, and current studies are underway for other species of mosquitoes.

Most studies that have explored the sublethal effects of fungal infections on disease vectors were carried out mainly with the fungi *M. anisopliae* and *B. bassiana*. Females of

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An. gambiae infected with low and high doses of *M. anisopliae* showed a 49% and 56% decrease over eight gonotrophic cycles (Scholte *et al*. 2007). Similar results were observed in *An. gambiae* sprayed with *B. bassiana* spore solutions; exposed females laid significantly fewer eggs (~16% decrease) than noninfected females (Kamareddine *et al*. 2013). *Anopheles funestus* females exposed to *M. anisopliae* and *B. bassiana* showed a similar decreasing effect on eggs laid per female through three gonotrophic cycles (Mouatcho *et al*. 2011). Darbro *et al*. (2012) reported that *Ae. aegypti* females exposed 24 h. to *B. bassiana* decreased 39% their fecundity. Similarly, the reproductive capacity of *Ae. albopictus* was affected by the exposure of *M. anisopliae* spores, reducing in 42% the mean of laid eggs when compared with uninfected females (Shoukat *et al*. 2020a).

A sublethal (LC_{20}) and lethal (LC_{50}) exposition of *B. bassiana* decrease in 35% and 47% the fecundity of *Ae. albopictus* (Shoukat *et al*. 2020b). Also, Deng *et al*. (2019) reported a similar decrease (39%) in the fecundity of *Ae. albopictus* exposed to *B. bassiana* spores. Here, the decline in the fecundity rate for Reynosa strain of *A. tamarii* was 60% (compared to control). Thus, this additional fungal taxa have potential use as a biocontrol agent for the dengue vector.

There are numerous reports about the effect of fungal infection on the fertility of medically important mosquito eggs. One of the first reports was carried out in 2007 where *Ae. aegypti* eggs were exposed with 21 hyphomycetous fungi at four exposure periods (5, 10, 15, and 25 day); eggs exposed to *Cordyceps farinosa* for 25 day had >95% decrease in the hatching rate (Luz *et al*. 2007). Luz *et al*. (2011) evaluated the effect of topical infection of *M. anisopliae* and *B. bassiana* on *Anopheles gambiae* eggs. After a long exposure period (5 day), *M. anisopliae* could prevent eclosion of eggs, while *B. bassiana* reduced ~60% eggs eclosion. Also, Leles *et al*. (2012) reported that eggs of *Ae. aegypti* incubated with 3.3×10^5 conidia/g of *M. anisopliae* for 25 day had a hatching rate of 25%, while the hatching rate of unexposed eggs (controls) was 68.6%. Water and oil-in-water emulsion of *M. anisopliae* were reported to significantly reduce the larvae eclosion of *Ae. aegypti* eggs by direct and indirect exposition (Sousa *et al*. 2013). Furthermore, granular formulations of *Metarhizium brunneum* were reported effective in decreasing the hatching rate of *Ae. aegypti* eggs. At the highest concentration assessed (6.85×10^6 conidia per cup), only 23% of eggs hatched compared with the 92% in the control group (Flor-Weiler *et al*. 2019).

Other fungal species have also been reported to be effective in reducing viable egg hatchability. Rocha *et al*. (2015) exposed *Ae. aegypti* eggs to seven isolates of *Tolypocladium cylindrosporum*. The most effective isolate reduced the hatching rate to 30% after 15 day of incubation. Also, Flor-Weiler *et al*. (2017) reported that the infection of *Tolypocladium cylindrosporum* at 1×10^7 conidia/mL successfully stopped the eclosion of *Ae. aegypti* eggs, while in *Ae. albopictus*, the eclosion was reduced to 29% in exposed eggs after 21-day incubation.

Only Pelizza *et al*. (2013) have assessed the sublethal effect on fertility of mosquitoes that survived fungal infection. Larvae of *Ae. aegypti* exposed with zoospores of *Leptolegnia chapmanii* decreased viable eggs through six gonotrophic cycles to 41%. Here, females of *Ae. aegypti* infected with our most effective fungus isolate (*A. tamarii*) reduce by 37% the hatching rate of laid eggs.

Our data show the association between each of the three genera of saprophyte fungi and wild mosquitoes from five collecting sites (municipalities) of Mexico. The fungal isolate (Reynosa strain of *A. tamarii*) showed the highest lethal and sub-lethal effects on *Ae. aegypti* females. In addition, conidia of *Aspergillus* spp. were easier to obtain than those from other fungi; this means less time-consuming work and fewer resources for growing the fungus. As these fungi infect wild mosquitoes, native fungal isolates may have better control of local vector populations than foreign isolates.

A. tamarii is a non-aflatoxigenic species, a member of *Aspergillus* section *Flavi* (Habibi & Afzali 2021), which is widely used in food industries (Hong *et al*. 2015), with the potential to produce biomass-degrading enzymes (Monclaro *et al*. 2022). Some strains produce cyclopiazonic acid (CPA) and less toxic compounds, fumigaclavine and kojic acid (Dorner 1983; Janardhanan *et al*. 1984; Sillapawattana & Klungsupya 2022). Although it is an unusual cause of infection, it has been implicated as an opportunistic human pathogen in cases of onychomycosis, keratitis, and cutaneous infections in immuno-depressed individuals or injured in a traumatic event (Kimura *et al*. 2018; Kredics *et al*. 2007; Kristensen *et al*. 2005). However, the distribution of airborne *Aspergillus* spp. varies according to the weather conditions. Thus, the risks associated with using these species will vary by country (Pasqualotto 2009).

These findings demonstrate the feasibility of isolating fungi with potential pathogenicity against mosquitoes and showed the Reynosa strain of *A. tamarii* (UAMH 12497) to be a good prospect for the biocontrol of *Ae. aegypti*; however, environmental and human health concerns should be considered. Therefore, further research is necessary to consider these concerns.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Infection of *A. tamarii* in *Ae. aegypti.* A) *Ae. aegypti* mosquito after succumbing to *A. tamarii* conidia exposure and kept 7 d. in a wet chamber. B) Macro- and C) Micro-morphology of *A. tamarii* isolated from the carcass of dead *Ae. aegypti* after fungal infection.

Table S1 Molecular identification of fungi isolated from wild mosquitoes in Mexico.

Table S2 Number of occurrences and mosquito host of each fungus isolated.

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