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Pathogenicity of entomopathogenic fungi to eggs, larvae, and adults and their effects on development of *Aedes albopictus*

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Abstract. *Ramayanti I, Herlinda S, Muslim A, Hasyim H, Anwar C, Suwandi S, Damiri N, Irsan C, Verawaty M. 2023. Pathogenicity of entomopathogenic fungi to eggs, larvae, and adults and their effects on development of* Aedes albopictus. *Biodiversitas 24: 4766-4774.* No information is available on the effect of fungi on the development of *Aedes albopictus* and the effectiveness of fungi in killing its eggs, larvae, and adults. The aim of this research was to determine the pathogenicity of entomopathogenic fungi on the eggs, larvae, and adults of *Ae. albopictus* and to investigate the effects of the fungi on the development of *Ae. albopictus*. The fungal species identified molecularly were *Aedes albopictus*, *Beauveria bassiana*, *Metarhizium anisopliae*, *Penicillium citrinum*, and *Talaromyces diversus.* The results showed that entomopathogenic fungi negatively affected the development of *Ae. albopictus* and could decrease the eggs laid by adult females and shorten adult longevity. *M. anisopliae* was the most pathogenic fungal species (mortality rate 100%) for eggs, larvae, pupae, and adults of *Ae. albopictus*. However, *B. bassiana*, *P. citrinum*, and *T. diversus* were also found pathogenic to them. The effect of eggs treated with entomopathogenic fungi negatively affected the larval, pupal, and adult stages of *Ae. albopictus*. These findings highlighted that *M. anisopliae*, *B. bassiana*, *P. citrinum*, and *T. diversus* have insecticidal activity against *Ae. albopictus* eggs, larvae, pupae, and adults. Therefore, further investigation is needed to develop these fungal species into ovicides, larvicides, and adulticides to control *Ae. albopictus*.

Keywords: Asian tiger mosquito, *Beauveria bassiana*, *Metarhizium anisopliae*, *Penicillium citrinum*, *Talaromyces diversus*

INTRODUCTION

Aedes albopictus, the Asian tiger mosquito, is a very invasive species of mosquito that can spread rapidly and invade large parts of the world (Akiner et al. 2016). The spread of mosquitoes usually occurs through passive dispersal by wind or human-mediated means, such as maritime transport, land vehicles, and trade (Dhimal et al. 2015; Nugroho et al. 2019). The active dispersal of *Ae. albopictus* occurs by movement or flying against the wind (Akiner et al. 2016). *Ae. albopictus* has been notified in Sumatra, Kalimantan, Java, Nusa Tenggara, Sulawesi, Maluku, Papua (Lee and Ryu 2019), and West Java (Yuliani et al. 2021). *Ae. albopictus* is the most important mosquito because it is known to be a primary and secondary vector for the transmission of chikungunya virus (CHIKV) and dengue virus (DENV), as well as several other arboviruses (Dhimal et al. 2015; Ferreira-De-Lima and Lima-Camara 2018). The mosquito can also transmit diseases, such as *Dirofilaria immitis*, *Plasmodium lophurae*, *Plasmodium gallinaceum*, and *Plasmodium fallax* (Peach and Matthews 2022). Longevity of *Ae. albopictus* adults are longer than other mosquito species, so the duration of chikungunya transmission is also longer (Rezza 2014). The mosquito has a variety of habitats, such as urban, suburban, and forest. Some disposable containers also have a high risk of chikungunya and dengue mosquito habitats, such as metal-based material, dark in color and no cover (Yuliani et al. 2021), making the population easier during outbreaks and difficult to control (Bonizzoni et al. 2014).

Management strategies to reduce the burden of mosquito-borne diseases rely on controlling vectors or mosquitoes (Flor-Weiler et al. 2017). To reduce the burden and population density of *Ae. albopictus*, synthetic insecticides targeting larval, pupal, and adult mosquitoes, have been used extensively; however, some insecticides are resistant to *Ae. albopictus*, such as permethrin and temephos (Vontas et al. 2012). Furthermore, the routine use of synthetic insecticides causes other problems such as water, air, and soil pollution, human health problems, and the killing of non-target animals (Hamid et al. 2017). An environmentally friendly controls which does not require synthetic insecticides to control mosquito is the use of plant

extracts, such as papaya and pineapple peel extracts (Nur Athen et al. 2020), or the use of biological control using entomopathogens such as entomopathogenic fungi (Greenfield et al. 2015).

Previous studies indicated that entomopathogenic fungi were promising candidates for the control of immature stages and adult of *Culex* (Greenfield et al. 2015; Ramayanti et al. 2022), *Aedes* (Alkhaibari et al. 2016; Quintero-Zapata et al. 2022; Ramayanti et al. 2023), and *Anopheles* mosquitoes (Valero-Jiménez et al. 2014; Vivekanandhan et al. 2022). Entomopathogenic fungi are the most commonly used for the control of *Aedes* mosquitoes (Greenfield et al. 2015), and some species of them have been reported to be effective in killing the larvae of *Ae. albopictus* were *Beauveria bassiana* (Balsamo) (Lee et al. 2019), *Metarhizium anisopliae* (Metschnikoff) (Shoukat et al. 2020). *Metarhizium brunneum* was also effective against *Aedes* mosquito larvae (Alkhaibari et al. 2017). *Tolypocladium cylindrosporum* is pathogenic to *Ae. albopictus* eggs (Flor-Weiler et al. 2017). The entomopathogenic fungi from Indonesia, particularly from South Sumatra, have been reported to be effective in killing eggs, larvae, and adults of *Culex quinquefasciatus* (Ramayanti et al. 2022) and *Aedes aegypti* (Ramayanti et al. 2023). No information is available regarding the effect of fungi on the development of *Ae. albopictus* and the effectiveness of fungi in killing its eggs, larvae, and adults. It was first reported that entomopathogenic fungi from South Sumatra could affect and kill the eggs, larvae, and adults of *Ae. albopictus*. Therefore, this study aimed to determine the pathogenicity of entomopathogenic fungi from South Sumatra on the eggs, larvae, and adults of *Ae. albopictus* and to investigate the effects of the fungi on the development of *Ae. albopictus.*

MATERIALS AND METHODS

Fungal preparation

The fungal isolates used in this study were obtained from South Sumatra, Indonesia soil. The identified fungal species were *B. bassiana* TaAlPA isolate (GenBank accession number OM791688) from Alang-alang Lebar, Palembang (2°56′32″S 104°42′16″E), *B. bassiana* TaLmME isolate (GenBank accession number OM791687) from Lebak, Muara Enim (3°23′51″S 104°19′41″E) (Ramayanti et al. 2023), *Penicillium citrinum* BKbTp isolate (GenBank accession number MT448730) from Talang Patai, Pagar Alam (4°00′45″S 103°12′21″E), *Talaromyces diversus* MSwTp1 isolate (GenBank accession number MT448731) from Talang Dabok, Ogan Komering Ilir (3°00′18″S 104°40′05″E), *B. bassiana* BSwTd4 isolate (GenBank accession number MT448732) from Talang Patai, Pagar Alam (4°00′45″S 103°12′21″E), and *M. anisopliae* MSwTp3 isolate (GenBank accession number MT488733) from Talang Patai, Pagar Alam (4°00′45″S 103°12′21″E) (Herlinda et al. 2020). The fungal isolates were grown on the sabouraud dextrose agar medium. They were recultured on the broth medium (sabouraud dextrose broth) for the bioassay.

Mass-rearing of *Aedes albopictus*

Aedes albopictus eggs were obtained from P2B2 Research and Development Loka, the Health Research and Development Centre, Ministry of Health of Indonesia in Baturaja, South Sumatra, and mass rearing of Ae. albopictus had been conducted since 2017. For bioassay, *Ae. albopictus* rearing was conducted at the Entomology Laboratory, Faculty of Agriculture, Universitas Sriwijaya. The room temperature and relative humidity during mass rearing were 28 ± 1°C and 83 ± 1%, respectively. The lighting in the room was set to a photoperiod of 12 hours of light followed by 12 hours of darkness (Kauffman et al. 2017). The emerging larvae were kept in a disinfected plastic tray (20 x 30 x 3 cm), and the tray was filled with 1,000 mL of tap water for 400-500 larvae. The larvae were fed daily with dog biscuits until pupation (Vivekanandhan et al. 2018). The emerging pupae were placed in a disinfected transparent plastic cup (Ø 9 cm, height 13 cm) filled with 350 mL of tap water. Then, the cup placed in a disinfected transparent plastic cage (30 x 30 x 30 cm) to keep the emerging adults in the cage. At the top of cage, a 10% sucrose solution infused on cotton wool was hung for the adult male diet (Ramayanti et al. 2023). Adult females were fed on blood through direct sucking on the guinea pig for one hour twice a week (Snetselaar et al. 2014). The females took 4 days to become gravid. In addition, an ovitrap was placed in the cage for gravid female to lay eggs. The ovitrap was designed as a disinfected transparent plastic cup (Ø 9 cm, height 13 cm) with dark walls filled with water to a depth of 10 cm (Wu et al. 2013). An ovistrip was attached to the inside wall of the ovitrap. The ovistrip was a filter paper (25 x 4 cm) with chequered lines to make it easier to count the eggs laid. The eggs were collected daily for the further experiment.

Bioassay of fungal pathogenicity on *Aedes albopictus* eggs

The pathogenicity of fungal isolates to *Ae. albopictus* eggs, larvae, and adults were tested in a laboratory with an average temperature and relative humidity of 28.51°C and 83.18%, respectively. After culturing the fungal isolates on agar medium (SDA medium), fungal culture was re-grown on broth medium (SDB medium) to increase the density of fungal conidia (Gustianingtyas et al. 2020). During the 14-days of incubation of fungal culture on SDB medium, the fungal culture was shaken continuously for 7 days and then not shaken for 7 days. To increase fungal pathogenicity, 600 mL of fungal broth culture was added to 400 mL of a fermented aqueous extract of shrimp waste-enriched compost produced through two-step fermentation (Suwandi et al. 2018). The fungal broth culture enriched with the compost was incubated for 7 days before the bioassay. The bioassay was conducted following the method of Luz et al. (2011). Next, 10 mL of fungal broth culture (1 x 10^{10} conidia mL^{-1}) was applied to the ovistrip, while ovistrip of control was applied with 10 mL of sterile distilled water. Then, ovistrip was attached to the inside wall of the ovitrap containing 100 mL of water. The ovitrap was then placed in the disinfected transparent plastic cage (30 x 30 x 30 cm), and 30 gravid adult females were also placed in the

cage to allow the females to lay their eggs in the ovitrap. The oviposition period for the female adults was 4 x 24 hours (Blanford et al. 2012). The experiment was designed as a completely randomized design with 6 isolates and the control used as treatments and repeated three times; the female adults were given a 10% sucrose solution for their diet. The ovitrap containing the eggs was removed from the cage daily, and the number of eggs laid was also counted daily. Viable (hatched) eggs were monitored and recorded every 2 hours for 48 hours. Changes in egg morphology were also recorded daily. The unhatched eggs (abortion) were cultured in an SDA medium to detect and identify the microorganism causing the unhatched eggs. The emergence of larvae was observed daily, and the number of dead larvae was counted daily; dead pupae were also monitored daily until adults emerged.

Bioassay of fungal pathogenicity on *Aedes albopictus* larvae

The pathogenicity of fungal isolates to *Ae. albopictus* larvae were tested following the method of Alkhaibari et al. (2017). The 30 larvae of the thirdth instar were treated with 10 mL suspension (1 x 10^{10} conidia mL^{-1}) of entomopathogenic fungal isolate. The fungal suspension was placed in a disinfected transparent plastic cup (Ø 7 cm, height 9 cm) with 100 mL of water inside. Only 10 mL of sterile water was given to 30 larvae for the control. Larvae were exposed to the fungal suspension for 1 x 24 hours. The dead larvae were then monitored and recorded daily for 8 days. The experiment was designed as a completely randomized design with 6 isolates and the control, with all treatments replicated three times. The variables observed were the number of larval deaths and the morphological changes in larvae after treatment with fungi. Daily observations were also made of the time of larval death and the behavior of unhealthy larvae. By observing changes in larval behavior and morphology, the health of the larvae could be determined. Therefore, to determine the lethal time (LT_{50} and LT_{95}), the time of larval death was used. The corpses were cultured in an SDA medium to determine fungal infection and whether the fungus originated from the corpses.

Bioassay of fungal pathogenicity on *Aedes albopictus* adults

The bioassay to determine the pathogenicity of fungal isolates to *Ae. albopictus* adults were tested following the method of Shoukat et al. (2020). Thirty adults of three-day-old females and males of *Ae. albopictus* (1:1 ratio) per isolate were treated with fungal suspension (1 x 10^{10} conidia mL^{-1}) for 24 hours. The inner wall of disinfected transparent plastic cage (30 x 30 x 30 cm) was sprayed with 10 mL of fungal suspension. The cage was then air-dried for 2 hours (Mnyone et al. 2011). Next, 10 mL of water was sprayed onto the cage's inner wall for the control cage. The 10% sucrose solution diet was suspended from the top of the cage for the adults. Furthermore, all treatments were repeated three times using a completely randomized design. The number of dead adults was recorded 24 hours after fungal exposure and continuing until 7 days had elapsed. The dead adults had no signs of movement and were in a state of death (Shoukat et al. 2020). Daily observations were also made of the time of death of adults and the behavior of unhealthy adults; the time of adult death was used to determine the lethal time (LT_{50} and LT_{95}). Furthermore, to determine fungal infection and to confirm whether the fungus originated from the corpses, the corpses or dead adult was cultured in SDA medium.

Data analysis

Data on egg, larval, pupal, and adult mortality of *Ae. albopictus*, LT_{50}, and LT_{95} of larvae in each treatment were analyzed using analysis of variance (ANOVA). Parametric statistical analysis was used, so all data were tested for normal distribution using the Shapiro-Wilk test and for homogeneity of variance using Levene's test. The eggs laid were logarithmically transformed to homogeneous variance before being subjected to a one-way analysis of variance. An arcsin transformation was performed to homogenize the variance for egg, larval, pupal, and adult mortality. Data means were compared using Tukey's Honestly Significant (HSD) at a 5% significance level. P values have been added to the description of the results to clarify that the statements in the results section are based on a statistical procedure. SAS University Edition 2.7 9.4 M5 software was used for all statistical analyses. The malformations on *Ae. albopictus* immature stages and adults infected by the fungus were photographed.

RESULTS AND DISCUSSION

Fungal pathogenicity on *Aedes albopictus* eggs

The developmental time of all instar larvae ($p<0.0001$) of *Ae. albopictus* treated with entomopathogenic fungi (1 x 10^{10} conidia mL^{-1}) was significantly longer than the control (untreated fungus) (Table 1). In addition, treatments with the fungi significantly prolonged the stadia of *Ae. albopictus* egg and pupae ($P<0.0001$) (Table 2). However, the adult longevity of control *Ae. albopictus* was longer ($p<0.0001$) compared to other treatments (treated fungi). Consequently, the lifespan of *Ae. albopictus* control was significantly longer ($p<0.0001$) among other treatments. Nevertheless, there was a non-significant difference ($p=0.28$) in the sex ratio of *Ae. albopictus* adults of treated and untreated fungus.

Survival ability of *Ae. albopictus* pupae on control was the highest among other treatments treated with fungi. The lowest pupal survival ability was occurred in the treatment with *M. anisopliae* MSwTp3 isolate (Figure 1). The survival ability of *Ae. albopictus* adults in the control were also found to be highest among other treatments treated with fungi (Figure 2). Furthermore, *Ae. albopictus* adult longevity in the control was longer, so the adults were the longest searching and finding their hosts.

The effect of eggs treated with entomopathogenic fungi (1 x 10^{10} conidia mL^{-1}) on the egg laid, the egg, larval, and pupal mortality of *Ae. albopictus* was significantly different from those of control (Table 3). Eggs on ovitrap after 96

hours by 30 gravid females of *Ae. albopictus* treated with *T. diversus* MSwTp1 isolate were the least (981.33 eggs) ($p<0.0001$) as compared to other treatments, and this treatment was not significantly different from those of other treated fungi. However, all fungal treatments were significantly different from those of control. *Ae. albopictus* egg mortality in the control was the lowest (18.08%) and significantly different from that in the fungal treatments ($p<0.0001$). The treated egg (entomopathogenic fungi 1 x 10^{10} conidia mL^{-1}) also affected the survival next stages of *Ae. albopictus*, such as the emerging larvae, pupae, and adults. Larvae from the treated eggs showed significantly higher mortality ($p<0.0001$) than the control. Unemerged pupae and adults from the treated eggs were significantly higher ($p<0.0001$) than those of control. The highest unemerged pupae and adults were observed by *M. anisopliae* MSwTp3 isolate among other treatments ($p<0.0001$), but it was not significantly different from those by *B. bassiana* BSwTd4 isolate. The entomopathogenic fungi not only killed *Ae. albopictus* eggs, but also its next stages, such as larvae, pupae, and adults. The most pathogenic fungi for *Ae. albopictus* eggs were *M. anisopliae* MSwTp3 isolate, and *B. bassiana* BSwTd4 isolate.

The morphology of *Ae. albopictus* eggs infected by entomopathogenic fungi differed from that of healthy control eggs (Figure 3). An eggshell of the infected eggs was covered with white or greenish-white mycelia depending on the fungal species infecting the eggs. The infected eggs were shriveled and dry and generally empty inside. On the contrary, the mycelia did not cover the healthy eggs of the control. The unhatched healthy eggs were still flawless and filled with fluid.

Table 1. Length of different developmental times of egg and instar larvae of *Aedes albopictus* treated with entomopathogenic fungi (1 x 10^{10} conidia mL^{-1})

Fungal species	Isolates code	Length of different developmental time (days)				
		Egg	1st instar	2nd instar	3rd instar	4th instar
Control	-	1.94±0.03^{d}	2.06±0.05^{d}	2.95±0.17^{b}	2.77±0.14^{d}	3.08±0.05^{d}
Beauveria bassiana	TaAlPA	2.70±0.02^{bc}	4.38±0.05^{abc}	4.51±0.04^{a}	4.24±0.03^{bc}	4.22±0.01^{bc}
Beauveria bassiana	TaLmME	2.98±0.04^{a}	4.48±0.10^{ab}	4.58±0.03^{a}	4.61±0.03^{ab}	4.43±0.03^{ab}
Penicillum citrinum	BKbTp	2.86±0.03^{ab}	4.26±0.06^{bc}	4.28±0.04^{a}	4.16±0.06^{c}	4.11±0.02^{c}
Talaromyces diversus	MSwTp1	2.60±0.04^{c}	4.09±0.05^{c}	4.23±0.03^{a}	4.19±0.04^{bc}	4.03±0.03^{c}
Beauveria bassiana	BSwTd4	3.00±0.02^{a}	4.60±0.02^{ab}	4.65±0.03^{a}	4.58±0.02^{abc}	4.38±0.05^{ab}
Metarhizium anisopliae	MSwTp3	3.02±0.03^{a}	4.67±0.02^{a}	4.68±0.04^{a}	4.69±0.04^{a}	4.58±0.04^{a}
F-value		115.70*	201.80*	42.25*	63.09*	139.80*
P-value		4.10×10^{-11}	8.99×10^{-13}	3.52×10^{-8}	2.48×10^{-9}	1.12×10^{-11}
HSD value		0.05	0.08	0.11	0.10	0.05

Note: *: significantly different; values within a column followed by the same letters were not significantly different at $P < 0.05$ according to Tukey's HSD test. Original data were transformed using the square root before statistical analysis

Table 2. Length of pupal stadia and adult longevity, sex ratio, and total lifespan of *Aedes albopictus* treated with entomopathogenic fungi (1 x 10^{10} conidia mL^{-1})

Fungal species	Isolates code	Pupal stadia (days)	Adult longevity (days)	Total of developmental time (days)	Sex ratio
Control	-	2.33±0.27^{b}	28.67±0.27^{a}	43.80±0.52^{a}	0.43±0.02
Beauveria bassiana	TaAlPA	4.67±0.27^{a}	7.00±0.47^{b}	31.72±0.52^{bc}	0.31±0.07
Beauveria bassiana	TaLmME	5.00±0.47^{a}	6.33±0.27^{b}	32.41±0.71^{bc}	0.41±0.00
Penicillum citrinum	BKbTp	4.33±0.27^{a}	7.00±0.47^{b}	31.00±0.31^{bc}	0.44±0.04
Talaromyces diversus	MSwTp1	4.00±0.00^{a}	7.67±0.27^{b}	30.79±0.30^{c}	0.42±0.06
Beauveria bassiana	BSwTd4	5.33±0.27^{a}	6.67±0.72^{b}	33.21±0.44^{bc}	0.50±0.03
Metarhizium anisopliae	MSwTp3	5.67±0.27^{a}	6.33±0.27^{b}	33.64±0.45^{b}	0.47±0.01
F-value		11.05*	116.10*	53.75*	1.40^{ns}
P-value		1.27×10^{-4}	4.01×10^{-11}	7.21×10^{-9}	0.28
HSD value		0.39	0.46	0.24	0.13

Note: ns: not significantly different *: significantly different; values within a column followed by the same letters were not significantly different at $P < 0.05$ according to Tukey's HSD test. Original data were transformed using the square root before statistical analysis

Fungal pathogenicity on *Aedes albopictus* larvae

30 larvae of third instar of *Ae. albopictus* treated with 10 mL suspension (1 x 10^{10} conidia mL^{-1}) of the entomopathogenic fungal isolate had significantly higher mortality ($p<0.0001$) than control (Table 4). *M. anisopliae* MSwTp3 isolate induced the highest larval mortality rate (100% with LT_{50} 2.68 days and LT_{95} 8.45 days). This treatment was significantly different from those of other fungal species, namely *B. bassiana* TaAlPA (74.44% with LT_{50} 3.99 days and LT_{95} 9.77 days), TaLmME (85.56% with LT_{50} 4.13 days and LT_{95} 9.91 days), and BSwTd4 isolates (93.33% with LT_{50} 3.84 days and LT_{95} 9.61 days); *P. citrinum* BKbTp isolate (75.56% with LT_{50} 5.03 days and LT_{95} 10.81 days); and *T. diversus* MSwTp1 isolate (75.56% with LT_{50} 5.03 days and LT_{95} 10.81 days). This indicates that *M. anisopliae* MSwTp3 isolate was more pathogenic to *Ae. albopictus* larvae. However, five other fungal isolates used in the present research were also found to be pathogenic against *Ae. albopictus* larvae. These findings highlighted that *M. anisopliae* (MSwTp3 isolate) and the five other fungal isolates had larvicidal activity against *Ae. albopictus* larvae.

The fungus-infected larvae of *Ae. albopictus* showed specific morphology characteristics, especially morphology of unhealthy and dead larvae. The fungus-infected larvae showed lysis of the gut lumen, which turned white, and the larval abdomen was not clearly segmented. The epithelial lining was milky, and a ruptured anal segment was found (Figure 4). Conidia and mycelia were produced by the larval corpses when they were cultured on an SDA medium. While, the healthy control larvae possessed a transparent gut lumen with a recognizably different abdomen segment, and larvae also had a visible epithelial lining and an intact anal segment. The fungi did not infect the healthy larvae of control; no conidia or mycelia were found on the body of the corpses. Pupae became sick and died when they emerged from infected larvae. The bodies of the sick pupae were thinner, hardened, and straight; they had a black head. At the same time, the healthy pupae became bigger and fatter. The body was round, flexible soft, and bent like a comma shape. The head color was dark-brown (Figure 5).

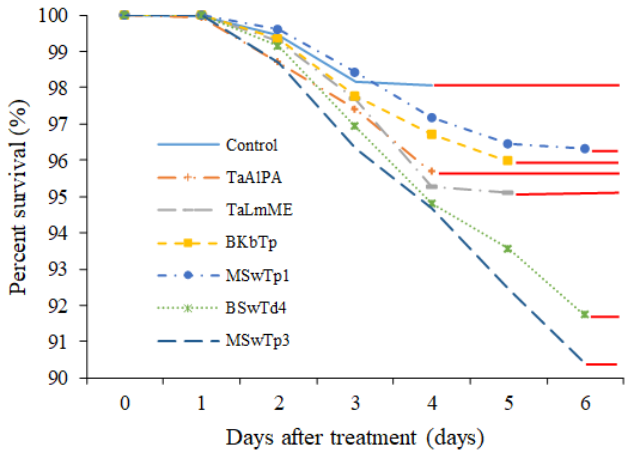

Figure 1. Survival ability of *Aedes albopictus* pupae: The pupae transformed into adults

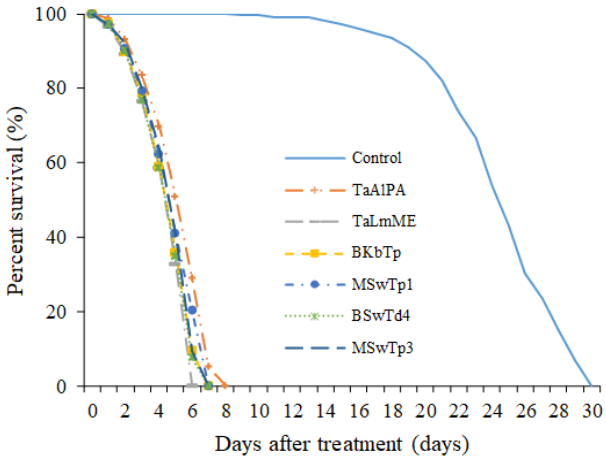

Figure 2. Survival ability of *Aedes albopictus* adults

Table 3. Effect of eggs treated with entomopathogenic fungi (1 x 10^{10} conidia mL^{-1}) on the egg laid, the egg, larval, and pupal mortality of *Aedes albopictus*

Fungal species	Isolates code	Eggs laid per female per 30 females [a)]	Egg mortality (%)[b)]	Larval mortality (%)[b)]	Unemerged	
					Pupae (%)[b)]	Adult (%)[b]
Control	-	1175.33 ± 11.06^{a}	18.08 ± 0.41^{c}	2.23 ± 0.15^{c}	1.11 ± 0.15^{c}	4,23± 0.20^{d}
Beauveria bassiana	TaAlPA	1000.67 ± 14.18^{b}	32.33 ± 0.69^{b}	25.43 ± 0.21^{b}	3.77 ± 0.16^{b}	11,25± 0.46^{c}
Beauveria bassiana	TaLmME	997.67 ± 3.47^{b}	34.02 ± 0.66^{b}	31.56 ± 1.28^{a}	4.98 ± 0.23^{a}	12,93± 0.36^{bc}
Penicillum citrinum	BKbTp	1007.00 ± 2.36^{b}	31.51 ± 0.86^{b}	24.61 ± 0.61^{b}	3.91 ± 0.19^{b}	11,42± 0.82^{c}
Talaromyces diversus	MSwTp1	981.33 ± 10.75^{b}	30.87 ± 0.31^{b}	23.92 ± 0.11^{b}	3.67 ± 0.19^{b}	11,78± 0.63^{c}
Beauveria bassiana	BSwTd4	1003.33 ± 3.78^{b}	38.91 ± 0.55^{a}	34.10 ± 1.40^{a}	5.12 ± 0.14^{a}	15.82± 0.53^{ab}
Metarhizium anisopliae	MSwTp3	1000.00 ± 4.50^{b}	39.07 ± 0.37^{a}	36.28 ± 0.65^{a}	5.33 ± 0.17^{a}	16.57± 0.74^{a}
F-value		39.55*	57.35*	246.00*	52.62*	43.25*
P-value		5.43×10^{-8}	4.69×10^{-9}	2.29×10^{-13}	8.30×10^{-9}	3.03×10^{-8}
HSD value		0.02	2.10	2.96	1.67	2.93

Note: *: significantly different; values within a column followed by the same letters were not significantly different at $P<0.05$ according to Tukey's HSD test, [a)]Original data were transformed using logarithmic transformation, [b)]Original data were transformed using Arcsin transformation before statistical analysis. Egg mortality data were corrected using the Abbott formula

Fungal pathogenicity on *Aedes albopictus* adults

Thirty adults of three-day-old females and males of *Ae. albopictus* treated with the fungal suspension (1 x 10^{10} conidia mL^{-1}) for 24 hours had significantly higher mortality rate ($p<0.0001$) than untreated control (Table 5). The highest adult mortality was recorded by *M. anisopliae* MSwTp3 isolate (75.56% with LT_{50} 3.36 days and LT_{95} 7.90 days). However, *M. anisopliae* treatment was not significantly different from those of other fungal species/isolates. Therefore, all fungal species/isolates used in the present study were pathogenic against *Ae. albopictus* adults. The findings highlighted that *M. anisopliae*, *B. bassiana*, *P. citrinum*, and *T. diversus* had insecticidal activity against *Ae. albopictus* adults.

When the dead fungal-infected adults and corpses were grown in the SDA medium they were covered with the conidia and mycelia of entomopathogenic fungi (Figure 6). The infected adults had asymmetrical wing shapes and a curled proboscis. The adults underwent mycosis in the abdomen and thorax and had a hard and stiff abdomen and thorax. Otherwise, the healthy control adults had symmetrical wing shapes and a straight black proboscis. There was no mycosis in the corpse's body. The abdomen of the healthy adults was elongated. Healthy adult corpses grown in an SDA medium were not covered with fungal conidia and mycelia of the entomopathogenic fungi.

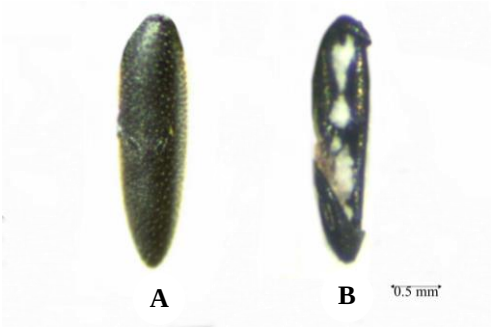

Figure 3. Morphology of *Aedes albopictus* eggs: healthy egg of control (A) and infected treated egg (B)

Table 4. Effect of larvae treated with entomopathogenic fungi (1 x 10^{10} conidia mL^{-1}) on larval mortality, LT_{50} and LT_{95} of *Aedes albopictus*

Fungal species	Isolates code	Larvae mortality (%)[a)]	LT_{50} (days)[b)]	LT_{95} (days)[b)]
Control	-	0.00 ± 0.00^{e}	17.69 ± 0.56^{a}	23.46 ± 0.77^{a}
Beauveria bassiana	TaAlPA	74.44 ± 0.91^{cd}	3.99 ± 0.20^{bc}	9.77 ± 0.41^{b}
Beauveria bassiana	TaLmME	85.56 ± 3.27^{bc}	4.13 ± 0.14^{bc}	9.91 ± 0.35^{b}
Penicillum citrinum	BKbTp	75.56 ± 2.40^{cd}	5.03 ± 0.07^{b}	10.81 ± 0.24^{b}
Talaromyces diversus	MSwTp1	64.44 ± 0.91^{d}	4.04 ± 0.11^{bc}	9.82 ± 0.22^{b}
Beauveria bassiana	BSwTd4	93.33 ± 1.57^{b}	3.84 ± 0.60^{bc}	9.61 ± 0.81^{b}
Metarhizium anisopliae	MSwTp3	100.00 ± 0.00^{a}	2.68 ± 0.11^{c}	8.45 ± 0.32^{b}
F-value		245.00^{*}	109.60^{*}	58.90^{*}
P-value		2.63×10^{-13}	5.94×10^{-11}	3.93×10^{-9}
HSD value		8.62	0.40	0.42

Note: *: significantly different; values within a column followed by the same letters were not significantly different at $P < 0.05$ according to Tukey's HSD test, [a)]Original data were transformed using Arcsin transformation before statistical analysis, [b)] Original data were transformed using square root transformation before statistical analysis

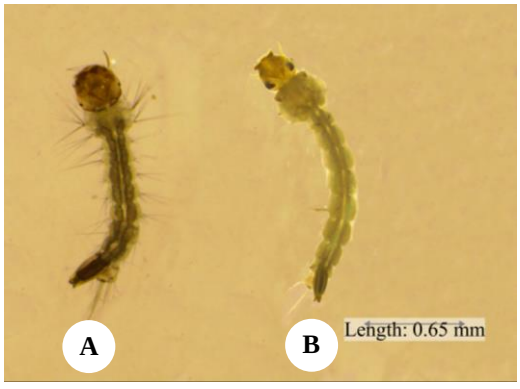

Figure 4. Morphology of *Aedes albopictus* larvae: healthy larvae of control (A) and infected treated larvae (B)

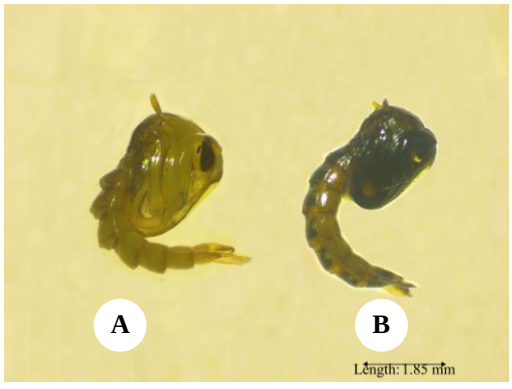

Figure 5. Morphology of *Aedes albopictus* pupae: healthy pupae of control (A) and infected treated pupae (B)

Discussion

The development of *Ae. albopictus* was affected by *B. bassiana* and *M. anisoplae*. Developmental time of instar larvae of *Ae. albopictus* treated with the entomopathogenic fungi was longer than the control (untreated fungus). However, fungi significantly shortened the egg, pupal stadia, and adult longevity. The entomopathogenic fungi from South Sumatra in present study could prolong larval stadia. Fungi reduce the conversion of digested and ingested food, stimulating the larvae to develop more slowly (Hussain et al. 2009). Similar results were obtained in *B. bassiana* and *Purpureocillium lilacinum*, they prolonged the developmental time of instar larvae of *Helicoverpa zea* (Lopez and Sword 2015), and also *B. bassiana* and *M. anisopliae* exhibited slower larval development of *Spodoptera frugiperda* (Lestari et al. 2022). The larval stadia and reduced adult longevity are prolonged and related to challenges between disease and insect immune system (Kalvnadi et al. 2018). The entomopathogenic fungi decreased *Ae.albopictus* adult longevity so that the duration of the adult searching and finding their hosts could be shorter in the field than the control adults.

The entomopathogenic fungi could decrease the eggs laid by females of *Ae. albopictus*. The fungi also killed the eggs and increased egg mortality. The effect of eggs treated with entomopathogenic fungi continued to affect the survival next stages of *Ae. albopictus* (the larval, pupal, and adult stages). The eggs that survived the fungal infection could produce larvae, but some of them were sick and mycosis. Furthermore, some pupae and adults produced from fungal-treated eggs also became sick and showed mycosis. *M. anisopliae* could induce the eggs of *Ae. aegypti* unhatched. Moreover, the hatched eggs could produce fungal-infected larvae (Leles et al. 2012). The results of the present study showed that the most pathogenic fungi to *Ae. albopictus* eggs were *M. anisopliae* MSwTp3 isolate, and *B. bassiana* BSwTd4 isolate. The morphology of *Ae. albopictus* eggs infected by the fungi had an eggshell covered with white or greenish-white mycelia. The infected eggs were flawed, shriveled, dry, and generally empty inside. Ramayanti et al. (2023) also reported similar morphology of eggs found on *Ae. aegypti* infected by entomopathogenic fungi. The empty and dry inside the eggs because the fungi absorb host insect fluids (Gabarty et al. 2014).

The third instar larvae of *Ae. albopictus* treated with *M. anisopliae* MSwTp3 isolate had the highest mortality (100%) and the shortest LT_{50} (2.68 days). However, the other fungal species, such as *B. bassiana*, *P. citrinum*, *T. diversus* were also found pathogenic against *Ae. albopictus* larvae. These findings highlighted that *M. anisopliae* and the other fungal species (*B. bassiana*, *P. citrinum*, and *T. diversus*) had larvicidal activity against *Ae. albopictus* larvae. The mortality rate of larvae treated with fungi in the present research was higher than in previous researches (Ramayanti et al. 2022; 2023) because broth fungal culture enriched with liquid compost was used in this study. The liquid compost can increase the fungal pathogenicity (Suwandi et al. 2018). In addition, the broth fungal culture could also increase the fungal pathogenicity due to blastospores producing fungi. The blastospores are more effective in killing mosquitoes than aerial conidia from a solid medium (Alkhaibari et al. 2017).

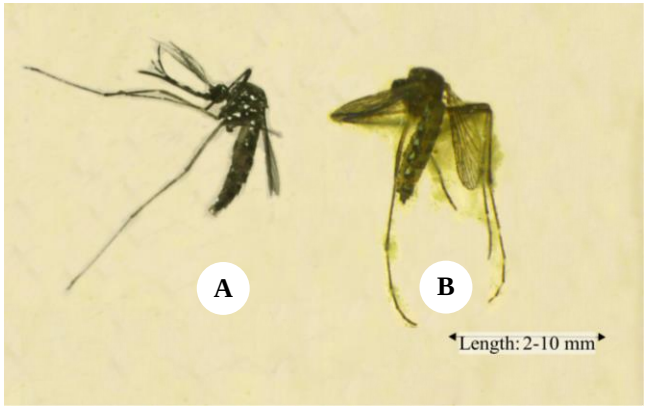

Figure 6. Morphology of *Aedes albopictus* adults: a healthy adult of control (A) and infected treated adult (B)

Table 5. Effect of adults treated with entomopathogenic fungi (1 x 10^{10} conidia mL^{-1}) on adult mortality, LT_{50} and LT_{95} of *Aedes albopictus*

Fungal species	Isolates code	Adult mortality (%)[a)]	LT_{50} (days)[b)]	LT_{95} (days)[b)]
Control	-	0.00 ± 0.00^{b}	13.07 ± 2.42^{a}	17.61 ± 3.13^{a}
Beauveria bassiana	TaAlPA	51.11 ± 2.40^{a}	4.08 ± 0.30^{b}	8.96 ± 0.73^{b}
Beauveria bassiana	TaLmME	65.56 ± 0.91^{a}	4.76 ± 0.12^{b}	9.95 ± 0.67^{ab}
Penicillum citrinum	BKbTp	48.89 ± 0.91^{a}	5.40 ± 0.27^{b}	9.30 ± 0.45^{b}
Talaromyces diversus	MSwTp1	44.44 ± 0.91^{a}	4.43 ± 0.18^{b}	8.97 ± 0.54^{b}
Beauveria bassiana	BSwTd4	70.00 ± 2.72^{a}	4.37 ± 0.38^{b}	8.92 ± 1.09^{b}
Metarhizium anisopliae	MSwTp3	75.56 ± 0.91^{a}	3.36 ± 0.07^{b}	7.90 ± 0.60^{b}
F-value		24.76*	12.60*	4.52*
P-value		1.08×10^{-6}	6.11×10^{-5}	94.10×10^{-2}
HSD value		26.68	0.79	1.02

Note: *: significantly different; values within a column followed by the same letters were not significantly different at $P < 0.05$ according to Tukey's HSD test, [a)]Original data were transformed using Arcsin transformation before statistical analysis, [b)]Original data were transformed using square root transformation before statistical analysis

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Similar to our findings, the mortality of *Ae. aegypti* larvae treated with entomopathogenic fungi were higher than their egg mortality (Ramayanti et al. 2023). The larvae mortality of the mosquitoes is higher than the egg mortality because the larval cuticle of the integument is thinner than those of the eggshell (Farnesi et al. 2015). The thinner the insect's cuticle, the easier it is for the fungus to penetrate its body (Ortiz-Urquiza and Keyhani 2013).

The results revealed that *Ae. albopictus* larvae infected with entomopathogenic fungi had abnormal morphology. The gut lumen of fungus-infected larvae was ruptured. The color of lysis gut lumen turned white. Therefore the larval abdomen was not clearly segmented. The larvae also had an epithelial lining with milky color and a ruptured anal segment. The conidia and mycelia of the fungi cover the infected larvae that eventually became corpses. The abnormal morphology of *Ae. albopictus* in the present research was similar to the morphology of *Ae. aegypti* larvae infected with *B. bassiana* (Ramayanti et al. 2023).

Ae. albopictus adults infected by the fungi could have asymmetrical wing shapes and a curled proboscis, and the mycosis occurred on the abdomen and thorax. Similar to this current finding, *Ae. aegypti* adults also underwent mycosis and had abnormal morphology after being treated with entomopathogenic fungi (Ramayanti et al. 2023). The fungus treated insect died due to the hyphal penetration into the insect body and poisoning by secondary metabolites produced by the fungi (Mancillas-Paredes et al. 2019). Moreover, the insect's body showed mycosis because the fungus absorbs the body fluids as it grows, and fungal conidia and mycelia cover the corpse (Gabarty et al. 2014).

In conclusion, the present finding showed that entomopathogenic fungi had a negative effect on the development of *Ae. albopictus*. The fungus can decrease the eggs laid by females of *Ae. albopictus* and shorten adult longevity. The most pathogenic fungal species to the eggs, larvae, pupae, and adults of *Ae. albopictus* was *M. anisopliae*. However, *B. bassiana*, *P. citrinum*, and *T. diversus* were also found pathogenic to the eggs, larvae, pupae, and adults of *Ae. albopictus*. The treated eggs (entomopathogenic fungi) continuously affect the survival next stages of *Ae. albopictus* (the larval, pupal, and adult stages). These findings highlighted that *M. anisopliae*, *B. bassiana*, *P. citrinum*, and *T. diversus* had insecticidal activity against *Ae. albopictus* eggs, larvae, pupae, and adults. Further investigation is needed to develop these fungal species into ovicides, larvicides, and adulticides to control *Ae. albopictus*.

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