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First report of entomopathogenic fungi from South Sumatra (Indonesia): pathogenicity to egg, larvae, and adult of *Culex quinquefasciatus*

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Abstract. *Ramayanti I, Herlinda S, Muslim A, Hasyim H. 2022. First report of entomopathogenic fungi from South Sumatra (Indonesia): pathogenicity to egg, larvae, and adult of* Culex quinquefasciatus. *Biodiversitas 23: 5695-5702.* Mosquito control has currently used many biocontrol agents, such as entomopathogenic fungi. So, the study aimed to determine the pathogenicity of the entomopathogenic fungi from South Sumatra to the eggs, larvae, and adults of *Culex quinquefasciatus.* The fungal isolates used were eight isolates from South Sumatra and have been identified molecularly. The fungal species that were the most pathogenic to the eggs of *Cx. quinquefasciatus* were *Beauveria bassiana* (BSwTd4, TaLmME, TaPsBA isolates), *Metarhizium anisopliae* (MSwTp3 isolate), *Penicillium citrinum* (BKbTp isolate), and *Talaromyces diversus* (MSwTp1 isolate). The *Cx. quinquefasciatus* eggs infected with the fungus not only could kill the eggs but also could continue to kill the emerging larvae, pupae, and adult. This is the first record that *B. bassiana*, *M. anisopliae*, *P. citrinum*, and *T. diversus* from South Sumatera Indonesia were pathogenic to the eggs of *Cx. quinquefasciatus* and had ovicidal activity. The most pathogenic fungal species to the larvae of *Cx. quinquefasciatus* were *M. anisopliae* (MSwTp3 isolate) and *B. bassiana* (BSwTd4 and TaLmME isolates). Three species of entomopathogenic fungi that were the most pathogenic to the adults of *Cx. quinquefasciatus* were *M. anisopliae* (MSwTp3 isolate), *B. bassiana* (BSwTd4 and TaLmME isolates), and *P. citrinum* (BKbTp isolate). Finally, The entomopathogenic fungi from South Sumatra have the negative effect on *Cx. quinquefasciatus* growth. The entomopathogenic fungi from South Sumatra have potential to be developed as the ovicide, larvicide, and adulticide. The future application of the fungi against the mosquito eggs, larvae, and adults can be used an ovitrap, fungal spores formulated in a synthetic oil, and fungus-impregnated black cloths, respectively.

Keywords: *Beauveria bassiana, Metarhizium anisopliae, Penicillium citrinum, Talaromyces diversus, Purpureocillium lilacinum*

INTRODUCTION

Filariasis or elephantiasis or lymphatic filariasis is an infectious disease caused by the parasitic filarial worms, such as *Wuchereria bancrofti* (Pratiwi et al. 2019) and *Brugia* sp. (Intarapuk and Bhumiratana 2021). This worm is transmitted by vector insects of the mosquitoes, especially *Culex* (Blut 2013). There are more than 38 species of mosquitoes that act as vectors of filariasis transmission (Famakinde 2018), including *Culex quinquefasciatus* (Simonsen and Mwakitalu 2013; Susilowati 2018), *Culex vishnui* (Nchoutpouen et al. 2019), *Mansonia africana* and *Mansonia unifo* (Ughasi et al. 2012). Indonesia is an endemic area for lymphatic filariasis (Ginandjar et al. 2018), especially in South Sumatra (Nurjazuli and Santjaka 2020). The consequences of this elephantiasis disease can cause physical disability, mental, social, and financial losses (Enciso et al. 2021). The higher population density of vector insects tends to be positively correlated with the higher transmission rate of this filarial worms and the higher the number of elephantiasis sufferers (Gordon et al. 2018; Ridha et al. 2020; Santoso et al. 2021).

To suppress and break the transmission of the lymphatic filariasis, the vector insects of transmission needs to be controlled and the population suppressed as low as possible so that the chain of transmission is broken. Some methods have been carried out to reduce the population density of filariasis vector insects. For example, *Cx. quinquefasciatus* has been controlled using a repellent insecticide (Aguiar et al. 2015). Control with botanical insecticides has also been carried out, for example the use of rosmarin leaf oil (*Rosmarinus officinalis* L) to kill the larvae of *Cx. quinquefasciatus* (Susilowati 2018). These vector insects are generally controlled with synthetic insecticides (Nchoutpouen et al. 2019). However, routinely spraying synthetic insecticides causes the new problems due to the higher level of *Cx. quinquefasciatus* resistance and it has been reported that this mosquito is resistant to permethrin, deltamethrin, DDT (dichloro-diphenyl-trichloroethane) (Nchoutpouen et al. 2019), and bendiocarb (Talipouo et al. 2021). Residues of the synthetic insecticides may cause the non-target animals killed, and

the insecticides induce the human health problems and the pollution on water, air, and soil (Hamid et al. 2017). The use of synthetic insecticides also causes the high operational costs for application or spraying (Chowański et al. 2014).

Currently, mosquito control has used many biocontrol agents, for example, entomopathogenic fungi (pathogens that cause insect disease or insect pathology). The use of entomopathogenic fungi has occurred, for example in Thailand, conidia of *Penicillium citrinum* has been found to be effective in killing the larvae of *Cx. quinquefasciatus* (Maketon et al. 2014). In India, the mycelia extract of *Beauveria bassiana* has been found to be effective in killing larvae of *Cx. quinquefasciatus* (Vivekanandhan et al. 2018). The entomopathogenic fungi found in Indonesia, especially in South Sumatra, have been tested and effectively killed some species of insect pests attacking plants (Herlinda et al. 2020a, 2020b; Gustianingtyas et al. 2021; Herlinda et al. 2021). Although many species of entomopathogenic fungi have been found in South Sumatra, there is no information on the effectiveness of these entomopathogenic fungi to kill the filariasis vector mosquito, *Cx. quinquefasciatus*. The previous study is only the pathogenicity of the entomopathogenic fungi to kill the egg, larvae, and adult of *Aedes aegypti* (Ramayanti et al. 2022). The novelty of this research is that the entomopathogenic fungi from South Sumatra and were first tested to kill eggs, larvae, and adults of *Cx. quinquefasciatus*. This research is feasible because the fungi as potential biological control agents are specific strains from South Sumatra so that they do not disturb the natural balance of existing microorganisms and *Cx. quinquefasciatus* is the main vector of filariasis which needs to be controlled. The purpose of this study was to determine the pathogenicity of the entomopathogenic fungi from South Sumatra to the eggs, larvae, and adults of *Cx. Quinquefasciatus*.

MATERIALS AND METHODS

Fungal preparation

The fungal isolates used for this current research were from the collection of the Laboratory of Entomology, Department of Plant Protection, Faculty of Agriculture, Universitas Sriwijaya, and they were identified molecularly. The fungal species identified were *B. bassiana* TaAlPA isolate (GenBank acc. no. OM791688), *B. bassiana* LtKrLH isolate (GenBank acc. no. OM791680), *B. bassiana* TaLmME isolate (GenBank acc. no. OM791687), and *B. bassiana* TaPsBA isolate (GenBank acc. no. OM791689) (Ramayanti et al. 2022), *P. citrinum* BKbTp isolate (GenBank acc. no. MT448730), *Talaromyces diversus* MSwTp1 isolate (GenBank acc. no. MT448731), *B. bassiana* BSwTd4 isolate (GenBank acc. no. MT448732), and *Metarhizium anisopliae* MT488733 isolate (GenBank acc. no. MT488733) (Herlinda et al. 2020a) (Table 1). The fungi originated from South Sumatra, Indonesia, with location Alang-alang Lebar, Palembang (2°56′32″S 104°42′16″E), Kota Raya, Lahat (3°46′38″S 103°35′25″E), Lebak, Muara Enim (3°23′51″S 104°19′41″E), Purwosari, Banyuasin (2°52′19″S 104°33′14″E), Talang Patai, Pagar Alam (4°00′45″S 103°12′21″E) and Talang Dabok, Ogan Komering Ilir (3°00′18″S 104°40′05″E). All fungal isolates were cultured on the agar medium, Sabouraud Dextrose Agar (SDA), and for bioassay they were also re-cultured on the liquid medium, SDB (Sabouraud Dextrose Broth).

Mass-rearing of *Culex quinquefasciatus*

Eggs of *Cx. quinquefasciatus* were gained from P2B2 Research and Development Loka, the Health Research and Development Center (the Balitbangkes), the Ministry of Health of Indonesia in Baturaja, South Sumatra, and *Cx. quinquefasciatus* mass-rearing has been carried out since June 2013. The *Cx. quinquefasciatus* mass-rearing for bioassay was carried out at the Laboratory of Entomology, Faculty of Agriculture, Universitas Sriwijaya. The room temperature and relative humidity during the mass-rearing were 29 ± 1°C and 84 ± 1%, respectively. The room lighting was set to photoperiod with 12 hours of light and 12 hours of dark (Kauffman et al. 2017). The emerging larvae were kept into a transparent plastic cup (Ø 7 cm, height 9 cm) that was disinfected, and the cup was filled in 50 mL of water (Ramayanti et al. 2022). The larvae were fed with dog biscuits (Vivekanandhan et al. 2018).

Table 1. Origin of the isolates of entomopathogenic fungi from South Sumatra, Indonesia, used in this research

Location, village or district/city	Isolate origin	Altitude (m)	Fungal species	Fungal isolate code	GenBank acc no.
Alang-alang Lebar, Palembang	Soil	23.0	*Beauveria bassiana*	TaAlPA	OM791688*
Kota Raya, Lahat	Insect	369.9	*Beauveria bassiana*	LtKrLH	OM791680*
Lebak, Muara Enim	Soil	33.5	*Beauveria bassiana*	TaLmME	OM791687*
Purwosari, Banyuasin	Soil	19.0	*Beauveria bassiana*	TaPsBA	OM791689*
Talang Patai, Pagar Alam	Soil	175.0	*Penicillium citrinum*	BKbTp	MT448730**
Talang Dabok, Ogan Komering Ilir	Soil	24.0	*Talaromyces diversus*	MSwTp1	MT448731**
Talang Patai, Pagar Alam	Soil	193.0	*Beauveria bassiana*	BSwTd4	MT448732**
Talang Patai, Pagar Alam	Soil	193.0	*Metarhizium anisopliae*	MSwTp3	MT488733**

Note: *)Ramayanti et al. (2022), **)Herlinda et al. (2020a)

The larvae within the plastic cup were put into a disinfected transparent plastic cage (50 x 50 x 50 cm) in order to keep the emerging adults remaining in the cage. The 10% sucrose solution infused on cotton wool for adult diet was hung on the top of the cage. Then, an ovitrap was put in the plastic cage where the adult mosquitoes were emerged from the pupae. The ovitrap was designed as a disinfected transparent plastic cup (Ø 9 cm, height 13 cm) that had dark wall and was filled with water to a depth of 10 cm (Wu et al. 2013).

The bioassay of fungal pathogenicity to egg, larvae, and adult of *Culex quinquefasciatus*

The bioassay to determine the pathogenicity of isolates of the entomopathogenic fungi from South Sumatra to the eggs, larvae, and adults of *Cx. quinquefasciatus* was carried out at the laboratory with the average temperature and the relative humidity, 29.79°C and 84.11%, respectively. After the fungal isolates were cultured on the SDA medium, then the fungal cultures were re-grown on the SDB medium in order to increase the fungal conidial density (Gustianingtyas et al. 2020). During the process of growing the fungal culture on the SDB medium (the liquid medium) for 14 days, the culture was shaken continuously for 7 days and then not shaken for 7 days. The conidia harvested from the liquid medium were calculated for getting conidial density used for bioassay below.

The bioassay to determine the pathogenicity of isolates of the entomopathogenic fungi from South Sumatra to the eggs of *Cx. quinquefasciatus* was carried out following the method of Luz et al. (2011). The liquid fungal culture with a concentration of 1 x 10^{10} conidia mL^{-1} was poured 10 mL into the ovitrap containing 100 mL of water. At the same time, the control was only poured 10 mL of sterile distilled water. This experiment was designed using a completely randomized design with 8 isolates and control as treatments. The experiment was repeated three times. Then, the ovitrap was put in the disinfected plastic cage, and 30 gravid female adults were also put in the cage so that the female adults could lay their eggs in the ovitrap. The duration for the female adults laying their eggs was 4 x 24 hours (Blanford et al. 2012). The female adults were also provided with 10% sucrose solution for their diet. The ovitrap containing the eggs laid was replaced daily from the cage, and then the number of eggs laid was also counted every day. The viable eggs (the hatched eggs) were monitored and recorded every 2 hours for 48 hours. The egg morphology changing were also recorded daily. The abortion or unhatched eggs were cultured in SDA medium in order to detect and to identify the microorganism that caused abortion or unhatched. The larvae emerging were observed daily and the dead larvae were counted every day. The dead pupae were also monitored every day until adult emerging.

The bioassay to determine the pathogenicity of isolates of the entomopathogenic fungi from South Sumatra to the larvae of *Cx. quinquefasciatus* was carried out following the method of Alkhaibari et al. (2017). The 30 third-instar larvae were treated with 10 mL suspension of the entomopathogenic fungal isolate, the fungal suspension was put in a disinfected transparent plastic cups (Ø 7 cm, height 9 cm) with 100 mL of water inside. The 30 control larvae were only exposed to 10 mL of sterile water. All treatments were replicated three times and the experiment was designed using a completely randomized design with 8 isolates and control as treatments. The duration of fungal exposure to the larvae was 1 x 24 hours, and then the dead larvae were monitored and recorded daily for 8 days. The variables observed were the number of larval deaths and the morphology changes of larvae after being treated with the fungi. The time of larval death and the behavior of unhealthy larvae were also observed every day. The health of the larvae identified by observing the changes of the larvae behavior and morphology. The time of larval death was used to determine LT_{50} (the Lethal Time) and LT_{95}. The cadaver or dead larvae were cultured in SDA medium to determine the fungal infection and to confirm whether the fungus emerged from the cadavers.

The bioassay to determine the pathogenicity of isolates of the entomopathogenic fungi from South Sumatra to the adults of *Cx. quinquefasciatus* was carried out following the method of Blanford et al. (2012) and Shoukat et al. (2020). Fifteen female and 15 male adults (total of 30 adults) per isolate were treated with the fungal suspension for 24 hours. The adults used in this experiment were 3 days old. Ten mL of the fungal suspension (1 x 10^{10} conidia mL^{-1}) were sprayed on the inner wall of disinfected transparent plastic cage (50 × 50 × 50 cm). Then, the cage was air-dried for 2 hours (Mnyone et al. 2011). Then, 30 adults were put in the plastic cage. For the plastic cage of control, ten mL of water was sprayed on the inner wall of the cage. All treatments were repeated three times using a completely randomized design. The adults of fungal treatment and control were given the diet of 10% sucrose solution hang on the top of the cage. After fungal exposure for 24 hours. The number of dead adults was started to be recorded after 24 hours of fungal exposure until 7 days after exposure. The dead adults were characterized with no movement occurred (Shoukat et al. 2020). The time of adult death and the behavior of unhealthy adults were also observed daily. The time of adult death was used to determine of LT_{50} and LT_{95}. The cadaver or dead adult was cultured in SDA medium to determine the fungal infection and to confirm whether the fungus emerged from the cadavers.

Data analysis

The data of egg, larval, and pupal mortality of *Cx. quinquefasciatus*, LT_{50} and LT_{95} of the larvae; adult mortality, LT_{50} and LT_{95} of *Cx. quinquefasciatus* of each treatment were analyzed using ANOVA (analysis of variance). We implemented the parametric statistical analysis, and therefore all data were tested for normal distribution using the Shapiro-Wilk test and for variance homogeneity by Levene's test. Logarithmic transformation was performed to homogenous variance for the eggs laid before being subjected to one-way analyses of variance. Arcsin transformation was performed to homogenous variance for the egg, larval, pupal, adult mortality. The mean of the data was compared using Tukey's Honestly

Significant (HSD) at a 5% level of significance. To make a clear understanding that the statements under results section based on a statistical procedure, P values have been added to the result description. All statistical analyses were calculated using software of SAS University Edition 2.7 9.4 M5. The malformation of eggs, larvae, pupae, and adults of *Cx. quinquefasciatus* infected by the fungus were presented in photograph.

RESULTS AND DISCUSSION

The bioassay of fungal pathogenicity to egg of *Culex quinquefasciatus*

Obtained findings reported that eggs laid on the ovitrap by the gravid *Cx. quinquefasciatus* female of control (untreated fungal) was the least (1469.67 eggs/female per 96 hours) among those of fungal treatments. Egg mortality of *Cx. quinquefasciatus* of control was the lowest (16.76%) and significantly different from those of fungal treatments (Table 2). All isolates used were pathogenic to the eggs of *Cx. quinquefasciatus*. Egg mortality of *Cx. quinquefasciatus* caused by *B. bassiana* isolate BSwTd4 was the highest (39.94%) and was not significantly different from those caused by *B. bassiana* isolate TaLmME (38.86%), and *M. anisopliae* isolate MSwTp3 (38.75%), *B. bassiana* isolate TaPsBA (36.91%), *P. citrinum* isolate BKbTp (37.04%), and *T. diversus* isolate MSwTp1(35.66%). Thus, the most pathogenic fungal species against eggs of *Cx. quinquefasciatus* were *B. bassiana* (BSwTd4, TaLmME, TaPsBA isolates), *M. anisopliae* (MSwTp3 isolate), *P. citrinum* (BKbTp isolate), and *T. diversus* (MSwTp1 isolate). The effect of eggs treated with the fungus still affected the emerging larvae, the larvae resulted higher mortality than those of controls, as well as the pupae from treated eggs produced higher mortality than those of controls.

The morphology of *Cx. quinquefasciatus* eggs infected with the entomopathogenic fungi showed differences from the healthy eggs from the control group. The body liquid of *Cx. quinquefasciatus* infected eggs had feculent color without embryo inside, while the healthy eggs from the control group had clearly visible color with embryo inside. The color of anterior and posterior infected eggs was darker than those of the healthy eggs.

The bioassay of fungal pathogenicity to larvae of *Culex quinquefasciatus*

The third-instar larvae of *Cx. quinquefasciatus* treated with the entomopathogenic fungi (1 x 10^{10} conidia mL^{-1}) had significantly higher mortality compared to the control larvae (untreated larvae) (Table 3). All fungal isolates used in the current study were pathogenic to the third-instar larvae of *Cx. quinquefasciatus* due to the mortality caused by the fungi being more than 60%. The larval mortality caused by *M. anisopliae* isolate MSwTp3 (100% with LT_{50} 2.02 days and LT_{95} 7.15 days) was the highest and not significantly different from mortality caused by *B. bassiana* isolate BSwTd4 (98.89% with LT_{50} 2.51 days and LT_{95} 7.61 days) and *B. bassiana* isolate TaLmME (97.78% with LT_{50} 2.75 days and LT_{95} 7.85 days).

The most pathogenic fungal species to the third-instar larvae of *Cx. quinquefasciatus* were *M. anisopliae* (MSwTp3 isolate) and *B. bassiana* (BSwTd4 and TaLmME isolates). Obtained findings highlighted that *M. anisopliae* (MSwTp3 isolate) and *B. bassiana* (BSwTd4 and TaLmME isolates) possessed larvicidal activity.

The fungal infected larvae of *Cx. quinquefasciatus* showed typical morphology, particularly the morphology of sick and dead larvae. The sick larvae underwent lysis of the gut lumen with white color and the larvae abdomen had no distinct segment. The epithelial lining possessed milky color with a ruptured anal segment (Figure 2). The healthy larvae of control had a transparent gut lumen with a visible or recognizably different segment of abdomen. The healthy larvae also had a visible epithelial lining and an undamaged anal segment. The larval cadavers of fungal treatment cultured on SDA medium produced conidia and mycelia covering the cadaver body, while the healthy larvae were not infected or covered by fungal conidia or mycelia. The pupae emerging from the infected larvae generally became sick and died. The sick pupae body were thinner, hardened, and straight shaped and had the black head. The healthy pupae had fatter, round, flexible and soft body, bent like a comma shape and their head had dark-brown in color (Figure 3).

The bioassay of fungal pathogenicity to adult of *Culex quinquefasciatus*

The adults of *Cx. quinquefasciatus* treated with the entomopathogenic fungi (1 x 10^{10} conidia mL^{-1}) had significantly higher mortality compared to the control adults (untreated adults) (Table 4). The adult mortality caused by *M. anisopliae* isolate MSwTp3 (100% with LT_{50} 3.25 days and LT_{95} 6.70 days) was highest and not significantly different from those caused by *B. bassiana* isolate BSwTd4 (100% with LT_{50} 3.46 days and LT_{95} 6.76 days) and *B. bassiana* isolate TaLmME (98.89% with LT_{50} 3.70 days and LT_{95} 7.15 days), and *P. citrinum* isolate BKbTp (98.89% with LT_{50} 3.96 days and LT_{95} 7.41 days). Nevertheless, all fungal isolates in this study were pathogenic to the adults of *Cx. quinquefasciatus* due to the mortality caused by the fungi being more than 60%. The most pathogenic fungal species to the adults of *Cx. quinquefasciatus* were *M. anisopliae* (MSwTp3 isolate), *B. bassiana* (BSwTd4 and TaLmME isolates), and *P. citrinum* (BKbTp isolate). This research findings highlighted that *M. anisopliae* (MSwTp3 isolate), *B. bassiana* (BSwTd4 and TaLmME isolates), and *P. citrinum* (BKbTp isolate) had adulticidal activity.

The adults of *Cx. quinquefasciatus* treated with the entomopathogenic fungi became sick and finally died. The typical symptoms of sick and dead infected adults were malformation. The unhealthy adults had the asymmetrical wing shapes, the dried and mycosis body, and a spiral shape proboscis (Figure 4).

Table 2. 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 *Culex quinquefasciatus*

Species	Isolate code	Eggs laid per female per 96 hours [a)]	Egg mortality (%)[b)]	Larval mortality (%)[b)]	Pupal mortality (%)[b)]
Control	-	1469.67±14.46^{b}	16.76±0.82^{d}	17.59±0.11^{d}	0.99±0.15^{d}
Beauveria bassiana	TaAlPA	1511.00±11.09^{ab}	32.70±0.71^{bc}	33.33±0.45^{c}	2.86±0.14^{c}
Beauveria bassiana	LtKrLH	1482.67±13.74^{b}	31.06±0.42^{c}	30.58±0.55^{d}	2.60±0.06^{c}
Beauveria bassiana	TaLmME	1616.67±9.48^{a}	38.86±0.23^{a}	40.02±0.12^{a}	5.06±0.12^{ab}
Beauveria bassiana	TaPsBA	1574.33±15.59^{ab}	36.91±0.25^{ab}	35.23±0.37^{c}	3.37±0.06^{c}
Penicillum citrinum	BKbTp	1556.33±26.22^{ab}	37.04±1.17^{ab}	37.72±0.31^{b}	3.75±0.32^{bc}
Talaromyces diversus	MSwTp1	1563.67±26.02^{ab}	35.66±0.95^{abc}	34.41±0.28^{c}	3.17±0.12^{c}
Beauveria bassiana	BSwTd4	1637.33±3.60^{a}	39.94±0.08^{a}	41.20±0.28^{a}	6.31±0.49^{a}
Metarhizium anisopliae	MSwTp3	1613.33±29.58^{a}	38.75±1.26^{a}	40.15±0.02^{a}	5.49±0.12^{a}
F-value		6.20*	63.5*	345.2*	45.41*
P-value		6.50 x 10^{-4}	1.29 x 10^{-11}	2.0 x 10^{-16}	2.25 x 10^{-10}
HSD value		0.04	2.98	1.94	1.96

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 prior to statistical analysis

Table 3. Effect of larvae treated with entomopathogenic fungi (1 x 10^{10} conidia mL^{-1}) on larval mortality, LT_{50} and LT_{95} of *Culex quinquefasciatus*

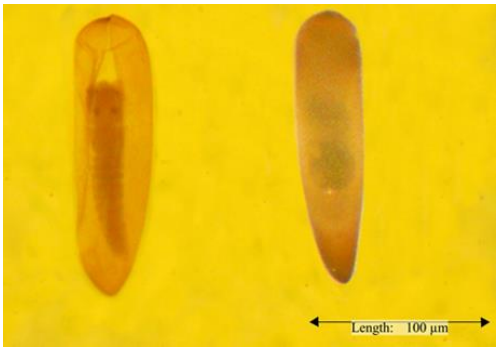
Species	Isolate code	Larvae mortality (%)[a)]	LT_{50} (days)[b)]	LT_{95} (days)[b)]
Control	-	0.00±0.00^{f}	14.98±0.43^{a}	20.21±0.51^{a}
Beauveria bassiana	TaAlPA	84.44±2.40^{cd}	3.97±0.16^{b}	9.08±0.28^{bc}
Beauveria bassiana	LtKrLH	78.89±2.40^{de}	4.21±0.17^{b}	9.31±0.28^{bc}
Beauveria bassiana	TaLmME	97.78±0.91^{ab}	2.75±0.07^{cd}	7.85±0.15^{cd}
Beauveria bassiana	TaPsBA	80.00±3.14^{cde}	4.05±0.11^{b}	9.15±0.12^{bc}
Penicillum citrinum	BKbTp	92.22±0.91^{bc}	3.78±0.51^{bc}	8.88±0.62^{bcd}
Talaromyces diversus	MSwTp1	64.44±0.91^{e}	5.04±0.15^{b}	10.14±0.26^{b}
Beauveria bassiana	BSwTd4	98.89±0.91^{a}	2.51±0.10^{d}	7.61±0.21^{cd}
Metarhizium anisopliae	MSwTp3	100.00±0.00^{a}	2.02±0.07^{d}	7.15±0.07^{d}
F-value		155.00*	116.60*	79.77*
P-value		5.31 x 10^{-15}	6.52 x 10^{-14}	1.80 x 10^{-12}
HSD value		10.62	0.33	0.30

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 prior to statistical analysis, [b)] Original data were transformed using square root (sqrt) transformation.

Table 4. Effect of adults treated with entomopathogenic fungi (1 x 10^{10} conidia mL^{-1}) on adult mortality, LT_{50} and LT_{95} of *Culex quinquefasciatus*

Species	Isolate code	Adult mortality (%)[a)]	LT_{50} (days)[b)]	LT_{95} (days)[b)]
Control	-	0.00±0.00^{d}	11.99±0.40^{a}	15.44±0.42^{a}
Beauveria bassiana	TaAlPA	88.89±1.81^{b}	4.64±0.03^{c}	8.09±0.06^{bc}
Beauveria bassiana	LtKrLH	82.22±0.91^{b}	4.84±0.02^{bc}	8.29±0.02^{b}
Beauveria bassiana	TaLmME	98.89±0.91^{a}	3.70±0.04^{de}	7.15±0.06^{d}
Beauveria bassiana	TaPsBA	87.78±0.91^{b}	4.63±0.01^{c}	8.08±0.03^{bc}
Penicillum citrinum	BKbTp	98.89±0.91^{a}	3.96±0.05^{d}	7.41±0.06^{cd}
Talaromyces diversus	MSwTp1	63.33±1.57^{c}	5.37±0.06^{b}	8.82±0.09^{b}
Beauveria bassiana	BSwTd4	100.00±0.00^{a}	3.46±0.10^{de}	6.76±0.21^{d}
Metarhizium anisopliae	MSwTp3	100.00±0.00^{a}	3.25±0.10^{e}	6.70±0.10^{d}
F-value		23.11*	229.30*	183.60*
P-value		5.85 x 10^{-8}	2.00 x 10^{-16}	1.19 x 10^{-15}
HSD value		24.55	0.15	0.15

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 prior to statistical analysis, [b)]Original data were transformed using square root (sqrt) transformation.



A B

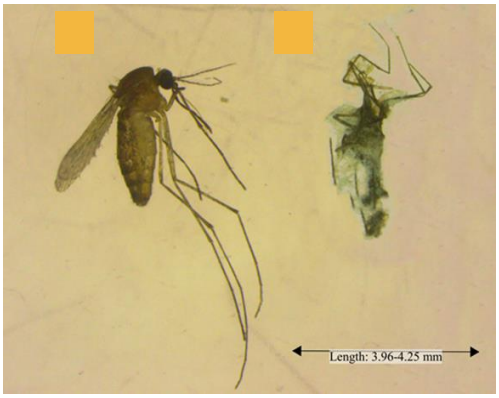
Figure 1. Morphology of the *Culex quinquefasciatus* eggs: a healthy egg of control (A) and an infected treated egg (B)

A B

Figure 2. Morphology of the *Culex quinquefasciatus* larvae: a healthy larvae of control (A) and an infected treated larvae (B)

A B

Figure 3. Morphology of the *Culex quinquefasciatus* pupae: a healthy pupae of control (A) and an infected treated pupae (B)



A B

Figure 4. Morphology of the *Cx. quinquefasciatus* adults: a healthy adult of control (A) and an infected treated adult (B)

If the unhealthy adults died, their cadavers grown in SDA medium could be covered with the fungal conidia and mycelia. On the contrary, the healthy adults of control possessed the symmetrical wing shapes, and a straight shaped black proboscis, and no mycosis on the cadaver body. The healthy adults had the elongate abdomen. The cadavers from the healthy adults grown in SDA medium were not covered with the fungal conidia and mycelia.

Discussion

The eggs laid by the gravid *Cx. quinquefasciatus* female of control (untreated) were the least among those of fungal treatments. However, the eggs laid by the fungal treated female were more than those of control because the ovitrap where they were laying eggs had water with dyed color due to added with the fungal suspension. The gravid female *Culex* mosquitoes preferred to lay eggs in dyed water (Day 2016; Perea and Callaghan 2017). Although the treated eggs laid by female *Cx. quinquefasciatus* in this study were less than 50%, but the treated eggs induced the sick larvae and produced the mortality of emerging larvae and pupae. Besides, the mycosis on the dead larvae and pupae that failed to emerge from the eggs occurred. In this study, the ovitrap used to expose the fungi to the eggs of *Cx. quinquefasciatus* could effectively infected its eggs, larvae, pupae, and adults. The finding highlighted that the *Cx. quinquefasciatus* eggs infected with the fungus not only could kill the eggs but also could continue to kill the emerging larvae, pupae, and adult. The most pathogenic fungal species against the eggs of *Cx. quinquefasciatus* were *B. bassiana* (BSwTd4, TaLmME, TaPsBA isolates), *M. anisopliae* (MSwTp3 isolate), *P. citrinum* (BKbTp isolate), and *T. diversus* (MSwTp1 isolate). This is the first record that *B. bassiana*, *M. anisopliae*, *P. citrinum*, and *T. diversus* from South Sumatera Indonesia were pathogenic to the eggs of *Cx. quinquefasciatus* and had ovicidal activity. The entomopathogenic fungi have reported caused the eggs of mosquito unhatched and abortion and the emerging larvae and pupae could not carry on their life (Leles et al. 2012; Ramayanti et al. 2022). The obtained data also reported the embryo of eggs treated with the fungi were lysis and egg liquid becoming feculent color and no embryo inside the eggs.

The egg mortality of *Cx. quinquefasciatus* caused by the entomopathogenic fungi were lower than the larvae mortality caused by the fungi. The third-instar larvae of *Cx. quinquefasciatus* could be immediately killed by *M. anisopliae* (MSwTp3 isolate) and *B. bassiana* (BSwTd4 and TaLmME isolates) (LT_{50} < 3 days). The high mortality of the larvae treated with the entomopathogenic fungi in short time due to higher fungal suspension used (1 x 10^{10} conidia mL^{-1}) and the fungi cultured in the broth medium (SDB). The broth culture is able to grow the fungus and produce blastospores, and the blastospores are more effective compared to aerial conidia in killing the mosquitoes (Alkhaibari et al. 2017). The findings highlighted that both species of the fungi could be develop to be a larvicide for *Cx. quinquefasciatus* because they have highest level of larvicidal activity (97.78-100% of larvae mortality). The larvae mortality caused by the

entomopathogenic fungi was higher than the egg mortality caused by the entomopathogenic fungi because the cuticle of integument of the larvae is thinner than those of the eggs (Farnesi et al. 2015). The thinner was the cuticle of the insect integument, the easier the fungal conidia are penetrated into insect body (Ortiz-Urquiza and Keyhani 2013).

The results obtained that the larvae infected by the fungi could be sick or dead because their gut lumen ruptured or lysis by the fungi. The fungi also damaged the anal and abdomen segments so that the larva body became malformation. The larvae died due to the conidia of entomopathogenic fungi germinating and their hyphae penetrating into the insect integument, after that the hyphae entered to the body cavity (Boomsma et al. 2014). The hyphae developed to become blastospores in the larvae hemolymph (Mancillas-Paredes et al. 2019). The blastospores of the entomopathogenic fungi could produce secondary metabolites, such as bassiacridin (Quesada-moraga and Vey 2004) and beauvericin (Safavi 2012) secreted by *B. bassiana* and destruxin produced by *M. anisopliae* (Borisade et al. 2016). The secondary metabolites or toxin could disrupt normal cell metabolism of the insect (Mancillas-Paredes et al. 2019).

Three species of the entomopathogenic fungi that were the most pathogenic to the adults of *Cx. quinquefasciatus* were *M. anisopliae* (MSwTp3 isolate), *B. bassiana* (BSwTd4 and TaLmME isolates), and *P. citrinum* (BKbTp isolate). This research findings highlighted that besides *M. anisopliae* (MSwTp3 isolate) and *B. bassiana* (BSwTd4 and TaLmME isolates), *P. citrinum* (BKbTp isolate) was also pathogenic to the adults of *Cx. quinquefasciatus*. The results obtained that the fungal species that were pathogenic to adults differed from those that were pathogenic to eggs and larvae of *Cx. quinquefasciatus*. The fungal species that were pathogenic to the eggs of *Cx. quinquefasciatus* were *B. bassiana* (BSwTd4, TaLmME, TaPsBA isolates), *M. anisopliae* (MSwTp3 isolate), *P. citrinum* (BKbTp isolate), and *T. diversus* (MSwTp1 isolate), while the fungal species that were pathogenic to the larvae of *Cx. quinquefasciatus* were *M. anisopliae* (MSwTp3 isolate) and *B. bassiana* (BSwTd4 and TaLmME isolates). The entomopathogenic fungi could induce the adults of *Cx. quinquefasciatus* becoming malformation (e.g. asymmetrical wing, curled proboscis) and mycosis (after growing onto the SDA medium). The dead adults were caused by conidia germinating to be mycelia and penetrating into the adult body cavity producing secondary metabolites (via blastospores) disrupting normal cell metabolism of the insect (Mancillas-Paredes et al. 2019). After the adults died, the fungi still grow saprophytically on the cadavers of adults and the fungi induced the cadaver body to become mycosis (Gabarty et al. 2014). The future application of the fungi against the mosquito eggs, larvae, and adults can be used an ovitrap, fungal spores formulated in a synthetic oil, and fungus-impregnated black cloths, respectively.

The fungal species that were the most pathogenic to the eggs, larvae, and adults of *Cx. quinquefasciatus* were *B. bassiana* (BSwTd4 isolate), *M. anisopliae* (MSwTp3 isolate), and *P. citrinum* (BKbTp isolate), however *T. diversus* was also the most pathogenic to the eggs. This is the first record that *B. bassiana*, *M. anisopliae*, *P. citrinum*, and *T. diversus* from South Sumatera Indonesia were pathogenic to *Cx. quinquefasciatus*. So, the entomopathogenic fungi from South Sumatra negatively affect *Cx. quinquefasciatus* growth. The entomopathogenic fungi from South Sumatra have potential to be developed as the ovicide, larvicide, and adulticide.

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