Entomopathogenic fungi from South Sumatra (Indonesia) affecting development of Aedes aegypti

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Abstract. Entomopathogenic fungi can affect the mortality of adults, larvae, and eggs of Ae. aegypti. However, their effectiveness against the development of the mosquito has not been widely reported. This study evaluated the effect of entomopathogenic fungi from South Sumatra on the development of Ae. aegypti. Gravid I females of Ae. Aegypti, 11 isolates of Beauveria bassiana, and 4 separate isolates of Purpureocillium lilacinum, Talaromyces diversus, Penicillium citrinum, and Metarhizium anisopliae, respectively, were used for bioassays. These isolates could prolong the egg hatching time and increase the development of the first to fourth instar larval and pupae stages, shortened the adult longevity, and affected the mosquito's life cycle. The longest time to reach the hatching time (2.47 days) occured on the isolate MSwTp3 of M. anisopliae, which also was no significant differences in the length of the larval stages of first instar (3.63 days), second instar (4.59 days), the third instar (4.56 days), and the forth instar (4.60 days). The control pupae stage lasted 3.00 days and had a significant difference from the treated pupae, which reached 6.00 days, caused by M. anisopliae (isolate MSwTp3), T. diversus (isolate MSwTp1), P. citrinum (isolate BKbTp), and B. bassiana (isolates TaTsOI, TaAlPA, LtApPGA, LtKrLH, TaTtLH, TaLmMe , TaPsBA, and BSwTd4). The adult longevity of control was 31.00 days and differed significantly from the adult longevity treated with M. anisopliae (isolate MSwTp3) and P. citrinum (isolate BKbTp), which lasted only 4.33 days. The life cycle of control mosquitoes was 46.82 days, and significantly different from those treated with the fungi. The shortest life cycle caused by B. bassiana (isolate TaCjPGA) was 26.53 days. Finally, the entomopathogenic fungi from South Sumatra have negative effects on the development of Ae. aegypti.

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

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1. Introduction

Aedes aegypti is the most important mosquito because its role is as a vector of yellow fever dengue, and chikungunya viruses. The mosquito has spread in several provinces in Indonesia, such as Banjarmasin [1], Central Java [2], Jakarta [3], and South Sumatra [4]. Its spread caused the dengue, chikungunya, and yellow fever viruses transmitted rapidly [5]. The dengue could cause the the losses gaining several billion dollars per year [6]. To prevent the viruses becoming endemic [5], the vector need to be controlled.

Ae. aegypti is usually controlled by using synthetic insecticides due to fast action and easy application [7]. Nevertheless, the synthetic insecticides often caused Ae. aegypti resistant [1]. An alternative control that is eco-friendly is biological control using entomopathogenic fungi, such as Metarhizium anisopliae [8], Metarhizium brunneum [9], and Beauveria bassiana [10]. There is no information on the effect of entomopathogenic fungi from Indonesia on the development of Ae. aegypti. So that, the effect of entomopathogenic fungi on development of Ae. aegypti was evaluated.

2. Materials and method

2.1 Mass-rearing of Aedes aegypti

Ae. Aegypti eggs were gained from P2B2 Research and Development Loka, the Health Research and Development Center, the Ministry of Health of Indonesia in Baturaja, South Sumatra. The Ae. Aegypti cultures were incubated in a sterile and control room with 12:12 (L:D) h., 26 ± 1°C temperature, and 85 ± 10% RH using method of [11] at the Laboratory of Entomology, Faculty of Agriculture, Universitas Sriwijaya. The emerging larvae were fed with dog biscuits [12] and adults consumed 10% sucrose solution [13]. The newly emerged adults were kept in a cage with ovitrap inside. The eggs laid by the female adults were harvested daily for bioassay test.

2.2 Bioassay for assessing entomopathogenic fungal affecting the depelopment of Aedes aegypti

The fungal isolates used in this research were 15 isolates from collection of the the Laboratory of Entomology, Faculty of Agriculture, Universitas Sriwijaya. The fungal isolates consisted of 11 isolates of *B. basiana*, and 4 separate isolates of *Purpureocillium lilacinum*, *Talaromyces diversus*, *Penicillium citrinum*, and *M. anisopliae*, respectively. All isolates were identified molecularly and have been deposited in the GenBank. The fungi were cultured in SDB (Sabouraud Dextrose Broth) and were shaken for a week and then incubated at rest (not shaken) for a week as well. The treatments for this experiment were 15 fungal isolates and a control (steril water) and used a completely randomized design with three replications.

The bioassay was assessed by pouring 10 mL of a fungal suspension (1 x 10^{10} conidia/ml) into an ovitrap (a plastic cup containing 100 ml of water for adults laying eggs), while for the control only 10 mL of sterile distilled water was exposed to the ovitrap. Thirty gravid copulated female were placed in a cage with the ovitrap inside. The female adults were allowed to lay eggs for 4 x 24 hours. The ovitrap containing eggs was replaced from the cage and the eggs laid and the hatched eggs were counted. The other variables recorded were the

length of different developmental time of egg, the 1st instar, 2nd instar, and 4th instar. Length of pupal stadium and adult longevity, total of lifespan, and the sex of adults emerging were also recorded.

2.3 Data analysis

The data of the length of different developmental time of egg, the 1st instar, 2nd instar, and 4th instar, length of pupal stadium and adult longevity, total of lifespan, and the sex of adults emerging were analyzed using analysis of variance (ANOVA). Then, the analysis were followed by Tukey's Honestly Significant (HSD) at a 5% level of significance. The analysis were calculated using software of SAS University Edition 2.7 9.4 M5.

3. Results and Discussions

The result showed that all fungal isolates (11 isolates of *Beauveria basiana*, and 4 separate isolates of *P. lilacinum*, *T. diversus*, *P. citrinum*, and *M. anisopliae*, respectively) could prolong the egg hatching time significantly compared to the control (Table 1). The longest time to reach the hatching time, namely 2.47 days, was caused by the isolate MSwTp3 of *M. anisopliae*, which also made no significant differences in the length of the larval stages of first instar (3.63 days), second instar (4.59 days), the third instar (4.56 days), and the forth instar (4.60 days).

Table 1. Length of different developmental time of egg and instar larvae of *Aedes aegypti* treated with entomopathogenic fungi (1 x 10^{10} conidia/ml)

Species	Isolate	Length of different developmental time (days)				
		Egg	1st instar	2nd instar	3rd instar	4th instar
Control	-	1.95h	2.61h	2.88j	2.94h	2.44i
Beauveria basiana	LtTpOI	2.24def	3.13f	3.83g	3.84f	3.83h
Beauveria basiana	TaTsOI	2.21ef	3.21ef	4.07ef	4.06de	4.13def
Beauveria basiana	TaAlPA	2.23def	3.34cdef	4.14def	4.17cde	4.26cde
P. lilacinum	TaSkPA	2.21ef	2.88g	3.48h	3.80f	3.93fgh
Beauveria basiana	TaBrPGA	2.11g	3.18ef	4.05f	4.05e	4.11defg
Beauveria basiana	TaCjPGA	2.17fg	2.68gh	3.24i	3.54g	3.91gh
Beauveria basiana	LtApPGA	2.10g	3.23def	4.16def	4.03e	4.11defg
Beauveria basiana	LtKrLH	2.30de	3.43abcd	4.28bcd	4.26bc	4.32bcd
Beauveria basiana	TaTtLH	2.19fg	3.21ef	4.10ef	4.08de	4.10efg
Beauveria basiana	TaLmMe	2.32bcd	3.51abc	4.40b	4.42ab	4.45abc
Beauveria basiana	TaPsBA	2.30cd	3.37bcde	4.22cde	4.22cd	4.27cde
Penicillum citrinum	BKbTp	2.38abc	3.47abc	4.37bc	4.43ab	4.50abc
Talaromyces diversus	MSwTp1	2.41ab	3.53abc	4.36bc	4.47a	4.52ab
Beauveria basiana	BSwTd4	2.40ab	3.57ab	4.45ab	4.50a	4.55ab
Metarhizium anisopliae	MSwTp3	2.47a	3.63a	4.59a	4.56a	4.60a
F-value		18.09*	17.26*	69.27*	58.41*	50.62*
P-value		$1.63x10^{-11}$	$3.09 x10^{-11}$	$2 x10^{-16}$	$2 x10^{-16}$	$2 x10^{-16}$
HSD value		0.05	0.10	0.07	0.07	0.09

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

The fungal isolates increased the developmental time of the 1st to 4th instar larvae and pupae. However, the fungi shortened the adult longevity and the Ae. Aegypti life cycle (Table 2). The control pupae stage lasted 3.00 days and had a significant difference from the treated pupae, which reached 6.00 days caused by M. anisopliae (isolate MSwTp3), T. diversus (isolate MSwTp1), P. citrinum (isolate BKbTp), and B. bassiana (isolates TaTsOI, TaAlPA, LtApPGA, LtKrLH, TaTtLH, TaLmMe , TaPsBA, and BSwTd4). The longevity adults of control was 31.00 days and differed significantly from the treated adult longevity treated with M. anisopliae (isolate MSwTp3) and P. citrinum (isolate BKbTp), which had longevity of 4.33 days. The life cycle of control mosquitoes was 46.82 days, significantly longer from those treated with the fungi. The shortest life cycle in B. bassiana (isolate TaCjPGA) was 26.53 days. The adult longevity caused by M. anisopliae MSwTp3 isolate and P. citrinum BKbTp isolate was the shortest among other treatments. Meanwhile, the fungi did not affect the sex ratio of Ae. Aegypti. Finally, the entomopathogenic fungi from South Sumatra have negative effects on the development of Ae. aegypti.

These results highlighted that all fungal species could shorten the adult longevity and the Ae. aegypti life cycle. This demonstrated that the fungi were pathogenic to the Ae. aegypti. The eggs of Ae. aegypti that were infected by the fungi could produced the infected or sick larvae or the unhatched eggs or abortion eggs [14]. The infected or sick larvae could also produce infected or sick pupae and shorter adult longevity. The present study showed that M. anisopliae MSwTp3 isolate and P. citrinum BKbTp isolate were the most pathogenic to the Ae. aegypti adults and caused the shortest adult longevity. M. anisopliae could cause unhatched eggs and dead larvae of Ae. aegypti [14]. The entomopathogenic fungi could absorb the body fluids of the host insects [15] and make the insect hosts to become mycosis [16].

Table 2. Length of pupal and adult stadia, and total lifespan of Aedes aegypti treated with entomopathogenic fungi (1 x 1010 conidia/ml)

Species	Isolate	Length of stadia (days)			Sex ratio
		Pupae	Adult	Total of lifespan	
Control	-	3.00e	31.00a	46.82a	0.77
Beauveria basiana	LtTpOI	4.67c	6.00de	27.54d	0.87
Beauveria basiana	TaTsOI	6.00a	5.67def	29.34bc	0.78
Beauveria basiana	TaAlPA	6.00a	5.33efg	29.48bc	0.82
P. lilacinum	TaSkPA	5.33b	7.33b	28.97c	0.87
Beauveria basiana	TaBrPGA	5.67ab	6.33cd	29.50bc	0.84
Beauveria basiana	TaCjPGA	4.00d	7.00bc	26.53d	0.82
Beauveria basiana	LtApPGA	6.00a	5.67def	29.31bc	0.83
Beauveria basiana	LtKrLH	6.00a	5.00fgh	29.60bc	0.81
Beauveria basiana	TaTtLH	6.00a	6.00de	29.69bc	0.81
Beauveria basiana	TaLmMe	6.00a	4.67gh	29.78bc	0.84
Beauveria basiana	TaPsBA	6.00a	5.00fgh	29.39bc	0.78
Penicillum citrinum	BKbTp	6.00a	4.33h	29.49bc	0.78
Talaromyces diversus	MSwTp1	6.00a	5.00fgh	30.28b	0.79
Beauveria basiana	BSwTd4	6.00a	4.67gh	30.13b	0.75
Metarhizium anisopliae	MSwTp3	6.00a	4.33h	30.19b	0.76
F-value		21.47*	223.60*	106.20*	0.866ns
P-value		1.53×10^{-12}	2×10^{-16}	2×10^{-16}	0.612
HSD value		0.22	0.28	0.19	0.006

Note: ns = not significantly differen * = 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

4. Conclusions

The 11 isolates of *B. bassiana*, and 4 separate isolates of *P. lilacinum*, *T. diversus*, *P. citrinum*, and *Metarhizium anisopliae*, respectively could prolong the egg hatching time and increase the development of the 1st to 4th instar larval and pupae stages, shorten the adult longevity, and affect the mosquito's life cycle. Meanwhile, the sex ratio showed the the number of female mosquitoes was more than that of the males. Finally, the entomopathogenic fungi from South Sumatra have negative effects on the development of *Ae. aegypti*.

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