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EFFICACY OF *Bacillus thuringiensis* BIOLARVICIDE AND TEMEPHOS SYNTHETIC LARVICIDES ON *Culex quinquefasciatus* LARVAE

EFIKASI BIOLARVARSIDA *Bacillus thuringiensis* DAN LARVASIDA SINTETIS TEMEPHOS PADA LARVA *Culex quinquefasciatus*

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Abstract

An open environment with drainage and lush green grass around the hotel allows mosquitoes to breed. Mosquitoes from the genus *Culex* are one of the vectors for transmitting arboviruses and filariasis. Temephos is an active ingredient often used to control *Culex quinquefasciatus* and considered as environmental pollution. Therefore, it is necessary to develop environmentally friendly larvicides, such as the *Bacillus thuringiensis* biolarvicide. Many studies were conducted to control *Aedes aegypti* mosquito larvae using these microbial agents, but very little for controlling *Cx. quinquefasciatus* mosquito larvae. This study aims to compare the effectiveness of temephos and *B. thuringiensis* biolarvicides. *Cx. quinquefasciatus* larvae were divided into the insect sample group with temephos and the *B. thuringiensis* biolarvicide group at concentrations of 0.01, 0.02, and 0.03 mg/L. The number of dead larvae was calculated at 1, 2, 3, 4, 5, 6, and 24 hours. Data analysis was performed using probit analysis of lethal time (LT50 and LT90). From statistical analysis, *B. thuringiensis* as larvicides showed 100% mortality of mosquito larvae. *B. thuringiensis* biolarvicide can be used as a substitute for chemical larvicide since it is proven effective in killing *Cx. quinquefasciatus* mosquito larvae in 24 hours and is environmentally friendly.

Keywords: *B. thuringiensis*; Biolarvicide; *Cx. quinquefasciatus*; Temephos

Abstrak

Lingkungan terbuka dengan sistem pembuangan dan rumput yang hijau di sekitar hotel memungkinkan nyamuk berkembang biak. Nyamuk dari genus Culex adalah salah satu vektor yang mengirimkan arbovirus dan filariasis. Temephos adalah bahan aktif yang sering digunakan untuk mengendalikan Culex quinquefasciatus dan dianggap mencemari lingkungan. Oleh karena itu, perlu untuk mengembangkan larvasida yang ramah lingkungan, seperti Bacillus thuringiensis biolarvasida. Banyak penelitian yang dilakukan untuk mengendalikan larva nyamuk Aedes aegypti menggunakan agen mikroba ini, tetapi sangat sedikit untuk mengendalikan larva nyamuk Cx. quinquefasciatus. Penelitian ini bertujuan untuk membandingkan efektivitas temephos dan B. thuringiensis biolarvasida. Larva Cx. quinquefasciatus dibagi menjadi kelompok sampel serangga dengan temephos dan kelompok B. thuringiensis biolarvasida pada konsentrasi 0,01, 0,02, dan 0,03 mg/L. Jumlah larva yang mati dihitung pada 1, 2, 3, 4, 5, 6, dan 24 jam. Analisis data dilakukan analisis waktu letal probit (LT50 dan LT90). Analisis statistik, B. thuringiensis sebagai larvasida menunjukkan 100% kematian larva nyamuk. B. thuringiensis biolarvasida dapat digunakan sebagai pengganti larvasida kimia karena terbukti efektif dalam membunuh larva nyamuk Cx. quinquefasciatus dalam waktu 24 jam dan ramah lingkungan.

Kata kunci: *B. thuringiensis; Biolarvasida; Cx. quinquefasciatus; Temephos*

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INTRODUCTION

Mosquitoes are vector animals that are often found around us. An open environment with drainage and lush green grass around the hotel allows mosquitoes to breed. Mosquitoes of the *Culex* are one of the vectors that transmit arbovirus and filariasis (Ahdiyah & Purwani, 2015). Lymphatic filariasis is transmitted by different types of mosquitoes, for example by the *Culex* mosquito, widespread across urban and semi-urban areas (World Health Organization (WHO), 2023). According to the World Health Organization (WHO), in 2009, more than 1.3 billion people in 72 countries were affected by filariasis, with a prevalence of 65% in Southeast Asia, 30% in Africa, and 5% in other tropical regions (World Health Organization (WHO), 2010). These mosquitoes feed on carbon dioxide and are attracted to human body heat. Therefore, mosquitoes are often found outdoors, such as in parks.

Culex mosquitoes are nocturnal and have a maximum flight distance of 5 km (Webb et al., 2016). *Cx. quinquefasciatus* mosquitoes like dirty water, such as puddles, wastewater from bathing, sewage, and rivers full of garbage. They can also breed in all seasons. Eggs of *Cx. quinquefasciatus* are brown, long, and cylindrical and will develop into a larva in 8–12 days. Larvae breathe using a siphon and turn into pupae 2–3 days later.

The use of conventional insecticides is decreasing due to resistance, and the public is increasingly paying attention to the dangers of its use to health. Temephos is one of the chemical larvicides to control mosquitoes. It is considered as environmental pollution because of its dangerous chemical compounds and potentially cause resistance. In addition, using temephos can result in resistance from various species of mosquitoes, which are disease vectors (Nugroho, 2011). An environmentally friendly bio larvicide is needed to avoid the effects of chemical larvicides. *B. thuringiensis* is a positive, shaped, selective, non-toxic to other organisms, and environmentally friendly. It is used as a larvicide in water reservoirs and infects mosquito larvae within one hour of treatment. It can form endospores that produce protein crystals and work as a digestive toxin or poison for the larvae (Melanie et al., 2018).

B. thuringiensis, as a larvicide, is often used to control the *Aedes aegypti* mosquito. Research on the use of *B. thuringiensis* microbial agents against *Cx. quinquefasciatus* is still very little done. *Cx. quinquefasciatus* larvae are 2–4 times less sensitive than *Ae. Aegypti* in the same instar (Mahdalena & Ni'mah, 2019). The mode of action of *B. thuringiensis* poison is more unique than conventional insecticides. The proteolytically activated venom binds to receptors on the membrane of the insect midgut. After insertion into the membrane, pores are formed and cause swelling and cell lysis (Georghiou & Wirth, 1997). *B. thuringiensis* has an advantage over synthetic larvicides because of its high level of toxicity to the target organism. It became toxic to several lepidopteran, dipteran, or coleopteran species but safe to humans and other non-target organisms.

The success factor of this larvicidal test is the effectiveness of increasing the mortality of the mosquito larvae and its environmentally friendly nature. Based on the research conducted by Pei et al. (2002), it found that there was larval mortality due to *Bacillus sphaericus*. This suggests that it could be a promising alternative in managing resistance to *B. sphaericus* in *Cx. quinquefasciatus* larvae. In a study testing the effects of *B. thuringiensis* on *Culex pipiens* larvae, larval mortality was recorded at high LC50 concentrations calculated after 48 hours (Saliha et al., 2017). *B. thuringiensis* disrupted the fecundity and fertility of adult larvae derived from treated larvae. Many studies were conducted to control *Aedes aegypti* and *Cx. pipiens* mosquito larvae using these microbial agents, but very little for controlling *Cx. quinquefasciatus* mosquito larvae. Therefore, it also emphasizes more research to compare the effectiveness of temephos and *B. thuringiensis* in controlling *Cx. quinquefasciatus* mosquitoes, which are the vectors of filariasis. This study is expected to provide information on whether *B. thuringiensis* larvicide can be used as a replacement for chemical larvicides.

MATERIALS AND METHODS

Preparing Insect Sample

Samples of *Cx. quinquefasciatus* larvae were collected in sewage in a hotel in Pekalongan, Central Java Province, Indonesia.

Larvicide Effectiveness Test

This study used an experimental method with three repetitions of a randomized block design (RBD). *Cx. quinquefasciatus* larvae were divided into the insect sample group with temephos (abate 1%) at a concentration of 300 mg/L and the *B. thuringiensis* bio larvicide group at concentrations of 0.01, 0.02, and 0.03 mg/L. The exact number of samples represents each treatment group. Data was collected through observation and documentation. The number of dead larvae in each tub was calculated at 1, 2, 3, 4, 5, 6, and 24 hours. Dead larvae sink to the bottom, do not move, and do not respond to stimulation.

Data Analysis

Data analysis was performed using SPSS software with statistical analysis tests (One-Way Analysis of Variance (ANOVA)) and probit lethal time analysis (LT_{50} and LT_{90}). This process was carried out to determine whether the difference in treatment with temephos and *B. thuringiensis* bio larvicide significantly affected the mortality of *Cx. quinquefasciatus* mosquito larvae.

RESULTS

The observation of test mosquito larvae was repeated thrice at 1, 2, 3, 4, 5, 6, and 24 hours with temephos (abate 1%) at a concentration of 300 mg/L and the *B. thuringiensis* bio larvicide group at concentrations of 0.01, 0.02, and 0.03 mg/L. In the following, the observation and calculation results of *Cx. quinquefasciatus* larvae mortality are presented descriptively and statistically.

Table 1. Mortality percentage of Culex quinquefasciatus larvae on Bacillus thuringiensis biolarvicide and temephos larvicide

Treatment	Larvae mortality percentage (%) (mean ± standard deviation)						
	1 hour	2 hours	3 hours	4 hours	5 hours	6 hours	24 hours
Abate	26.6 ± 6.1	45.3 ± 12.8	65.3 ± 6.1	81.3 ± 16.6	89.3 ± 10	100 ± 0	100 ± 0
B. thuringiensis 0,01 mg/L	8 ± 4	16 ± 4	24 ± 4	32 ± 6.9	38.6 ± 2.3	48 ± 4	100 ± 0
B. thuringiensis 0,02 mg/L	16 ± 4	21.3 ± 2.3	28 ± 4	42.6 ± 4.6	53.3 ± 6.1	68 ± 10.5	100 ± 0
B. thuringiensis 0,03 mg/L	24 ± 4	32 ± 4	36 ± 9.4	56 ± 8	66.6 ± 6.1	81.3 ± 6.1	100 ± 0

Table 1 contains data on the percentage of *Cx. quinquefasciatus* larval mortality after 24 hours of observation at each concentration using temephos larvicide and *B. thuringiensis* biolarvicide, which were observed every 1, 2, 3, 4, 5, 6, and 24 hours. Based on the 24 hour observation, when using *B. thuringiensis* biolarvicide at a concentration of 0.01 mg/L for 6 hours, it can result in a larval mortality rate of 48%. At a concentration of 0.02 mg/L for 6 hours, the larval mortality rate is 68%. Furthermore, at a concentration of 0.03 mg/L for 6 hours, the larval mortality rate is 81%. As the concentration increases, the mortality of larvae also increases. Supported by research on *B. thuringiensis* against *Culex pipiens* larvae, the results indicate that *B. thuringiensis* has an impact on larval mortality based on concentration and exposure time. Low larvicidal activity was observed at a concentration of 23 mg/L. The effectiveness during the first hour showed that the *B. thuringiensis* biolarvicide killed 8–24% of the samples, and temephos killed 26.6%. Temephos at a concentration of 300 mg/L, killed all larval samples within 6 hours, while *B. thuringiensis* biolarvicide killed all samples within 24 hours. However, when using the *B. thuringiensis* biolarvicide at a concentration of 0.03 mg/L, it resulted in the highest percentage and worked faster than the other two groups of biolarvicides. The ANOVA test yielded a significance value of 0.00 ($P < 0.05$), indicating that there was an effect of *B. thuringiensis* larvicide concentrations on the mortality of *Cx. quinquefasciatus* larvae.

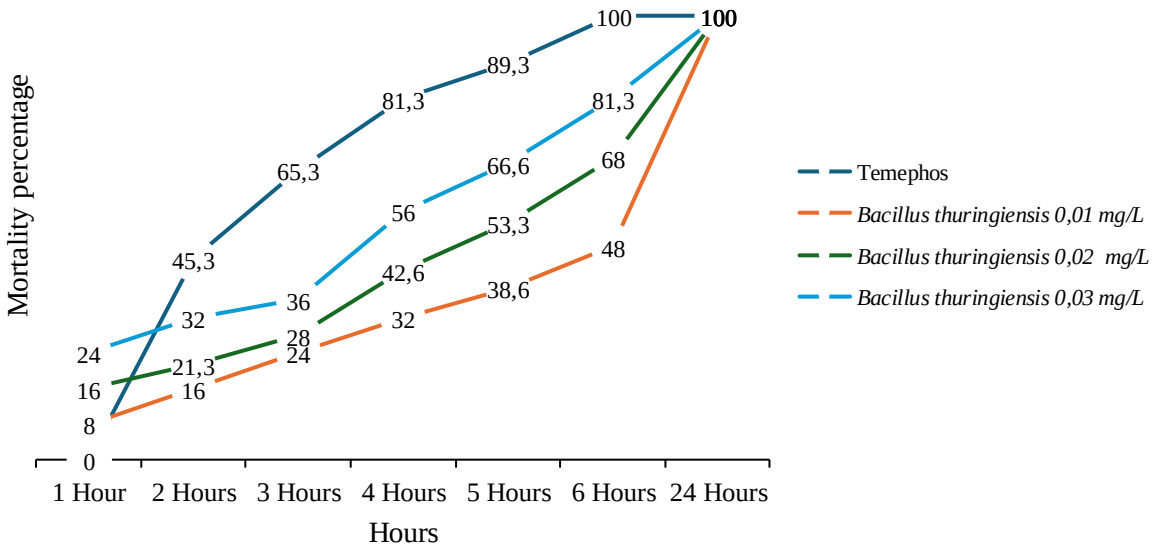

Figure 1. Mortality percentage of Culex quinquefasciatus larvae on Bacillus thuringiensis biolarvicide and temephos larvicide

Figure 1 shows the mortality of Cx. quinquefasciatus larvae from various concentrations experienced an increase in average with increasing concentrations. Therefore, the mortality of the larvae is directly proportional to the increase in concentration. Based on Figure 1, this study found that concentrations of 0.03 mg/L, 0.02 mg/L, and 0.01 mg/L are effective in achieving a larval mortality rate of 100%. Among the three concentration groups of B. thuringiensis biolarvicide, the one with a concentration of 0.03 mg/L yielded the highest percentage and acted more rapidly. Six hours of giving a concentration of 0.03 mg/L showed 81.3% mosquito mortality, 0.02 mg/L showed 68%, and 0.01 mg/L showed 48%. The results showed that the use of B. thuringiensis larvicides affected the mortality of Cx. quinquefasciatus above 90% 24 hours post contact at all concentration levels.

Table 2. Probit lethal time analysis of temephos dan Bacillus thuringiensis for Culex quinquefasciatus

Active ingredient	Lethal time	Estimate	Lower bound	Upper bound
Temephos	LT_{50}	2.26	1.643	2.743
	LT_{90}	4,81	4.193	5.864
Bacillus thuringiensis 0,01 mg/L	LT_{50}	6.000	4.953	8.732
	LT_{90}	11.009	8.429	19.192
Bacillus thuringiensis 0,02 mg/L	LT_{50}	4.648	3.929	5.804
	LT_{90}	5.804	7.225	13.006
Bacillus thuringiensis 0,03 mg/L	LT_{50}	3.618	2.881	4.401
	LT_{90}	7.808	6.412	11.029

The following are the results of the probit analysis to determine the LT_{50} and LT_{90} for each concentration. Table 2 shows the time to kill 50% and 90% of *Cx. quinquefasciatus* was shorter when using temephos than *B. thuringiensis*. The time needed by temephos to kill 50% of *Cx. quinquefasciatus* mosquito larvae were 2.26 hours and 4.81 hours for 90% of the samples. *B. thuringiensis* with a concentration of 0.01 mg/L killed 50% of the larvae for 6 hours and 90% for 11 hours. At a concentration of 0.02 mg/L, *B. thuringiensis* killed 50% of samples in 4.6 hours and 90% in 5.8 hours. At a concentration of 0.03 mg/L, it killed 50% of the samples in 3.6 hours and 90% in 7.8 hours.

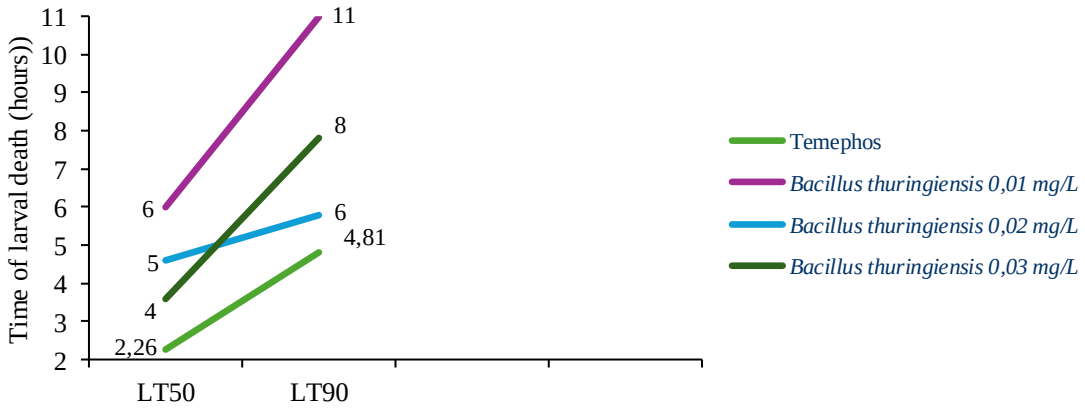

Figure 2. Probit charts LT50 and LT90 temephos and *Bacillus thuringiensis* for *Culex quinquefasciatus* for 24 hours

Based on Figure 2, the lower the LT_{50} and LT_{90} values, the less time it takes to kill the larvae. Therefore, the effectiveness of temephos larvicide was higher than *B. thuringiensis*. Decreasing LT_{50} and LT_{90} values in *Cx. quinquefasciatus* larvae correlated with the effectiveness of hourly Bti. *B. thuringiensis* at a concentration of 0.03 mg/L had lower LT_{50} and LT_{90} values than the other two concentrations. However, compared to temephos, it still has lower LT50 and LT90 values, which means it can kill all larvae samples in 4-5 hours. *B. thuringiensis* can only kill all larvae samples within 6–11 hours. *Cx. quinquefasciatus* larvae exposed to *B. thuringiensis* experienced decreased mortality and eventually died

DISCUSSION

The use of temephos is more effective and efficient. However, it has some drawbacks, such as not being environmentally friendly. The use of temephos can also cause resistance to larvicide. *B. thuringiensis* bio larvicide effectively kills *Cx. quinquefasciatus*, and is almost comparable to temephos larvicide, which can kill all mosquito larvae samples in 24 hours. The mortality of *Cx. quinquefasciatus* larvae increases with higher concentrations of treatment. The mortality is directly proportional to the concentration. Concentrations of 0.03 mg/L, 0.02 mg/L, and 0.01 mg/L were found effective in achieving a 100% larval mortality rate. Among these, 0.03 mg/L exhibited the highest and fastest mortality, reaching 81.3% in just six hours. The 0.02 mg/L concentration resulted in a 68% mortality, while the 0.01 mg/L concentration led to a 48% larval mortality. The freeze-dried, pure Cyt1A crystal powder from *B. thuringiensis* is toxic, producing respective 50% LC50 values of 11.332 mg/L (Kuppusamy & Ayyadurai, 2011).

Regarding a concentration of 83 mg/L, more than 80% of the larvae experienced mortality after 15 days of observation (Saliha et al., 2017). This result is in line with previous studies, which stated that the percentage of mosquito larvae mortality due to *B. thuringiensis* bio larvicide reached 90% at a concentration of 0.024 mg/L (Elqowiyya, 2015). *B. thuringiensis* produces protein crystals that are soluble and active in the alkaline environment of the larvae's gut. The protein crystals work as a digestive toxin for the larvae (Sihotang & Umniyati, 2018). In the target insect, the protein is activated by the insect's protein-digesting enzymes. The activated protein will attach to the receptor protein on the surface of the intestinal epithelial cells. This attachment causes the formation of pores in the cells resulting in cell lysis. As a result, insects will experience digestive disorders and die (Tampubolon et al., 2013). These results were reinforced by Hitipeuw (2022), who stated that the product Bio Larvicidal VectoBac WG with the active *B. thuringiensis* ingredient was effective as a larvicide in controlling *Culex* sp. *B. thuringiensis* produces numerous parasporal crystal toxins during sporulation that, upon proteolysis, bind to specialized midgut receptors, thus causing disruption of the gut epithelium, gut paralysis, toxemia, and eventual death of the host insect (Subramaniam et al., 2012).

The best *B. thuringiensis* concentration (0.03 mg/L) had the lowest LT50 and LT90 values but still took longer to kill all larvae compared to temephos (4–5 hours vs. 6–11 hours). *Cx. quinquefasciatus* larvae exposed to *B. thuringiensis* experienced reduced mortality and eventual death. This toxin not only causes cell lysis but also reduces the larvae appetite, causing them to stop eating (Muharsini & Wardhana, 2013). Meanwhile, the bacteria will continue to multiply in the larvae's body, so the larvae become infected. This mechanism also plays a role in the mortality of the larvae. Dead larvae appear black from front to back and swell due to disturbance of the osmotic pressure of the fluid (Elqowiyya, 2015). These results were reinforced by Hitipeuw (2022), who stated that the product Bio Larvicidal VectoBac WG with the active *B. thuringiensis* ingredient was effective as a larvicide in controlling *Culex* sp. *B. thuringiensis* produces numerous parasporal crystal toxins during sporulation that, upon proteolysis, bind to specialized midgut receptors, thus causing disruption of the gut epithelium, gut paralysis, toxemia, and eventual death of the host insect (Subramaniam et al., 2012).

Regarding a concentration of 83 mg/L, more than 80% of the larvae experienced mortality after 15 days of observation (Saliha et al., 2017). Based on the results, shows *Cx. quinquefasciatus* larval mortality after 24 hours of observation. *B. thuringiensis* biolarvicide at 0.01 mg/L for 6 hours results in 48% mortality, 0.02 mg/L for 6 hours achieves 68%, and 0.03 mg/L for 6 hours reaches 81%. During the first hour, *B. thuringiensis* killed 8–24% of samples, while temephos killed 26.6%. Temephos at 300 mg/L wiped out all larvae in 6 hours, whereas *B. thuringiensis* did it in 24 hours. Notably, *B. thuringiensis* at 0.03 mg/L was the fastest and most effective among the biolarvicides. *B. thuringiensis* has been gaining growing interest worldwide as a microbial insecticide with a high lethality against various species of mosquito larvae. It has also demonstrated a significantly safer margin concerning non-target aquatic organisms (Subramaniam et al., 2012). This result is in line with previous studies, which stated that the percentage of mosquito larvae mortality due to B. thuringiensis biolarvicide reached 90% at a concentratiom of 0.024 mg/L (Elqowiyya, 2015)

The *B. thuringiensis* larvicide is not harmful to the environment because it is degraded by ultraviolet light and is not toxic or toxic. In addition, it is non-pathogenic to non-target species such as birds, worms, and other aquatic organisms (Apriyani et al., 2019). Therefore, *B. thuringiensis* can be used as a substitute for chemical larvicide because it is proven effective in killing *Cx. quinquefasciatus* mosquito larvae in 24 hours and is environmentally friendly. *B. thuringiensis* have potential to be used as an ideal eco-friendly approach for the control of the major lymphatic filarial vector, *Cx. quinquefasciatus* (Kovendan et al. 2011).

CONCLUSION

This study demonstrates that *B. thuringiensis* is an effective larvicide for killing *Cx. quinquefasciatus* mosquito larvae. Although it is less effective than temephos, *B. thuringiensis* achieves 100% larval mortality within 24 hours. The advantages of *B. thuringiensis* include environmental sustainability and a lower risk of resistance development. At a concentration of 0.03 mg/L, a higher rate of larval mortality is achieved more rapidly within the first 6 hours. Therefore, *B. thuringiensis* is a viable and environmentally friendly alternative to chemical larvicides for eradicating *Cx. quinquefasciatus* mosquito larvae within 24 hours.

REFERENCES

Ahdiyah, I., & Purwani, K. I. (2015). Pengaruh ekstrak daun mangkokan (*Nothopanax scutellarium*) sebagai larvasida nyamuk *Culex* sp. *Jurnal Sains dan Seni ITS*, *4*(2), 32-36.

Apriyani, N., Setyaningrum, E., & Susanto, G. N. (2019). Pengaruh *Bacillus thuringiensis* israelensis sebagai larvasida vektor demam berdarah dengue (dbd) terhadap ikan guppy (*Poecilia reticulata*). *Journal of Biological Research Bio Wallacea*, *6*(1), 927-935.

Elqowiyya, A. I. (2015). Efikasi larvasida *Bacillus thuringiensis israelensis* terhadap kematian larva *Culex quinquefasciatus* dari daerah Bekasi (Undergraduate thesis). Fakultas Kesehatan dan Ilmu Kesehatan, UIN Syarif Hidayatullah Jakarta, Banten, Indonesia.

Georghiou, G. P., & Wirth, M. C. (1997). Influence of exposure to single versus multiple toxins of *Bacillus thuringiensis* subsp. israelensis on development of resistance in the mosquito *Culex*

quinquefasciatus (*Diptera: Culicidae*). *Applied and Environmental Microbiology*, *63*(3), 1095-1101.

Hitipeuw, D. (2022). Efikasi larvasida potensial *Bacillus thuringiensis* terhadap kematian larva *Aedes*, *Anopheles* dan *Culex* (Doctoral dissertation). Universitas Diponegoro, Semarang, Indonesia.

Kovendan, K., Murugan, K., Vincent, S., & Kamalakannan, S. (2011). Larvicidal efficacy of *Jatropha curcas* and bacterial insecticide, *Bacillus thuringiensis*, against lymphatic filarial vector, *Culex quinquefasciatus* Say (*Diptera: Culicidae*). *Parasitology Research*, *109*, 1251-1257.

Kuppusamy, C., & Ayyadurai, N. (2011). Synergistic activity of Cyt1A from *Bacillus thuringiensis* subsp *israelensis* with *Bacillus sphaericus* B101 H5a5b against *Bacillus sphaericus* B101 H5a5b-resistant strains of *Anopheles stephensi* Liston (*Diptera: Culicidae*). *Parasitology Research*, *110*, 381-388. doi: 10.1007/s00436-011-2502-5.

Mahdalena, V., & Ni'mah, T. (2019). Potensi dan pemanfaatan mikroorganisme dalam pengendalian penyakit tular nyamuk. *Spirakel*, *11*(2), 72-81.

Melanie, M., Rustama, M. M., Sihotang, I. S., & Kasmara, H. (2018). Effectiveness of storage time formulation of *Bacillus thuringiensis* against *Aedes aegypti* larvae (Linnaeus, 1757). *Jurnal Cropsaver*, *1*(1).

Muharsini, S., & Wardhana, A. H. (2013). Efficacy of micro-encapsulated of local isolate *B. thuringiensis* as bio-insectiside for control of myiasis caused by *Chrysomya bezziana* larvae. *Jurnal Ilmu Ternak dan Veteriner*, *19*(1), 67-73.

Nugroho, A. D. (2011). Kematian larva *Aedes aegypti* setelah pemberian abate dibandingkan dengan pemberian serbuk serai. *Kemas: Jurnal Kesehatan Masyarakat*, *7*(1), 91-96.

Pei, G., Oliveira, C. M., Yuan, Z., Nielsen-LeRoux, C., Silva-Filha, M. H., Yan, J., & Regis, L. (2002). A strain of *Bacillus sphaericus* causes slower development of resistance in *Culex quinquefasciatus*. *Applied and Environmental Microbiology*, *68*(6), 3003-3009.

Tampubolon, D. Y., Pangestiningsih, Y., Zahara, F., & Manik, F. (2013). Pathogenicity test *Bacillus thuringiensis* and *Metarhizium anisopliae* against mortality *Spodoptera litura* fabr (*Lepidoptera: Noctuidae*) in the laboratory. *Online Journal of Agroecotechnology*, *1*(3), 783-793.

Saliha, B., Wafa, H., & Laid, O. M. (2017). Effect of *Bacillus thuringiensis* var krustaki on the mortality and development of *Culex pipiens* (*Diptera: Cullicidae*). *International Journal of Mosquito Research*, *4*, 20-23.

Sihotang, H., & Umniyati, S. (2018). Toxisitas temephos, minyak atsiri jahe (*Zingiber officinale* Roxb), dan *Bacillus thuringiensis* ssp. israelensis(Bti) terhadap larva nyamuk *Ae. aegypti* dari Sumatra Utara. *Berita Kedokteran Masyarakat*, *34*(3), 127-136.

Subramaniam, J., Murugan, K., & Kovendan, K. (2012). Larvicidal and pupcidal efficacy of *Momordica charantia* leaf extract and bacterial insecticide, *Bacillus thuringiensis* against malarial vector, Anopheles stephensi Liston (*Diptera: Culicidae*). *Journal of Biopesticides*, *5*, 163.

Webb, C., Doggett, S., & Russell, R. (2016). *A guide to mosquitoes of Australia*. Clayton South: Csiro Publishing.

World Health Organization (WHO). (2023). Lymphatic filariaris. Retrieved from https://www.who.int/news-room/fact-sheets/detail/lymphatic-filariasis.

World Health Organization (WHO). (2010). *The global program to eliminate lymphatic filariasis: progress report 2000–2009 and strategic plan 2010–2020*, Geneva: World Health Organization.