Proceeding of The 6th Annual Basic Science International Conference
Received, , Accepted, , Published online, .

Bioassay MOSNON™ as Biolarvacide Towards *Aedes aegypti* Larvae

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Abstract— DHF is an arboviral type disease through *Aedes aegypti* and *Aedes albopictus* as vector's transmition virus on human. There are two methods for controlling *Aedes aegypti*'s larvae, they are controlling larvae using chemistry's larvacide and biology's larvacide (biolarvacide). Biolarvacide have more advantages than chemistry's larvacide. Its more specific to target organism, doesn't has bad effect on environment, and save for human health. So, application of *Bacillus thuringiensis* from Mosnon™ product is certainly save. Objective of this study is to investigate the toxicity of Mosnon™ product based on LC_{50} value in the multi location method. The methods is *Aedes aegypti*'s eggs were hatched into a container. Adult mosquitoes then maintained (rearing) in Barraub box to produce eggs (F2) which is then dried for storage. F2 of third larvae instar then used to toxicity test of the Mosnon™. Mosnon™ product are divided into 7 different concentrations of 0; 0.1; 0.25; 0.5; 1; 2; and 4 ppm. Each concentration and *Aedes aegypti*'s larvae as many as 50 individuals entered in each container contain of 5 L of well water and placed in three different locations. Toxicity of Mosnon™ was done by counting the number of dead larvae on a long exposure time of 24, 48, 72, and 96 hours then analysis using Probit analysis to determine LC_{50} value. Results shows that LC_{50} obtained on concentration of 0.02 ppm with exposure time of 48 hours at the location behind FMIPA UB building. This value is assessed as having lowest concentration that kills 50 % of the third instar larvae of *Aedes aegypti*.

1. INTRODUCTION

Dengue fever remains a major topic in some tropical and subtropical countries. World Health Organization explain that approximately 50 to 100 million cases of dengue fever occur each year, including 500,000 cases of dengue fever which resulted in 22,000 deaths each year [1]. The incident case of dengue fever increased thirty-fold over a period of fifty years back [2]. The rapid increase of dengue patients each year make dengue fever as the main focus of global public health problem [1]. In Indonesia, dengue fever case have been outbreaks periodically, especially in 1998 and 2004. The case was widespread almost in all 33 provinces in Indonesia [3]. Indonesian health ministry said that in 2015 there were four provinces declared outbreaks area for dengue fever (DHF), they are province of East Java, South Borneo, Central Borneo and Southeast Celebes. East Java Province has most cases among the four other provinces, its has 18 districts / cities [4].

Dengue Hemorrhagic Fever (DHF) is an arboviral disease caused by a virus and infected by *Aedes aegypti* and *Aedes albopictus* [2]. Dengue virus is better known by the acronym DENV. Its belongs to the *Flavivirus* genus of the Family Flaviviridae. The genome is about 10.7 kb and its a ssRNA virus type. Based on the research, namely the four serotypes of DENV has DENV-1, DENV-2, DENV-3 and DENV-4 [5]. The clinical symptoms of complex DENV infection are thrombocytopenia, abnormal blood clotting, and blood plasma leakage in children as well as bleeding until organ damage in adult patients that can lead to death [6].

Therefore, the dengue vector control needs to be done. One of the Indonesian Government's efforts in controlling the dengue vector is by environmental management systems. That was done by three program, such as bury, drain, and Closing (3M) container which has potency as a breeding ground for dengue vector. These efforts have not been effective enough in dealing with outbreaks of dengue [4].

An appropriate vector control for DHF's vector is controlling the larvae to prevent larvae develop to adult mosquitoe. The control has also been promoted by the government through a program distributing of free chemical larvicides in every home. Continuously use of chemical larvicides with improper dose of organophosphate and temephos group can increase the resistance of larvae through detoxification ability of active compounds that indicate the presence of mutations in the larvae. Besides, the residual impacts on environments and humans. The impacts are the death of aquatic organisms that play role as natural enemies of the larvae [7], an increased risk of hypothyroidism, induces the formation of lipid peroxidation, damage to the vessel endothelial function, changes

in modulus of elasticity in the aorta, increasing the risk of stunted growth in children, reducing the production of IGF-1 (insuline-like Growth Factors 1) in pregnant women resulting viscosity of the arterial system of children [8], so its necessary to find an alternative solution or other larvicides that are safe for the environment as well as for humans. One of them is a natural larvicidal (biolarvacide). Its utilised toxin from microorganisms such as *Bacillus thuringiensis* or *Bacillus sphaericus*, as well as products Mosnon™.

Mosnon™ is biolarvacidal, manufactured by the Japanese company, PT. Kyushu Medical Co., LTD. located in hyakunen-Kouen 1-1 Kurume, Fukuoka, Japan. Active ingredient of Mosnon™ is *Bacillus thuringiensis* D142. The mode of action of Mosnon™ is mosquito larvae will eat the crystal protein from *Bacillus thuringiensis* D142, conditions of pH and enzymes inside larvae's midgut will change the form of the crystal protein toxin from protoxin form become an active toxin. Toxin then binds to the midgut cells in the digestive cells and causes the cell lysis and eventually cause a hole in the intestine. The hole makes the larvae will not eat and eventually die [9]. The objective of this study is to investigate the toxicity of Mosnon™ product based on LC_{50} value in the multi location method.

2. METHODS

2.1 Chemicals

The material in this study is Mosnon™ produced by PT. Kyushu Medical Co., LTD., Located in hyakunen-Kouen 1-1 Kurume, Fukuoka, Japan. Based on the official website of PT. Kyushu Medical Co., LTD., Mosnon™ is an insecticide contain of active microorganisms and protein produced by *Bacillus thuringiensis* D142. *Bacillus thuringiensis* D142 can produce insecticidal proteins that are safe for environment [9].

2.2 Procedures

Aedes aegypti's eggs Provincial Health Office of East Java (Dinkes Provinsi Jawa Timur) were hatched into a container with a diameter of 40 cm large and water level is ¾ of the container's height. Adult mosquitoes then maintained (rearing) in Barraub box to produce eggs (F2) which is then dried for storage. A total ± 3,150 of F2 eggs were hatched inside the three containers with a diameter of 40 cm and water level is ¾ of the container's height. The hatched larvae fed with dogfeed 2.5 g per day and illuminated with a 10 watt electricity lamp until develop into third larvae instar. F2 of third larvae instar then used to toxicity test of the Mosnon™. Mosnon™ product are divided into 7 different concentrations of 0; 0.1; 0.25; 0.5; 1; 2; and 4 ppm. Each concentration and *Aedes aegypti*'s larvae as many as 50 individuals entered in each container contain of 5 L of well water and placed in three different locations. Each container was done by counting the number of dead larvae on a long exposure time of 24, 48, 72, and 96 hours. Toxicity Mosnon™ known by Probit analysis using SPSS for Windows Release 20.0 to determine LC_{50} value.

3. RESULTS AND DISCUSSION

Figure 1 Shows a reaction inside larvae when a toxin produced by *Bacillus thuringiensis* in Mosnon™ product into the intestine tract of *Aedes aegypti* larvae. The arrows indicate the parts of the intestine that had been broken and its contents have been out. *Bacillus thuringiensis* belongs to *Gram*'s positive bacteria, aerobic, lophotric type flagells. It can form endospores in conditions that are less favorable. In addition to forming endospores, *Bacillus thuringiensis* also produces a toxin called ICP (insecticidal Crystal Protein) or δ-endotoxin. ICP toxins are divided into two groups, namely *cry* toxins and *cyt* toxins [10]. The mode of action of *Bacillus thuringiensis cry* toxin is as follows: crystal protein (protoxin) ingested by mosquito larvae, into the digestive and hydrolyzed by alkaline conditions. Once hydrolyzed, protoxin interact with the protease enzyme and produce an active toxin. Active toxin then integrated into the plasma membrane to form a hexagonal pore through the cell digestion. The toxin affects the osmotic balance and ATP, so that eventually the cell lysis [11]. *cyt* toxin mechanism different from *cry* toxin, *cyt* toxin does not bind to the receptor protein but directly bind to lipid membrane and create pores in membranes. Pore formed by means of degrading lipid membrane such as detergents-like which degrade fat [10].

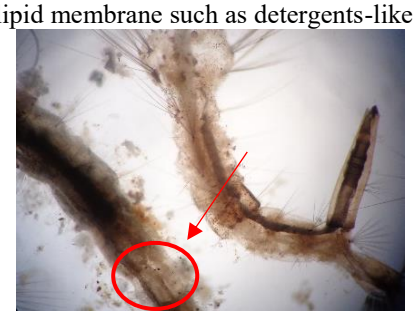


Figure 1 Mosnon™ reaction toward *Aedes aegypti* larvae

The ability of *Bacillus thuringiensis* in killing the larvae of *Aedes aegypti* further advanced tests have been conducted to determine the power to kill or toxicity to larvae. Based on these test results obtained as shown in Figure 2 which shows the relationship between the LC_{50} and the exposure time of Mosnon™. Based on these figures, the lowest concentration that kills 50 % of *Aedes aegypti* larvae is 0.02 ppm. While the statistical correlation that is obtained is the location Behind UB building has little resemblance to the location of the FMIPA UB's garden, and FMIPA UB's garden also has little resemblance to the Biology's garden (table 1). The similarity can be seen by the value of pH, relative humidity and ambient temperature at each location (Figure 3).

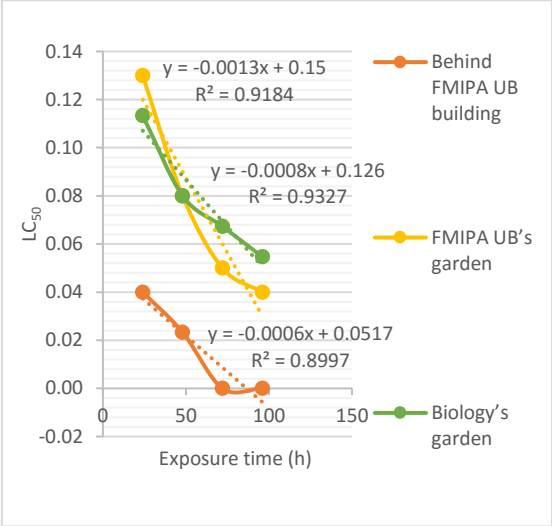
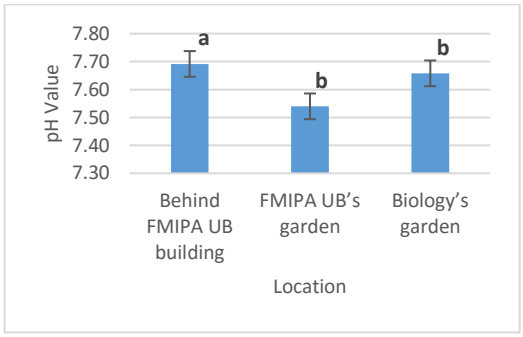


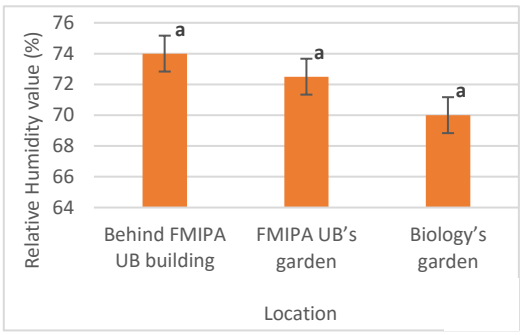
Figure 2 Relationship between LC_{50} and exposure time from Mosnon™

Table 1 ANOVA analysis of each location and exposure time based on Mosnon™ LC_{50}

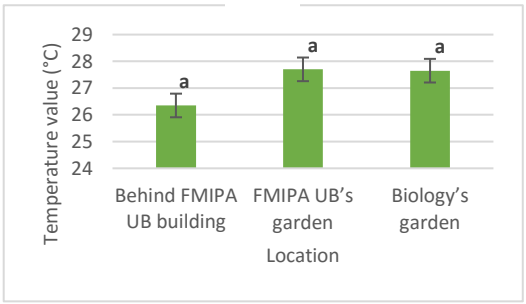
Location	Concentration (ppm)			
	LC_{50}— 24 h	LC_{50}— 48 h	LC_{50}— 72 h	LC_{50}— 96 h
Behind FMIPA UB building	0.04 (aA)	0.02 (aA)	-	-
FMIPA UB's garden	0.13 (abA)	0.08 (abA)	0.05 (abA)	0.04 (abA)
Biology's garden	0.11 (bA)	0.08 (bA)	0.07 (bA)	0.05 (bA)



(a)



(b)



(c)

Figure 3 Abiotic factor of each location: (a) pH value; (b) relative humidity value (%); (c) ambient temperature (°C)

4. CONCLUSIONS

Mosnon™ can kill *Aedes aegypti* larvae through *cry* and *cyt* toxin that has high specificity to diptera order. Bioassay result shown that 0.02 ppm concentration of Mosnon™ product has ability to kill 50 % *Aedes aegypti* larvae.

5. REFERENCES

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