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学位論文の要旨（論文の内容の要旨）
Summary of the Dissertation (Summary of Dissertation Contents)

論　文　題　目
Dissertation title
Biological Control for Suppressing Human Diseases: a Case Study of *Bacillus thuringiensis* Isolated Indigenously from East Java as a Natural Enemy against *Aedes aegypti*

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This study aims to investigate safe strategy to control *Aedes aegypti* mosquito using the most potential *Bacillus thuringiensis* isolated indigenously from East Java as a natural agent, to understand the current condition of biological control in East Java and to formulate some conclusions, which can be taken for future consideration by the local government.

East Java, as one of the province in Indonesia, has been growing very fast where the human population and the city are increasing very significantly compared to other regions in Indonesia. East Java is one of the very dense populated areas; unfortunately it still has various health problems. Actually, it is still difficult to be resolved, especially related to dengue hemorrhagic fever (DHF). The case snumber of DHF is rising every year. The Indonesian government has been reducing the number of DHF patients with various methods. Among others, by sprinkling mosquito larvicides (ABATE) in some containers to kill mosquito larvae at the surface of water, by fogging, and conducted the volunteer person to monitors the number of mosquito larvae / *Jumantik (Juru Pemantau Jentik)*. Unfortunately, all is not yet effective way to control mosquito. Based on this historical background, this study was therefore conducted.

Chapter 1 discusses the general introduction and literature review. Chapter 2 concern on association between climatic variability, DHF incidence, and distribution of *Aedes aegypti* as a vector of DHF in East Java. Chapter 3 is the main chapter, which focuses deeply on indigenous natural agent selection to control *Aedes aegypti* larvae. Chapter 4 focuses on formulating the safe strategy to control *Aedes aegypti* using the most potential indigenously *Bacillus thuringiensis* isolated from East Java.

The first section of Chapter 2 determines the spatial distribution of *Aedes aegypti* in East Java from 2008 to 2010 and to characterize the temporal patterns of *Aedes aegypti* in the tenth regions of East Java for 3 years (2008, 2009, 2010) and its association with local meteorological variables. For spatial distribution, the studies used the average of DHF incidence rate (IR) data in nine districts in East Java over a monthly period (January - December) from 2008 to 2010. The results of statistical analysis showed there was a significant relationship between the average IR and the number of *Aedes aegypti*. Mosquitoes were captured by using a bait in door has the most closely related to the average IR cause *Ae. aegypti* is an *anthropophilic* mosquito, taking its blood meals preferentially from humans. *Ae. aegypti* was more often found at indoor than outdoor. The highest CI and BI were recorded at 36.11 and 70.59 % respectively in Nganjuk District. The highest *Ae. aegypti* house index was 51% in Bangkalan District. In these districts, there are many good habitats for *Ae. aegypti* larvae. All locations of East Java has higher House Index (HI) than the WHO standard for high DHF risk area (i.e.10 % HI).The peak season to sucking blood of *Ae. aegypti* is occurred in the morning and in the evening.

The second section of chapter 2 discusses association between climatic factors (maximum and minimum temperature, rainfall, humidity, light duration, wind velocity) associated with DHF incidence in Nganjuk District, East Java Province, Indonesia from 2005 to 2010. The results of this study indicated the climatic variability is clearly associated with the dengue incidence rate (IR). The maximum air temperature, humidity, rainfall and light duration have played an important role in the transmission of DHF in Nganjuk District. The spearman correlation analyses showed that in humidity and rainfall have positive correlation with DHF incidence; on the contrary a decreased value of maximum air temperature and light duration would have impact on increased IR. The result of regression analysis indicated that IR of DHF was affected by the maximum air temperature, minimum air temperature, and rainfall in the rainy season; however in the dry season, the IR was affected by wind velocity and rainfall.

The third section of chapter 2 investigates the observation which was undertaken to determine the distribution pattern of *Aedes aegypti* and risk factors of DHF between regency and cities in Mojokerto 2012 and correlation between elevation of sampling location and distribution of mosquito. Mosquitoes are one of the insects that have an important role as vectors of disease agents. The diseases transmitted by mosquitoes are still public health problems in Indonesia especially in East Java Province, for example Dengue Hemorrhagic Fever (DHF). DHF is a very alarming disease because the occurrence of this epidemic is no longer just confined to certain geographic locations. At present, Mojokerto is a district in East Java region known as endemic for DHF in Indonesia. Number of cases in this district tends to rise and expand distributed. The spread pattern of dengue cases in this district is not certainty known. Sampling was conducted in two locations in the Mojokerto district, namely Prajurit Kulon for urban and Dlanggu for rural. We used survey for mosquito larvae by WHO standard and for mosquito eggs by ovitrap. The coordinates of sampling locations recorded using GPS and then identification of mosquitoes is performed at the Laboratory of Ecology and Animal Diversity, University of Brawijaya. Quantitative data were analyzed to determine the abundance, relative abundance, freq uency, relative frequency and IVI (Importance Value Index). Mosquito distribution patterns were analyzed with Morisita index. The research findings indicated that there are five mosquitoes species consist of *Aedes aegypti*, *Aedes albopictus*, *Aedes laniger*, *Culex bitaeniorchynchus* and *Culex quinquefasciatu s*. *Aedes aegypti* is the dominant species in urban area while *Culex quinquefasciatus* is the dominant species in rural area. Morisita index showed that mosquito dispersal on patterns in Mojokerto district is uniform. The elevation of the area and density of *Aedes aegypti* has a positive correlation. The first section of chapter 3 focuses on maintain the sustainability of the local bacteria which have effectively controlled population of mosquito larvae; it is necessary to observe the toxicity tests for local bacteria from other places around East Java. *B.t huringiensis* is an important insect pathogen that is highly toxic to mosquito larvae and related dipterans. The original *B.thuringiensis* exploration efforts in Indonesia were carried out because the *B. thuringiensis* crystal protein has an arrow host spectrum. Therefore, the ideal effort for killing Indonesian mosquitoes would be using *B. thuringiensis* isolated from Indonesia. This research investigated the toxicity of indigenous *Bacillus thuringiensis* isolated from East Java for controlling *Aedes aegypti* larvae. The result of the study disclosed that *B. thuringiensis* Brht isolate from Surabaya district has the highest percentage of *Aedes aegypti larvae* mortality at 24 hour ($LC_{50\text{-}24h}$ = $1.215x10^8$ cells/ml). It also described that these isolate is more effective than the reference *B.thuringiensis* (*B. thuringiensis* var. Israelensis HD 567).

The second section of chapter 3 investigates the toxicity of indigenous *Bacillus thuringiensis* isolates from Malang City for controlling *Aedes aegypti* larvae. Soil samples were taken from Purwantoro and Sawojajar sub districts, and bacterial isolation was performed using *B. thuringiensis* selective media. Phenotypic characteristics of the isolates were obtained with the simple matching method. The growth and prevalence of spores were determined by the Total Plate Count method, and toxicity tests were also performed on the *Aedes aegypti* third instar larval stage. The percentage of larval mortality was analysed using probit regression. The LC_{50} was analysed by ANOVA, and the Tukey HSD interval was 95%. Six isolates were obtained among 33 selected bacterial isolates (PWR4-31, PWR4-32, SWJ4-

2b, SWJ4-4b, SWJ-4k and SWJ5-1) that have a similar phenotype to reference *B.thuringiensis*. Based on the dendrogram, all of the bacterial isolates were 71% similar. The three isolates that had a higher prevalence of reference *B.thuringiensis* were PWR4-32, SWJ4-4b and SW5-1, of which there was a 52.44%, 23.59%, 34.46% spore prevalence, respectively. These three indigenous isolates from Malang City (PWR4-32, SWJ4-4b, SWJ5-1) successfully killed *Aedes aegypti* larvae. The PWR4-32 isolates were the most effective at killing the larvae. The study concluded that the six indigenous *B. thuringiensis* isolates (PWR4-31, PWR4-32, SWJ4-2b, SWJ4-4b, SWJ4-4k and SWJ5-1) among the 33 bacterial isolates that were found in the Sawojajar and Purwantoro sub districts were toxic to third instar *Ae. aegypti* larvae. The PWR4-32 isolates were identical to the reference *B.thuringiensis* and had 88% phenotype similarity. The PWR4-32 isolates had the highest spore prevalence (52.44%), and the early stationary phase occurred at 36 hs. The PWR4-32 isolates were the most effective at killing *Ae. aegypti* larvae ($LC_{50\text{-}72\ hs}$=2.3x10^8 cells/ml).

The third section of chapter 3 evaluates the toxicity of indigenous *Bacillus thuringiensis* isolated from East Java on non-target organism *Trichogaster pectoralis* (Class: Actinopterygii). The study resulted that three isolates of *Bacillus thuringiensis* from Bangkalan, Madiun and Lamongan have no direct effect on *trichogaster pectoralis.* Aquatic organisms, such as fresh water fish are generally unaffected (Eder and Iris, 2010). This large safety margin of preparations of *Bacillus thuringiensis* for non-target organisms indicated that their suitability for mosquito control programs in areas where protection of the natural ecosystem is important.

The chapter 4 reviews the safe strategy concept of biological control using indigenous *Bacillus thuringiensis*, the current status, and developmental trends of biological control using *B. thuringiensis,* mode of action. *Bacillus thuringiensis* provides effective alternatives to broad-spectrum larvicides in many situations with little or no environmental impacts. The advantages of microbial control agents' us age are numerous. These include safety for humans and other non-target organisms, reduction of pesticide residues in the aquatic environment, increased activity of most other natural enemies and increased biodiversity in aquatic ecosystem. This phenomenon indicates that indigenous *B. thuringiensis* from East Java isolates have its potential to become bio control of *Aedes aegypti* larvae.