The Detection of Aedes Aegypti Mosquito Resistance With Biochemical Test Based on Non-specific Esterase Enzyme Activity and Monooksigenase Enzyme in Ternate City

Amalan Tomia1, Upik Kesumawati Hadia, Susi Sovianas, Elok Budi Retnani2

1Universitas Muhammadiyah Maluku, Ternate, Indonesia
2Division of Parasitology and Medical Entomology, Faculty of Veterinary Medicine, Universitas Pertanian Bogor, Bogor 16680, Indonesia

Email : alga.tte@yahoo.co.id, upikke@gmail.com

Abstract. Broadly speaking, the aim of this research is to examine and analyze the effect of Efficiency, Fulfilment, Reliability, Privacy Variables on Customers’ Satisfaction on account opening services via the online system "Jenius BTPN". Determination of a sample of 100 respondents through a stratified random sampling approach with research locations at BTPN Bank. The research approach uses quantitative while the data analysis method uses multiple linear regression. Analysis test equipment using SPSS, version 22. The results of the study indicate that the Efficiency does not affect customer satisfaction (Sig 0.475> 0.05); Fulfillment affects customer satisfaction (sig 0.003 <0.01); Reliability does not affect satisfaction (sig 0.700> 0.05); Privacy has a significant effect on customer satisfaction (sig 0.000 <0.05); Privacy is the most dominant variable influential. R2 test is 65.9%

1. Introduction

The control of Dengue Fever (DF) depends on its vector control program i.e. Aedes aegypti. According to Chin [1], unsuccessful handling of dengue fever is due to the inability to break the chain of transmission through its vector. The chemical insecticide which has been used since 1972 is the main choice in the prevention program of DF vector during Extraordinary Occurrence (Kemenkes RI. 2014). The DF vector control program in Ternate City has used temefos insecticide since 1980 while the use of malation was started in 1990. In 2009, malation was reported to be ineffective and replaced with insecticides with an active ingredient of sipermetrin from the piretroid group, while temefos is still used to control larvae. However, the result is not maximal because the case of DF in Ternate City still occurs. The constraints that arise in the control of dengue vectors in Ternate City are due to the resistance of the Ae. Aegypti population against the applied insecticides. The trigger factor for the occurrence of resistance is the use of inappropriate insecticide dosage which did not eradicate the population because the same insecticide group was used for a long time [2]. Insect resistance generally occurs after a long and continuous use of insecticides i.e. for 2-20 years [3]. According to Ponlawat et al. [4], the increase of DF cases can occur due to the intensive use of one type of insecticide for a long period of time to control the mosquitos causing the DF vector that eventually leads to their resistance.
It is highly important to carry out the resistance monitoring activity as a side effect of insecticide use as a consideration in taking the policy of DF vector control [5]. According to WHO in 2000, the detection of resistance can be carried out by biochemical test based on the quantity of enzyme responsible for the process of resistance occurrence. The biochemical test can provide information on the status of the resistance more quickly and can show the resistance mechanisms measured on individual insects by using non-specific esterase [6].

Monooxygenase and nonspecific esterase enzymes are enzymes involved in the metabolism of xenobiotic compounds in insect bodies. The increased activity of monooxygenase enzyme is an indicator of resistance to pyrethroid synthetic insecticide group [7]. Increased nonspecific esterase enzyme activity serves to identify mechanisms underlying the occurrence of insect resistance to insecticides from the organophosphate group [8]. Currently, in Ternate City there are no data on Ae. Aegypti resistance, both on their adults and larvae, and the data are very important for the success of the elimination of DF. Therefore, it is necessary to conduct a research on the resistance status. This study aimed to analyze the resistance status of Ae. aegypti by using the biochemical method on temefos, malation and sipermetrin used in the DFvector dengue control program in Ternate City.

2. Research Methods

This research was conducted in 20 urban villages from 4 Districts (South Ternate District, Ternate Tengah District, North Ternate District and Ternate Island District) in Ternate City. The study was conducted for 5 months. There were ovitraps used in this study installed in 20 urban villages spread over the four districts in Ternate City. The Aedes spin mosquito eggs obtained from each urban village were dried and taken to the Entomology Laboratory of R&D Center of P2B2 Banjarnegara, and the Biochemical testing was conducted. The nonspecific esterase enzyme activity testing was performed based on the [4] that is one larvae of the first four instar larvaes of each field population was crushed by using a homogenizer glass the temperature of 40°C to make homogenate, and it was dissolved with 0.5 ml of PBS (Phosphat Buffer Sulfate). The homogenate was then transferred into the microplate using a micro-pipette of 50 μl of substrate material (containing 3 mg α naphthyl acetate dissolved in 0.5 ml of acetone). As much as 100 μl was taken and inserted into the PBS up to 10 ml volume and left undisturbed for 60 minutes. After the reaction for 10 minutes ended, the initial red color gradually turned blue. The reaction was stopped by the addition of 50 μl of 10% acetic acid into each microplate containing homogenate. The absorbence value of the final color intensity of the product can be measured by the Elisa reader machine with a wavelength (λ) of 450 nm [5]. The reading in the Elisa reader consists of qualitative and quantitative forms. The qualitative result is the colour change on the well plate, indicating that there was a resistant reaction. The quantitative result is the magnitude of the concentration and absorbence value (AV) on the insecticide tested measured by the reading of absorbence value (AV) at the wavelength (λ) of 450 nm. The AV values of <0.700, 0.700-0.900 and > 0.900 indicates susceptible, Tolerant and resistance respectively WHO in 2000. The testing of monooxygenation enzyme activity was conducted using the method conveyed by WHO (2000) i.e. the mosquito larvae were homogenized individually in 200 μl of phosphate buffer (pH 7.4). To prevent enzyme damage, the mosogenic homogeneity samples were stored at -85°C for use at the next testing stage. A total of 20 μl aliquot of homogenate of each mosquito was inserted into the microplate well and 80 μl of phosphate buffer was added. Subsequently, to each well was added 200 μl of TMB substrate and 25 μl of 3% H2O2 solution were added to each well. The microplate wells were incubated for 2 hours at the room temperature. From the intensity of the color produced at each well, its absorbance was measured on a microassay reader machine with a wavelength of 630 nm. Measurement of the monooxygenase enzyme activity was qualitatively characterized by the occurrence of red color changing to blue. Quantitatively, the reading of absorbance value (AV) was measured with a wavelength of 630 nm. The AV value <0.165 indicates susceptible, and the AV value> 0.165 indicates resistance WHO in 2000.
3. Results and Discussion

Biochemical test is a highly essential technique to detect mosquito vulnerability to insecticide based on the quantification of enzymes that work on the process of decreasing insect vulnerability status. The superiority of biochemical tests is that the vulnerability status information can be obtained faster and can indicate the mechanism of vulnerability reduction measured on individual insects. Two mechanisms for reducing the vulnerability status of the insects toward the synthetic pyrethroid and organophosphates insecticides can show the increase of the monooxygenase and nonspecific esterase (Est) enzyme activities [9]. The activity of nonspecific esterase enzymes was applied to investigate the increased activity of nonspecific esterase enzymes in the body of adult or larval mosquitoes [10]. The activity of esterase enzymes in the mosquito body measured from the absorption of color in the Absorbance Value unit (AV) is shown in Table 1.

| No | Isolates                     | The increase of nonspecific esterase enzyme activity (%) | Description: AV = Absorbance value |
|----|------------------------------|--------------------------------------------------------|-----------------------------------|
| 1  | South Ternate Dist (6 urban villages) | AV < 0.700: 83.3, AV 0.700-0.900: 11.1, AV > 0.900: 5.6 | |
| 2  | Central Ternate District (5 urban villages) | AV < 0.700: 73.3, AV 0.700-0.900: 26.7, AV > 0.900: 0.0 | |
| 3  | North Ternate District (5 urban villages) | AV < 0.700: 86.7, AV 0.700-0.900: 0.0, AV > 0.900: 13.3 | |
| 4  | Ternate Island District (4 urban villages) | AV < 0.700: 58.3, AV 0.700-0.900: 16.7, AV > 0.900: 25.0 | |

The results of the biochemical test showed that the increase of nonspecific quantitative esterase enzyme activity in 20 urban villages from the 4 districts in Ternate City were 25.0% in Ternate Island District, 13.3% in North Ternate District, 5.6% in South Ternate District and 0% in Central Ternate District.

![Image of biochemical reaction results](image-url)

**Figure 1.** The biochemical reaction results in the form of color changes to see the presence of the nonspecific esterase enzyme activity

The results of the biochemical test showed that qualitatively there was an activity of monooxigenase enzyme which is indicated by the occurrence of color change into a greenish color at the microplate well.
Figure 3. Quantitatively, the Absorbance Value (AV) as a result of the reading with microassay reader at the wavelength (λ) of 630 nm showed the resistance at to 4 districts, and the AV value can be seen in Table 2.

Table 2. The resistance status of *Ae. aegypti* mosquito against the pyrethroids and the mechanisms playing a role in the biochemical test

| No  | isolates                     | The increase of monooxsigenase enzyme activity(%) |
|-----|------------------------------|---------------------------------------------------|
|     |                              | susceptible AV<0.165 | resistance AV>0.165 |
| 1   | South Ternate Dist (6 urban villages) | 16.7 | 83.3 |
| 2   | Central Ternate District (5 urban villages) | 13.3 | 86.7 |
| 3   | North Ternate District (5 urban villages) | 20.0 | 80.0 |
| 4   | Ternate Island District (4 urban villages) | 8.3  | 91.7 |

Note : AV = Absorbance value

The results of the biochemical test showed that the increase of monooxsigenase enzyme activity in 20 urban villages from the 4 districts in Ternate City were 91.7% in Ternate Island District, 86.7% in Central Ternate District, 83.3% in South Ternate District and 80% in North Ternate District.

Figure 2. The biochemical reaction results in the form of color changes to see the presence of the monooxsigenase enzyme activity

**Discussions**

Esterase is a detoxification enzyme known to play a role in insect resistance mechanisms against insecticides from the organophosphate group. It is classified into the hydrolase enzyme group, a large group of enzymes that catalyzes the hydrolysis reaction of aliphatic compounds, aromatic esters, choline and organophosphorus esters [11]. Malation insecticide is an insecticide from the organophosphate class having two carboxylic acid ester groups, so this compound can be hydrolyzed by carboxyl esterase enzyme. The carboxyl esterase enzyme may hydrolyze one or both carboxylic groups composing the malation compound. If the carboxylic group composing the malation compound undergoes a change, then this insecticidal compound will lose its function. Resistance caused by enzyme activity occurs when the enzyme blocks the insecticide compound to reach its target side [12]. Prior study explains that the occurrence of resistance to temefos was influenced by metabolic factors in which detoxification enzymes were formed, especially the esterase and by cuticle thickening factor and mutation changes [13]. The Biochemical test result using the nonspecific esterase enzyme showed that 0-25% of *Ae.aegypti* isolates was resistant to the organophosphates (malation and temefos). The biochemical test result is different from the bioassay test results using susceptibility test kit using impregnated paper made from the active materials of malation and larvacide using temefos. The result of bioassay test showed that the *Ae. aegypti* isolates from Ternate City were resistant
Differences in the biochemical test results using the nonspecific esterase enzymes with the susceptibility test results showed that phenotypically Ae.aegypti mosquito isolates were resistant, but biochemically, they were still susceptible to insecticides from the organophosphate group. The insecticide use in Ternate City to overcome the DF vector has been discontinued since 2009 (9 years). This condition can cause the esterase in enzyme that acting as an insecticide target point experienced gene mutation so that the resistance toward the insecticide occurred. This kind of test was ever conducted in 2009 with the molecular diagnostic testing, and he found the VGSC gene mutation at the point of Val1016Ile as a targeted resistance mechanism of pyrethroid synthetic insecticide site on dengue vector (Ae aegypti) in Palembang. Ahmad et al. in 2009 reported that isolates collected from Bandung, Surabaya, Jakarta and Pala were still susceptible to malation despite their use of more than 32 years, due to the replacement of the insecticide malation use (organophosphates) by insecticides from other classes. Widiarti et al. (2010) reported that there was a decrease in resistance in the control of malaria vector of An. sundaicus and An. aconitus in some areas of East Java against the pyrethroid insecticides, a result of selective suppression of agricultural insecticides and cross-resistance from DDT (organochlorine group). Resistance occurred because the Ae. aegypti mosquitoes were able to develop their immune system against insecticides frequently used. They were also able to increase the production of detoxification enzymes such as esterase, glutathione S-transferase and insecticide receptor modification [14]. The resistance of Ae. aegypti against temefos had occurred in many places such as in Phnom Penh and Brazil (Braga et al., 2004). [15] explained that there was a tendency of Ae.aegypti resistance to malation although its resistance level was lower than temefos. Monoxygenase is one of the enzymes involved in the xenobiotic metabolism (insect poison) in the insect body that can cause insect resistance against insecticides.

The biochemical test yielded the same result as the bioassay test against sipermetrine (synthetic pyrethroid class) resistant to Ae. aegypti isolates in Ternate City. This suggests that the phenotypic and biochemical isolates of Ae.aegypti show similar results. The high resistance is caused by the widespread use of sipermetrine since 2009 by the Ternate Municipal Health Office in the DF vector control program. The speed of the rise of resistance is known to be related to the biological characteristics of vector species in each local population, type and level of selective suppression of insecticides [2]. The biochemical test with the monoxygenaseenzyme at a wavelength (λ) of 245 did not show significant color changes as a result of the increase of biochemical enzyme (Figure 3), but quantitatively it shows resistance reaction to Ae. Aegyptimosquitoes(Table 2). Brengues et al. in 2003 [16] in his study also found the occurrence of resistance without any increase in biochemical enzymes. This result is different from that of Sunaryo research in 2013 in Tembalang District, Semarang City, in which resistance toward Ae Aegyptimosquitoes occurred at the sampling site, and the resistance was marked by the biodegradation process in the mosquitoes’ body by the monoxygenase enzyme followed by the color change in the plate wells. The monoxygenase enzyme can detoxify the insecticide from the pyrethroid class and is also involved in both the bioactivation and insecticidal detoxification of the organophosphate group [17]. The occurrence of Ae. aegypti mosquito resistance against insecticide in Ternate City resulted from the exposures of synthetic pyrethroid and organophosphate insecticides used by the Ternate City Health Service Office and use of household insecticides with active ingredients from the synthetic pyrethroid class by the community. Similarly, Sunaryo in 2013 [18] found that mosquito resistance to pyrethroid synthetic insecticide in Tembalang District, Semarang City occurred because 54.67% of households chose to use household insecticides in their area to remove mosquito disruption in their home environment. Insecticide resistance is inherited to the next generation in mosquito population. The occurrence of resistance is due to the enzyme activity that blocks the insecticide compound to reach the target side. Resistance is caused by the selection process as result of the pressure from the environmental factors and the pressure of the long period of insecticide use hasmade the population previously non-resistant (susceptible) become resistant. Therefore, the control program of dengue vector by using insecticide becomes harder. The Ministry of Health in 2012 explains that resistance is a hindrance in the successful vector control with insecticides. The problems occurring Ternate City are due to the use of excessive insecticides applied in the long term. The control of DF vector as an effort to suppress dengue fever case in Ternate City with insecticide usage is not effective, so the local government must adopt alternative controls other than insecticide usage.
4. Conclusion

The biochemical test results showed that the nonspecific esterase enzyme activity on the 20 Ae. aegypti isolates in 20 urban villages in Ternate quantitatively has increased the enzyme esterase of 0-25% in which they were resistant to the insecticide group of organophosphate, and in the monoxygenase enzyme activity, there was an increase of the enzyme by 80-91.7% in which they were resistant to the peritroid insecticide group.

References

[1] J. Chin, “Manual pemberantasan penyakit menular,” penterjemah I, 2006.
[2] Widiarti et al., “The Resistance Map Of Dengue Haemorragic Fever Vector Aedes Aegypti Agains Organophosphates, Carbamates And Pyrethroid Insecticides In Central Java And Yogyakarta Province,” Bul. Penelit. Kesel., vol. 39, no. 4, pp. 176–189, 2011.
[3] V. Sridhar, D. Lokeshwari, K. R. Latha, and A. K. Chakravarthy, “MEETING REPORT: Insecticide resistance management: reflections and way forward,” Curr. Sci., vol. 107, no. 10, pp. 1640–1642, 2014.
[4] S. Selvi, M. A. Edah, W. A. Nazni, H. L. Lee, and A. H. Azahari, “Characterization on malathion and permethrin resistance by bioassays and the variation of esterase activity with the life stages of the mosquito Culex quinquefasciatus,” Trop. Biomed., vol. 24, pp. 63–75, 2007.
[5] W. H. Organization, “Techniques to detect insecticide resistance mechanisms (field and laboratory manual),” World Health Organization, 1998.
[6] W. Widiarti, D. T. Boewono, and M. Mujiono, “Uji Biokimia Untuk Identifikasi Mekanisme Resistensi Ganda Vektor Malaria Terhadap Insektisida Di Jawa Timur,” J. Penyakit Tidak Menular Indones., vol. 1, no. 1, pp. 23–33, 2009.
[7] S. Pimsamarn, W. Sompeng, S. Akksilp, P. Paeporn, and M. Limpawitthayakul, “Detection of insecticide resistance in Aedes aegypti to organophoshat and synthetic pyrethroid compounds in the north-east of Thailand,” 2009.
[8] W. Widiarti, S. J. Mardihusodo, and D. T. Boewono, “Uji Biokimia Kerentanan Anopheles Aconitus terhadap Insektisida Organofosfat (Fenitrothion) dan Karbamat (Bendiocarb) di Kabupaten Jepara,” Indones. Bull. Heal. Res., vol. 29, no. 3, 2001.
[9] T. Charoeviriyaephyaph, P. Rongnoparat, P. Chantarumporn, and M. J. Bangs, “Biochemical detection of pyrethroid resistance mechanisms in Anopheles minimus in Thailand,” J. vector Ecol. J. Soc. Vector Ecol., vol. 28, no. 1, pp. 108–116, 2003.
[10] H. L. Lee, “A rapid and simple method for the detection of insecticide resistance due to elevated esterase activity in Culex quinquefasciatus,” Trop. Biomed., vol. 7, no. 1, pp. 21–28, 1990.
[11] C. J. Jackson et al., “Structure and function of an insect α-carboxylesterase (αEsterase7) associated with insecticide resistance,” Proc. Natl. Acad. Sci., vol. 110, no. 25, pp. 10177–10182, 2013.
[12] J. Tang, “Competition and innovation behaviour,” Res. Policy, 2006.
[13] I. A. Braga, J. B. P. Lima, S. da S. Soares, and D. Valle, “Aedes aegypti resistance to temephos during 2001 in several municipalities in the states of Rio de Janeiro, Sergipe, and Alagoas, Brazil,” Mem. Inst. Oswaldo Cruz, vol. 99, no. 2, pp. 199–203, 2004.
[14] E. P. Lima et al., “Insecticide resistance in Aedes aegypti populations from Ceará, Brazil,” Parasit. Vectors, vol. 4, no. 1, p. 5, 2011.
[15] C. Kudeshia, P. Sikdar, and A. Mittal, “Spreading love through fan page liking: A perspective on small scale entrepreneurs,” Comput. Human Behav., vol. 54, pp. 257–270, 2016.
[16] C. Brengues et al., “Pyrethroid and DDT cross-resistance in Aedes aegypti is correlated with novel mutations in the voltage-gated sodium channel gene,” Med. Vet. Entomol., vol. 17, no. 1, pp. 87–94, 2003.
[17] J. G. Scott, “Insect cytochrome P450s: thinking beyond detoxification,” Recent Adv. Insect Physiol. Toxicol. Mol. Biol., vol. 1, pp. 17–124, 2008.
[18] S. Sunaryo, B. Ikawati, and R. Rahmawati, “Status resistensi vektor demam berdarah dengue (Aedes aegypti) terhadap malathion 0,8% dan permethrin 0,25% di Provinsi Jawa Tengah,” Indones. J. Heal. Ecol., vol. 13, no. 2, pp. 146–152, 2014.