



Resistance Status of *Ae.aegypti* to Insecticide in Ternate City, North Maluku

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ABSTRACT

Dengue Haemorrhagic Fever (DHF) remains as one of the serious health problems given priority of Indonesia. Vector resistance to insecticides is one of the problems in DHF vector control program in Indonesia. This study aimed to analyze the status of vector resistance Ae. Aegypti to insecticides were already used in DHF vector control programs in Ternate City. The results showed that the larvae Ae. aegypti from 20 sub-districts, 19 sub-districts were resistant to temephoslarvacide (Lethal Concentration (LC) 95 > 0.02 mg / L), only Ae. aegypti larvae from Makassar Barat which has showed susceptible (LC95: 0.012 mg / L). Resistance Ratio (RR) analysis to Ae.aegyptilarvae to temephosalso showed resistance (RR: 6-69). Result of resistance status to adult mosquito Ae.aegyptishowed that Ae. aegypti of 19 districts has been resistant to malathion (mortality 24 h <80%), only Ae. aegypti from Sulamadaha has a tolerant status of Malation (mortality 24 h = 89.3%). Resistance status of Ae.aegypti to Cypermethrin showed that 20 sub-districts were resistant (mortality 24 h <80%). Therefore, DHFvector control programs in Ternate City should be done immediately by insecticide rotation with carbamate group.

Keywords: Resistance Status, *Ae. aegypti*, Insecticide, Ternate City.

INTRODUCTION

Dengue Haemorrhagic Fever (DHF) remains as one of the serious health problems given priority of Indonesia. *Ae. aegypti* as the main vector in dengue virus transmission in Indonesia. Dengue virus that causes DHF is included in the Arthropod Borne Virus (Arbovirosis), Flaviviride family, and has 4 types of serotypes: Den-1, Den-2, Den yan-3, Den-4 (Gubler 2002).⁽¹⁾ Until now no vaccines or drugs for DHF have been found. Therefore, the control of DHF disease depends on its vector control, ie *Ae. aegypti* (Linnaeus) and *Ae. albopictus* (Skuse). DHF vector control program in Ternate City used

larvacide and adultisida. Since 2005, temephos has been used to *Aedes*larvae control program in Ternate City. Vector control program *Ae. aegypti* used malation (2005) and then in 2009 replaced with cypermethrin insecticide. However, the case of DHF in Ternate City still showed improvement. The Insecticides used for a long period can trigger of resistant insect species (IRAC 2006).⁽²⁾ Resistance is theincrease the endurance of an insect population against a previously lethal insecticide of resistance and can be passed down from generation to generation (Rong et al., 2012).⁽³⁾

Vector resistance to insecticides is one of the problems in DHF vector control program in Indonesia. The success of DHF vector control programs in the Ternate City depends on the resistance status of *Ae. aegypti*. Data on the status of resistance of *Ae.aegypti* is a very important inputs for DHF vector control programs. This study aims to determine the resistance status of *Ae.aegypti* to the malathion and cypermethrin that have been used in DHF vector control programs. This study also aims to determine the resistance status larvae of *Ae.aegypti* to temephosin Ternate City, North Maluku. The results are expected as the evidence base DHF vector control policy effectively, efficiently and on target in the area.

MATERIALS AND METHODS

Study Area: This research was conducted in Ternate City, North Maluku Province. The city of Ternate is astronomically located between 00 25'41.82 " - 1021'21.78 " LU and 12607'32.14 " - 127026'12 BT. Ternate City Area 5709.58km², consisting of 5547.55 km² of land area and sea 5547.55 km². Ternate Island is astrato volcano with regusol soil type conditions. The topography of Ternate City is dominated by coastal areas, mountainous and hilly terrain. The elevation of Ternate City is grouped into three categories: Low (0 – 499 meter), Medium (500 - 699 meter) and High (more than 700 meter). Ternate city has tropical climate with two seasons, north-west and east-south with two times transition each year. This area is characterized by tropical weather with average temperature 32°C, minimum temperature 24°C. During 2016, the highest number of rain days in June, maximum wind speed 31 knots, occurred in August. Larvae and adult mosquitoes were collected from 20 urban villages in Ternate City.

Sampling Method. Determination of sample in 20 sub-districts in Ternate City. Respondents in this study are the population: 17.631 families, the sample population is taken based on Lemeshow *et al.* (1997).⁽⁴⁾

$$n = \frac{N}{1 + N(e)^2}$$

Keterangan :

n = Number of sampel

N = Total population

e = The level of significance : 0.05

Total sample population:

$$n = \frac{17631}{1 + 17631(0,05)^2} = 391 \text{ sample}$$

Collection of larvae *Ae.aegypti*, species identification: Larvae of *Ae. aegypti* collection using 400 ovitrap mounted on 20 sub-district from Ternate City. Trapping of ovitrap done for 5 days then calculated ovitrap index (IO). Data of the breeding places characteristics and density of *Aedes* larvae were recorded. The eggs of *Aedes* sp rearing at the Laboratory of Parasitology and Entomology of Health of the Faculty of Veterinary Medicine-IPB. The larvae of *Aedes* sp. accommodated in the tray and maintained in the laboratory. Identify mosquito species morphologically following the identification keys of O'Connor and Soepanto.

Resistance Test of Larvae *Aedes* sp. Insecticides used in larvacidal testing are temephos solution (organophosphate). The susceptibility test used is the WHO standard. A total of 25 larvae placed on 1 ml of temephos solution were added to 249 ml of water. The larvae were contacted with different concentrations of temephos 0.005mg/L, 0.025mg/L, 0.125mg/L and 0.625mg/L (WHO 2013).⁽⁵⁾ The tests were performed with three repetitions each time and using susceptible mosquitoes from colony the Division of Parasitology and Health Entomology of the Faculty of Veterinary Medicine-IPB as a comparison. Mortality was calculated after 24 hours of observation, then calculated Lethal Concentration ((LC₅₀ and LC₉₅) and Resistance Ratios (RR₅₀ and RR₉₅).

Resistance Test of *Aedes* spp. DHF vector resistance test method was used a reference from

WHO Susceptibility Test.⁽⁵⁾ This method used *Ae. aegypti* from rearing larvae natural collection numbered 80-100 non-blood feed females aged 3-5 days. The test method of resistance of *Ae. aegypti* that were used as follows: (1) Aspirated 20-25 *Ae. aegypti* using aspirator into a tube with a green dot as many as four tubes, (2) Prepared 3 tubes with red dot then inserted to special impregnated paper for *Aedes* spp. which was Malathion 0.8% and Cypermethrin 0.05%, (3) Prepared one tube with a green dot and then put the non-insecticide paper as a control, (4) mosquitoes in the tube dot green color was contacted to the red dot tube for 1 hour, the same treatment also for mosquito control, (5) Upon contact, the mosquitoes was transferred back into the green dot tube by blowing, then mortality mosquito test was observed and recorded at 30 minutes, 1 hour and 24 hours after contact, (6) After 24 hours observation, all mosquitoes were inserted into the ependorf 1.5 ml tube with silica gel, (7) During the observations temperature and humidity of the room were also recorded. Mortalitas dihitung setelah 24 jam pengamatan, selanjutnya dihitung *Lethal consentase* (LC_{50} dan LC_{95}) dan *Rasioresistensi* (RR_{50} dan RR_{95}).

The observation result of mortality mosquito test after 24 hours using resistance criteria by the WHO (2013) were as follows: mortality mosquitoes test > 98% = susceptible; mortality mosquitoes test 80-97% = tolerance (resistance suspected); mortality mosquitoes test <80% = resistant (resistant individuals present).

Geographic Information System (GIS) of Status Resistance *Ae. aegypti*. The digital map of Ternate City is obtained from the National Coordinating Agency for Surveys and Mapping (Bakosurtanal) with a scale of 1: 25.000. The process of entering the status of the vulnerability data using Microsoft Excel is then incorporated into the ArcGIS 3.8 program.

Data analysis Data were analyzed by descriptive and analytic based on the characteristics. Descriptive analysis with frequency distribution tables, numerical descriptions, graphics and images; While the analytic analysis using appropriate

statistical tests. The susceptibility test to *Ae. aegypti* against temephos, malathion and cypermethrin was measured by probit regression analysis, LT_{50} , LT_{95} , and resistance ratio (RR).

RESULTS AND DISCUSSION

Ovitrap Index (IO) of *Ae. aegypti*

The ovitrap survey results in 20 sub-districts can be seen in Figure 1. The mean value of the ovitrap index (IO) ranges from 20-60%. The high value of IO is due to the behavior of the community that is water storage especially in the dry season. Boewonoet. al. (2006) suggests that indoor ovitrap gene rates more trapped eggs than ovitrap mounted outdoor.⁽⁶⁾ The highest IO values in the home are associated with *Ae. aegypti* life cycle (biting, resting and laying eggs) in indoor. Hii (2009) states that *Ae. aegypti* mosquito lives near human (host).⁽⁷⁾

The results of this study are in line with research conducted by Rudnick (1986) in Malaysia which states that *Ae. Aegypti* most commonly found indoor, man made breeding places and indoor biting.⁽⁸⁾ Research that has been done in Babakan Village, Bogor Distric, found that larvae *Ae. Aegypti* is more dominant indoor and *Ae. albopictus* in outdoor (Wahid, 2011).⁽⁹⁾ *Ae. aegypti* is in need of optimal environmental conditions for its breeding. Harrington et al. (2005) in his research conducted that in Thailand and Puerto Rico states that urban areas with densely populated people are the mainsites for breeding *Aedes* sp.⁽¹⁰⁾

The value of IO as one of the surveillance tools *Aedessp.* can describe the density of mosquitoes in a region. Szea et al. (2007) has conducted research in Hong Kong using IO as one of the early warning systems of DHF disease transmission.⁽¹¹⁾ The percentage of positive ovitrapin forms the *Aedes* mosquito exposure rate, while the number of eggs is used to estimate the population of adult female mosquitoes (Maroto et al. 2005).⁽¹²⁾

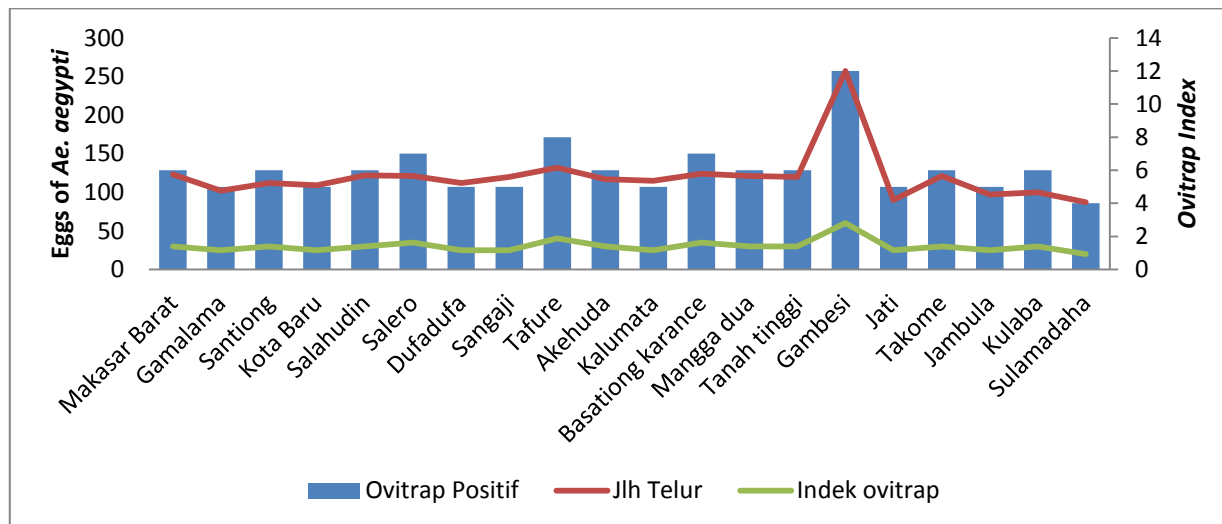


Figure 1 Ovitrap Index (IO) of *Ae. Aegypti* in Ternate City, North Maluku

Resistance Status of Larvae *Ae. aegypti* to Temephos Larvacides

Results of susceptibility test *Ae. Aegypti* against temephos in 20 sub-districts can be seen in Table 1. Lethal concentration (LC) analysis of 4 doses of temephos showed that from 20 sub-districts in Ternate City, only West Makassar urban village has $LC_{95} \leq 0.02$ mg / L. The larva *Ae. aegypti* in Kelurahan Makassar Barats susceptible to temephos. While the larvae *Ae. aegypti* from 19 sub-district are categorized resistant to temephos because it has $LC_{95} > 0.02$ mg/L. WHO (2013) has determined that the standard larvae *Ae. Aegypti* susceptible to temephos if $LC_{95} \leq 0.02$ mg/L. In general, the larvae of *Ae.aegypti* in Ternate City have resistance to larva temephos.

Ratio Analysis of Resistance (RR) at 95% Lethal Concentration, showed that larvae *Ae. aegypti* from 20 sub-districts in Ternate City have resistance to temephos ($RR > 1$). Resistance of *Ae. aegypti* in Ternate City because of the use of temephos in Ternate City more than 10 years. Temephos application interval is scheduled every month. According to Setiyaningsih *et. al.* (2015), factors affecting the occurrence of larval resistance to temephos are long-term use.⁽¹³⁾ Ahmad *et. al.* (2006) reported that *Ae. aegypti* from Surabaya has been resistant to temephos, although still in the range of low resistance with resistance ratio (RR) 4.39.⁽¹⁴⁾ Resistance of *Ae. Aegypti* against temephos has also

been reported in Bangkok with RR_{50} and $RR_{95} < 10$ and $LC_{95} > 0.01$ mg/L (Komalamisra *et. al.* 2011).⁽¹⁵⁾ Resistance of larvae *Ae. aegypti* to temephos is also reported by Bisset *et. al.* (2011) which states that larvae of *Ae. aegypti* from 15 sample sites in Havana-Cuba City has been resistant.⁽¹⁶⁾ Similarly, Loke *et. al.* (2010), found that the larvae of *Ae. Aegypti* collected from Shah Alam Selangor Malaysia area was resistant to temephos (0.02 mg/L) with LC_{50} of 0.007040-0.033795 mg/L and a resistance ratio of 1.2-6.7 times.⁽¹⁷⁾ Larvae of *Ae. aegypti* in Brazil in 2001 has been resistant to temephos (Braga *et. al.* 2004).⁽¹⁸⁾

Table 1 Resistance Status of Larvae *Ae.aegypti* against Temephos Larvacides in Ternate City, North Maluku

No	Sub-district	Mortality observation 24 h (%)				Lethal concentration**		Status
		0.005	0.025	0.125	0.625	Lc ₉₅	RR ₉₅	
1	Makasar Barat	0	42.7	93.3	100	0.012	6.0	susceptible
2	Gamalama	0	100	100	100	0.087	43.5	resistant
3	Santiong	28	100	100	100	0.087	43.5	resistant
4	Kota Baru	0	86.7	100	100	0.112	56.0	resistant
5	Salahudin	0	78.7	100	100	0.086	43.0	resistant
6	Salero	0	72	100	100	0.089	44.5	resistant
7	Dufa-dufa	78.7	100	98.7	100	0.073	36.5	resistant
8	Sangaji	0	14.7	100	100	0.123	61.5	resistant
9	Tafure	12	88	100	100	0.103	51.5	resistant
10	Akehuda	40	100	100	100	0.083	41.5	resistant
11	Kalumata	2.7	2.7	100	100	0.108	54.0	resistant
12	Bast. karance	0	6.7	90.7	100	0.138	69.0	resistant
13	Manggadua	2.7	100	100	100	0.102	51.0	resistant
14	Tanah tinggi	60	97.3	100	100	0.069	34.5	resistant
15	Jati	46.7	76	100	100	0.091	45.5	resistant
16	Gambesi	2.7	20	96	100	0.121	60.5	resistant
17	Takome	2.7	22.7	98.7	100	0.115	57.5	resistant
18	Jambula	74.7	100	98.7	100	0.067	33.5	resistant
19	Kulaba	66.7	98.7	98.7	100	0.065	32.5	resistant
20	Sulamadah	0	6.7	98.7	100	0.111	55.5	resistant
21	Control positive	92	100	100	100	0.002		susceptible

Notes :

*Resistance Status :Susceptible ($LC_{95} \leq 0.02$ mg/L), Resistant ($LC_{95} > 0.02$ mg/L)

** LC_{95} = The concentration required to kill 95% of the sample.

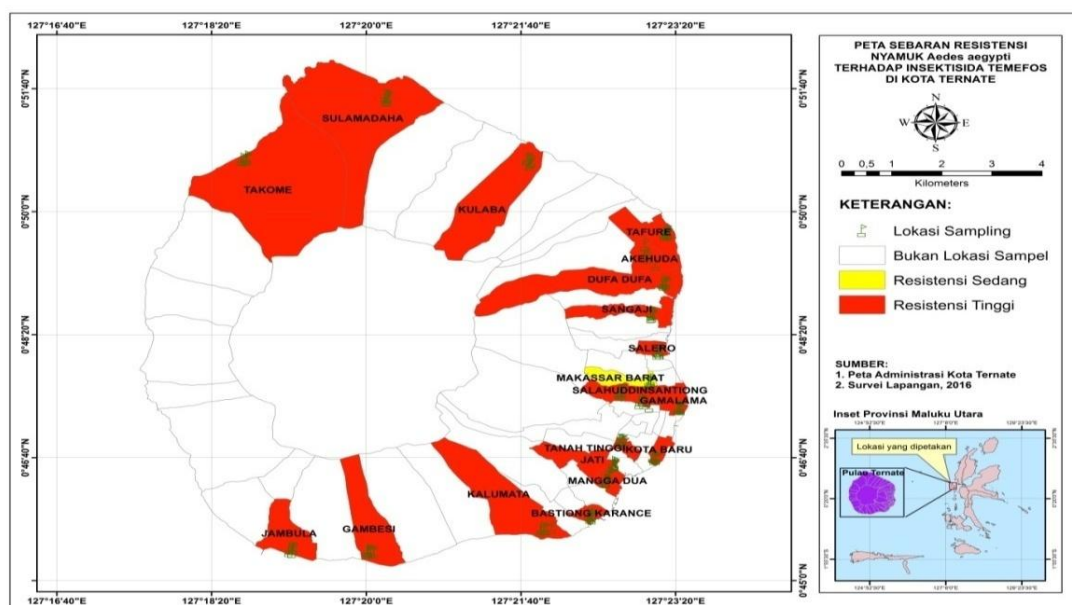


Figure 2 Mapping of resistance status of larvae *Ae. Aegypti* to temephos larvacides in Ternate City, North Maluku.

Resistance Status of *Ae.aegypti* to Malathion Insecticides

Based on the observations of mortality of *Ae. aegypti* after 24 hours indicates that from 20 sub-districts in Ternate City, only Sulamadaha sub-district has a mortality of 24 h > 80% that is 89.3%. *Ae. aegypti* in Sulamadaha sub-district has a tolerant status against malathion. While, the other 19 sub-districts have resistance to malathion because of the percentage mortality of *Ae. aegypti* <80%. Results of susceptibility test *Ae. aegypti* to malathion insecticides in Ternate City can be showed in Table 2.

Based on the result of probit analysis it is seen that Sulamadaha and Mangga Dua have moderate resistance status. While the other 18 sub-districts have very high resistance status because $RR_{95} > 10$. The time required to kill 50% of mosquitoes ranges from 1.86 hours to 69.54 hours while the time required to kill 95% of mosquitoes ranges from 22.48 to 69.54 hours. The longest time needed to knock down the 95% mosquitoes is 69.54 hours (Jambula). The shortest time required to knock down 95% of mosquitoes is 1.86 hours (Salahudin). Malathion insecticides belong to the organophosphate group. Malathion has been used

for more than 20 years for DHF vector control in Ternate City. In Year 2009, malathion has been replaced using cypermethrin insecticide. This is what causes *Ae. aegypti* in Ternate City has resistant to temephos. The results of this study are in line with research conducted by Widiarti *et. al.* (2011) states that *Ae. aegypti* in some districts in Central Java and Yogyakarta have been resistant (mortality < 80%).⁽¹⁹⁾ Mapping of resistance status of *Ae. aegypti* to malathion in Ternate City, North Maluku can be seen in Figure 3.

Several research results have been conducted in various regions also stated that *Ae. Aegypti* has resistance to malathion. The results of research conducted by Sukowati (2010) states that *Ae. aegypti* in Denpasar has resistance to malathion.⁽²⁰⁾ Resistance of *Ae. aegypti* has also been detected in the working area of Sam Ratulangi-Manado Airport Health Office (Soenjono, 2011).⁽²¹⁾ The results of Ambarita *et. al.* (2015) states that *Ae. aegypti* in South Sumatra Province has been resistant to malathion as a result of its continuous use of DHF vector control programs.⁽²²⁾

Table 2. Resistance status of *Ae. Aegypti* to malathion insecticides in Ternate City, North Maluku

No	Sub-district	Mortality (%)	Lethal time**		Resistance Ratio***		Status
			LT ₅₀	LT ₉₅	RR ₅₀	RR ₉₅	
1	Makasar Barat	30.7	27.96	43.17	27.25	18.36	Resistant
2	Gamalama	57.3	17.53	38.60	17.08	16.42	Resistant
3	Santiong	8.0	46.84	75.50	45.66	32.11	Resistant
4	Kota Baru	33.3	28.43	57.71	27.71	24.55	Resistant
5	Salahudin	50.7	22.25	36.86	21.69	15.68	Resistant
6	Salero	2.7	56.32	91.00	54.89	38.70	Resistant
7	Dufadufa	34.7	25.72	49.19	25.07	20.93	Resistant
8	Sangaji	6.7	56.68	96.69	55.25	41.13	Resistant
9	Tafure	36.0	24.27	46.90	23.66	19.95	Resistant
10	Akehuda	76.0	12.18	28.03	11.87	11.92	Resistant
11	Kalumata	68.0	10.29	28.15	10.04	11.97	Resistant
12	Bast. karance	41.3	24.73	56.57	24.10	24.06	Resistant
13	Manggadua	78.7	7.65	22.48	7.46	9.56	Resistant
14	Tanah tinggi	8.0	65.69	123.10	64.03	52.36	Resistant
15	Gambesi	54.7	17.92	37.75	17.46	16.06	Resistant
16	Jati	9.3	30.75	39.11	29.97	16.64	Resistant
17	Takome	30.7	32.33	84.68	31.51	36.02	Resistant

18	Jambula	2.7	69.54	110.73	67.78	47.10	Resistant
19	Kulaba	14.7	39.77	69.74	38.76	29.66	Resistant
20	Sulamadaha	89.3	1.86	19.23	1.81	8.18	Tolerant
21	Control positive	100.0	1.03	2.35			Susceptible

Notes :

Resistance Status :mortality mosquitoes test > 98% = susceptible;
 mortality mosquitoes test 80-97% = tolerant (resistance suspected);
 mortality mosquitoes test < 80% = resistant (resistant individuals present).

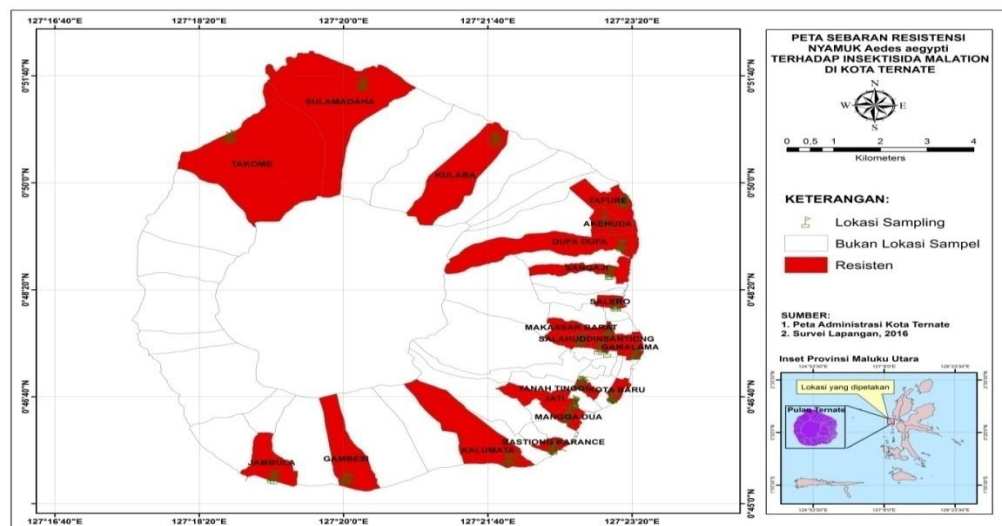


Figure 3 Mapping of resistance status of *Ae. Aegypti* to malathion in Ternate City, North Maluku

Resistance Status of *Ae.aegypti* to Cypermethrin Insecticides

Results of susceptibility test *Ae. aegypti* to malathion insecticides in Ternate City can be seen in Table 3. Based on the observations of mortality of *Ae. Aegypti* after 24 hours indicates that *Ae. aegypti* of 20 sub-districts in Ternate City has been resistant to cypermethrin because it has a mortality < 80%. Based on result of probit analysis seen that *Ae. aegypti* from 20 sub-districts in Ternate City hav

every high resistance status because of RR_{50} and $RR_{95} > 10$. The time required to knock down 50% of mosquitoes ranges from 20.92 to 72.98 hours while the time required for knock down 95% of mosquitoes ranges from 38.17-134.08 hours. The longest time needed to turn off the 95% mosquitoes in the High land colony (134.08 hours). Instead the shortest time required to knock down is 95% of the Salahudin mosquito (38.17).

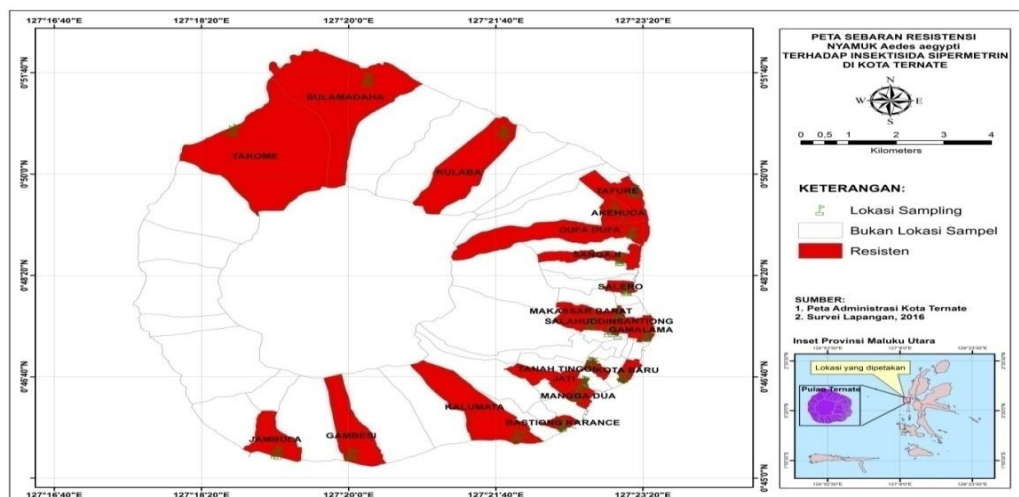


Figure 4 Mapping of resistance status of *Ae. Aegypti* to cypermethrin Ternate City, North Maluku

Tabel 3. Resistance status of *Ae. Aegypti* to cypermethrin insecticides in Ternate City, North Maluku

No	Sub-district	Mortality (%)	Lethal time**		Resistance Ratio***		Status
			LT ₅₀	LT ₉₅	RR ₅₀	RR ₉₅	
1	Makasar Barat	32.0	27.85	43.29	72.16	45.81	Resistant
2	Gamalama	38.7	25.54	41.83	66.18	44.26	Resistant
3	Santiong	2.7	49.49	72.28	128.23	76.48	Resistant
4	Kota Baru	24.0	29.68	45.32	76.88	47.95	Resistant
5	Salahudin	58.7	20.92	38.17	54.19	40.39	Resistant
6	Salero	5.3	54.49	89.25	141.17	94.45	Resistant
7	Dufadufa	9.3	43.14	70.89	111.75	75.01	Resistant
8	Sangaji	2.7	37.02	48.17	95.89	50.97	Resistant
9	Tafure	21.3	32.94	52.83	85.34	55.91	Resistant
10	Akehuda	18.7	46.94	90.92	121.59	96.21	Resistant
11	Kalumata	14.7	41.38	74.52	107.19	78.86	Resistant
12	Bast. karance	6.7	55.71	95.03	144.32	100.56	Resistant
13	Manggadua	9.3	51.43	90.90	133.23	96.19	Resistant
14	Tanah tinggi	8.0	72.98	134.95	189.07	142.80	Resistant
15	Gambesi	4.0	62.21	104.08	161.17	110.14	Resistant
16	Jati	2.7	37.02	48.17	95.89	50.97	Resistant
17	Takome	41.3	26.48	86.01	68.60	91.01	Resistant
18	Jambula	0	0	0	0	0	Resistant
19	Kulaba	45.3	21.88	94.28	56.68	99.76	Resistant
20	Sulamadaha	9.3	44.76	74.74	115.96	79.09	Resistant
21	Control positive	100.0	0.39	0.95			Susceptible

Notes :

Resistance Status :mortality mosquitoes test > 98% = susceptible;

mortality mosquitoes test 80-97% = tolerant (resistance suspected);

mortality mosquitoes test < 80% = resistant (resistant individuals present).

Resistance of *Ae. aegypti* in the Ternate City against cypermethrin occurs due to its continuous use since 2009. The results of this study are in line with research conducted by Nuanong *et. al.* (2007) which states that there has been resistance of *Ae. Aegypti* and *Ae. albopictus* from several cities in Thailand to cypermethrin insecticide with mosquito mortality < 70%.⁽²³⁾ Continuous insecticide use for DHF vector control in Ternate City causes vector resistance. Vector resistance to insecticide is greatly influenced by the intensity of the use of insecticide for a long time (\pm 2-20 years) (Georghio and Melon, 1983).⁽²⁴⁾ DHF vector control program in Ternate City should be immediately rotated insecticide carbamate group because the results of this study has shown that *Ae. Aegypti* has been resistant to organophosphate and pyrethroid. DHF control strategy should be more emphasis on preventive

strategy, that is intensification of reduction of breeding places. According to Hadi (2006) stated that the key of vector control of DHF is to improve education and public knowledge about the concept of one health that is increasing the role of cross program and cross sector, intensification of reduction of breeding places and community empowerment activities.⁽²⁵⁾

CONCLUSIONS

Larvae of *Ae. aegypti* from 20 sub-districts in Ternate City, 19 sub-districts were resistance to temephos (Lethal Concentration (LC) 95 > 0.02 mg/L), only larvae of *Ae. aegypti* from Makassar Barat has showed susceptible (LC₉₅: 0.012 mg/L). Larvae of *Ae. aegypti* has occurred resistance to temephos (Resistance Ratio (RR): 6-69). Resistance status of *Ae. aegypti* showed that *Ae. aegypti* of 19

sub-districts has been resistance to malathion (mortality 24 h <80%), only *Ae. aegypti* from Sulamadaha has a tolerant status against malathion (mortality 24 h = 89.3%). Resistance status of *Ae. aegypti* to cypermethrin showed that 20 districts were resistance (mortality 24 h <80%). Therefore, the vector control of DHF in Ternate City should be immediately insecticide rotation with carbamate group.

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