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PENELITIAN | *RESEARCH*

Temephos Resistance in *Culex quinquefasciatus* Population from Pabean Subdistrict Pekalongan

Resistensi Terhadap Temephos Pada Populasi Culex quinquefasciatus dari Kecamatan Pabean, Kota Pekalongan

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Abstrak. *Pekalongan adalah satu di antara daerah dengan kejadian filariasis limfatik sejak beberapa tahun yang lalu dan Culex quinquefasciatus merupakan vektor utama penyakit ini di Pekalongan. Penelitian ini bertujuan untuk mengetahui status kerentanan terhadap temephos dan menggambarkan aktivitas esterase pada populasi Cx. quinquefasciatus di Pekalongan. Penelitian dilakukan pada bulan April-Juli 2018. Uji kerentanan insektisida dilakukan pada larva tangkapan liar Cx. quinquefasciatus dari Pekalongan dengan dosis temephos yang diskriminatif (0,02 ppm). Aktivitas Esterase larva ini diuji secara biokimia untuk mempelajari mekanisme resistensi. Pada semua ulangan, angka kematian akibat temephos bervariasi dari 70% sampai 75%, menunjukkan bahwa Cx. quinquefasciatus di tempat penelitian resisten terhadap temephos. Hasil uji biokimia menunjukkan peningkatan aktivitas alfa dan beta esterase pada populasi Cx. quinquefasciatus. Hasil yang disajikan di sini memberikan laporan dan informasi dasar tentang status resistensi terhadap temephos pada Cx. quinquefasciatus di Pekalongan. Hal ini seharusnya menjadi perhatian bagi dinas kesehatan dalam manajemen pengendalian vektor.*

Kata Kunci*: Culex quinquefasciatus; resistensi temephos; esterases; Pekalongan*

Abstract. Pekalongan is one of the lymphatic filariasis endemic areas since many years ago and *Culex quinquefasciatus* is its main vector in Pekalongan. However, information about the insecticide resistance of *Cx quinquefasciatus* in this area is still limited. This research aimed to determine the susceptibility status of *Cx. quinquefasciatus* population in Pekalongan to temephos and describe their esterase activity. The research was conducted in April-July 2018 in Pabean, Pekalongan City. Insecticide susceptibility assays were performed on F1 generation of wild-caught larvae *Cx. quinquefasciatus* from Pekalongan which exposed to the discriminating doses of temephos (0.02 ppm). Esterase activity of these larvae was tested by biochemistry assay to describe the mechanisms of resistance. The results of this study showed that the mortality of *Cx. quinquefasciatus* which exposed to temephos was varied from 70 to 75%, indicated that *Cx. quinquefasciatus* in the area of study were resistant to temephos. While results from biochemistry assays demonstrated the increase of alpha and beta esterase activity in *Cx. quinquefasciatus* population. The results of this study provide the report and baseline information about the temephos resistance status of *Cx. quinquefasciatus* in Pekalongan. This should be an awareness for district health office in vector control management.

Keywords*: Culex quinquefasciatus*; temephos resistance; esterases; Pekalongan

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INTRODUCTION

Lymphatic filariasis is one of the parasitic diseases caused by filarial worms and it is transmitted through the bite of various types of mosquitoes. In Indonesia, there are three species of worms that have identified as the causative agent of Lymphatic filariasis: *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*. Pekalongan city is one of the endemic areas of lymphatic filariasis in Indonesia. Lymphatic filariasis disease in Pekalongan City is caused by *Wuchereria bancrofti* worm and transmitted by Culex *quinquefasciatus* mosquito as its vector. The first chronic filariasis case was found in Pekalongan City in 2002. Then it was followed by Mass Blood Survey (MBS) which found Microfilaria rate (Mf rate)>1% in that area.[1] Wahyudi reported that in Pekalongan city from 2004 to 2010 has found 179 clinical cases and 27 chronic cases of lymphatic filariasis.[2]

Previous study which conducted by Ramadhani et al (2017) mentioned that transmission of filariasis in Pekalongan City was influenced by the existence of *Cx. quinquefasciatus* mosquito breeding sites, the low coverage of Mass Drug Administration (MDA) (60.3%), the lack of population awareness on the cleanliness of the environment as well as the presence of filaria worm larvae in mosquitoes. It is also revealed that the MDA program which applied for five years has not been effective in breaking the transmission chain of filariasis in Pekalongan City.[1] Vector control program should be an important prevention effort to minimize the transmission of filariasis. However, filariasis vector control in Indonesia has not been carried out as a routine preventive program, including in Pekalongan City.

The use of insecticides in mosquitoes control programs around the world is regularly disputed, particularly because of the insecticide resistance problems. The adaptation of mosquitoes threatens the prevention of epidemics due to the absence of an alternative of insecticides. The mosquitoes can mutate to resist themselves from insecticide, so they can survive from exposure to chemical ingredients. Insecticide resistance in mosquito which develops from mutations could be transferred to their offspring, and this should be an awareness for vector control program in the following years. Mutations in mosquitoes which develop the ability to resist organophosphates (OPs) usually survive by environmental selection.[3] In general, insecticide resistance in insect population is based on three mechanisms: (1) decrease of target site sensitivity, (2) change of cuticle layer which reduces penetration of insecticide compound to body of insect, and (3) increase of detoxification enzyme.[4]

Insecticide resistance of mosquito usually develops based on one or more of those three mechanisms. In several cases, mutation in mosquitoes increases when being exposed in toxic environment. There are some genes encode esterase which play a role to metabolize insecticides before the active ingredient can inhibit acetylcholinesterase synapses. In the case of resistance, this esterase is produced in excess by a process of amplification of the number of copies of the genes which encode them in the genome or an increase in their expression. Some resistance alleles have up to 50 copies of esterase gene, while the sensitive allele contains only one copy.[3]

Temephos, belongs to the family of organophosphate (OP) group, usually applied to control immature mosquitoes vectors due to its cost-effectiveness and community acceptance.[5] A recent study showed their effectiveness as a larvicide for mosquito control.[3] Many countries used this insecticide in mosquito control. However, massive use of temephos had led to the development of resistance especially in *Aedes aegypti* population in Indonesia, even though *Ae. aegypti* larvae from Pekalongan still tolerance to this insecticide.[6]

Culex quinquefasciatus is widely distributed in Pekalongan and plays a significant role as filariasis vector, but no data are available on its resistance status to temephos. This present study aims to find out the insecticide resistance on temephos by susceptibility test and to detect the increase of esterase enzyme activity in *Cx. quinquefasciatus* larvae. Information on the mechanisms underlying the resistance of a vector population will be useful as a basis for policy in determining the basic consideration of the selection of insecticide active ingredients by the District Health Office. Resistance of *Cx. quinquefasciatus* larvae against temephos has been reported in various countries,[3,7] but has not been reported in Pekalongan City. Currently temephos was not used in *Cx. quinquefasciatus* control program as a vector of lymphatic filariasis in Pekalongan City. The results of this study hopefully will be useful as basis information for consideration of the selection of active insecticide materials by the local District Health Office in filariasis vector control program.

MATERIALS AND METHODS

This research was conducted from April to November 2018 using cross sectional design. The area of study was carried out in Pabean Sub-

Figure 1. *Culex quinquefasciatus* larvae collection site in Pekalongan City

district, Pekalongan City, Central Java Indonesia. This area was determined based on its endemicity status of filariasis (based on Pekalongan health office report).

Culex quinquefasciatus larvae samples were collected from some puddles in this village. Furthermore, *Cx. quinquefasciatus* larvae from the field were reared in the laboratory until it becomes imago and selected based on morphological characteristic for *Cx. quinquefasciatus* as the subject of this study. The adult *Cx. quinquefasciatus* were reared in the laboratory until it becomes the first generation larvae (Filial 1/F1). The area of study could be seen in Figure 1.

Preliminarily larval bioassays were conducted on late III or early IV instar healthy larvae of *Cx. quinquefasciatus* at diagnostic concentrations of temephos (0.02 mg/L).[8] Experiments were performed by placing 25 larvae in a 500 ml beaker glass containing 249 ml aquadest and 1 ml temephos solution.

Three replicates for the treated group and one for the control (using aquadest) were used for larval bioassay at room temperature (27±1°C) and 70% humidity. The proportion of dead larvae after a 24-hour holding period was calculated. Data on susceptibility test results were used to determine the susceptibility status of *Cx. quinquefasciatus* larvae against temephos with the following classification: susceptible (mortality ranged from 98-100%), tolerant or need confirmation (mortality ranged from 80-<98%), and resistant (mortality less than 80%).[8]

A total of 30 larvae of F1 *Cx. quinquefasciatus* larvae were also examined biochemically to detect esterase enzyme activity. Before the test was performed, the mosquito larvae samples were prepared individually by homogenated in 200 µl phosphate buffer (*pH* 7.4). The biochemical test was performed by referring to WHO[9] procedure as follows: The homogenates of each mosquito test were taken as much as 2x20 µl and placed into two different wells on the ELISA microplate. Aquadest was used as negative control. In one well containing homogenate was added 200 µl of alpha naphthyl acetate work solution and at another well was added 200 µl of beta naphthyl acetate beta solution.

Microplate was incubated for 10 minutes at room temperature above the shaker at 190 rpm. Furthermore, at each well added by 50 µl coupling agent Fast Blue and incubated for 10 minutes at room temperature above the shaker at 190 rpm. The test was then carried out using a microplate reader and the absorbance value was read at 450 nm. The microplate assay for non-specific esterase hydrolyzing α and β naphthyl acetate substrate were interpreted in correspondence with the experimental evidence for absorbance value of the final color intensity of the enzymatic reactions obtained by Mardihusodo[10] for α esterase, that were: (1) reactions which were colorless/faint blue showed no increased esterase activity were read at AV < 0.700; (2) reactions which were greenish blue showed increased esterase activity were read at AV > 0.700. For β esterase interpretation in correspondence with the experimental evidence for absorbance value of the final color intensity of the enzymatic reactions obtained by Zulhasril and Lesmana[11] that were: (1) reactions which were colorless/faint pink showed no increased esterase activity were read at AV <

0.400; (2) reactions which were reddish pink showed increased esterase activity were read at AV > 0.400. The number of mosquitoes with increased esterase activity were displayed as percentage. Result of experiment then presented in tabular form. This study was carried out with ethical approval from Faculty of Medicine Jenderal Soedirman University, Medical and Health Research Ethics Committee Number 1914/UN23.07.5.1/PP.1/2018.

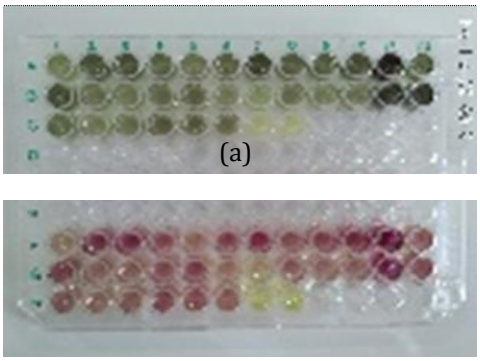
RESULTS

Larvae mortality after a 24 hours exposure to temephos at discriminating doses indicated that the field *Cx. quinquefasciatus* populations tested can be considered resistant (mortality less than 80%) (Table 1).

Tabel 1. Mortality of *Cx. quinquefasciatus* in Pekalongan toward temephos exposure

Group	n	% Mortality
Treatment	100	73.75 ± 2.5
Control	50	0

The mortality of *Cx. quinquefasciatus* larvae reached 73.75% of total tested larvae, which indicated that mosquito in the area of study categorized as resistant to temephos. While for biochemical assay showed an elevated α and β esterase activity in *Cx. quinquefasciatus* from Pekalongan (Figure 2).



(a)

(b)

Figure 2. Detection of α Esterase (a) and β Esterase (b) in Biochemical Assay

In Figure 2, the presence of color differences between the wells containing sample homogenates with wells containing negative controls reflected the activity of the α and β esterase enzymes. In the well containing negative control, the solution remains clear yellow, indicating that there was no reaction between the coupling agent (Fast Blue) and the naphtol compound. While for some wells that contain samples, the solution changes color which indicates the reaction between Fast Blue and the naphtol compound. The percentage of the increase of α and β esterase activity which measured by biochemical assay could be seen in Table 2.

Table 2. Number of *Cx. quinquefasciatus* larvae with increased α and β esterase activity collected from Pekalongan

Group	n	Increased α esterase	Increased β esterase
Cx. quinquefasciatus strain Pekalongan	30	30	96
Control (aquadest)	30	0	0

Based on Table 2, the increase esterase activity was more commonly found in β esterase than in α esterase.

DISCUSSION

Insecticide resistance detection is an important effort for vector control management. This study revealed that *Cx. quinquefasciatus* larvae in Pekalongan City categorized as resistant to temephos. This fact reflected that most of *Cx. quinquefasciatus* mosquito in the area of study would survive from temephos exposure. This result of study in accordance with previous study by Dellanay et al.[12] which showed the temephos resistance status of the Tunisian *Culex pipiens*. This agrees with our results which showing a resistance status of *Cx. quinquefasciatus* to this larvacide in Pekalongan.

Culex quinquefasciatus in Pekalongan has never been exposed directly to temephos but surprisingly this population showed resistant status to temephos. Otherwise, population of *Ae. aegypti* in this location which are relatively more frequently exposed with temephos was still tolerance to this insecticide.[6] The reason for the resistant status of *Cx. quinquefasciatus* in this study is still unknown, it could be possibly related to the breeding site of *Cx. quinquefasciatus* condition which is highly polluted with industrial waste in contrast with *Ae. aegypti* whose breeding site is in clean water. It has been shown in previous study that selection by toxic substances can increases the number of enzymes that are responsible for detoxification. The frequency of mosquitoes carrying these mutations increases in a toxic environment.[13]

Based on biochemistry assay, *Cx. quinquefasciatus* from Pekalongan City showed higher levels of esterase activity. This indicated the increase level of esterase is playing an

important role in resistance mechanism in temephos selected strain in Pekalongan City. Esterase is the detoxification enzyme which involved mainly in insecticide resistance. In Malaysia, Low et al[14] had confirmed by using biochemical tests that a major factor resulting in resistance in *Cx. quinquefasciatus* was due to elevated levels of esterase which correlated directly with malathion (OP) resistance. In addition, Widiastuti and Ikawati also had found that resistance development to malathion in *Ae. aegypti* population in Pekalongan was associated with increased esterase activity.[15]

The contribution of overproduced alpha and beta esterase in the resistance to the temephos in *Cx. quinquefasciatus* population in Pekalongan City was confirmed by the result of biochemistry assay using microplate. The increase in esterase activity in *Cx. quinquefasciatus* population in Pekalongan City was more commonly found in β esterase than in α esterase. However, both α and β naphthyl substrates are carboxylic esters. Esterase in arthropods including *Cx. quinquefasciatus* can be classified according to alpha or beta esterases depend on their ability to hydrolyze the substrates alpha- and beta-naphthyl acetate respectively.[16] Esterase that hydrolyze alpha and beta-naphthyl acetate are designated α esterase and β esterase, respectively.[16] The esterase enzyme is capable to hydrolyze temephos which is one of the insecticides from organophosphate group. Grigoraki stated that Carboxyesterases (CCEs) from Aedes vectors associated with temephos resistance. His study showed that Carboxyesterases metabolize temephos oxon to a temephos monoester, so this insecticide loses its function. [17]

It should be noted that despite many studies reported the temephos resistance in mosquitoes, the involved mechanism is not well characterized. Our investigation reported that temephos resistance could be explained by the increased of detoxification by esterase enzymes. This study showed that *Cx. quinquefasciatus* population from Pekalongan were resistant to temephos. Metabolic mechanisms may be contributed to temephos resistance in *Cx. quinquefasciatus* populations because the biochemical assay showed that there was an evidence of elevated esterase activity in the population in study area. Within mosquito populations which resistant to insecticide with metabolic mechanisms, replacement of insecticides should be made by different chemical structures. Hence, an alternative vector control program to reduce the density of *Cx. quinquefasciatus* is urgently required in Pekalongan. In addition, the application of Sterile Insect Technique (SIT) also could be developed to break the chain of lymphatic filariasis transmission in this area.

CONCLUSION

The population of *Cx. quinquefasciatus* in Pekalongan City was resistant to temephos. Based on biochemistry assay, it was revealed that insecticide resistance in *Cx. quinquefasciatus* population to temephos was based on an enzymatic mechanism which indicated by the increase of enzymes α and β esterase activity. This result should be an awareness to local health officer for *Cx. quinquefasciatus* mosquito control management.

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AUTHORS CONTRIBUTION

Dyah Widiastuti is the main contributor in this article. All authors listed in this article have contributed according to their expertise showed in the following roles:

Conceptualization	**:**	DW
Data Curation	**:**	DW
Formal Analysis	**:**	DW
Funding Acquisition	**:**	SMW
Investigation	**:**	SN
Methodology	**:**	SN
Project Administration	**:**	SN
Resources	**:**	SMW
Software	**:**	SN
Supervision	**:**	DW
Visualization	**:**	DW, SN
Writing-Original Draft Preparation	**:**	DW

Writing-Review & Editing : DW, SMW

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