

Artikel 9. High Level of Resistance - 5664-16949-1-PB

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High Levels of Resistance in A *Culex quinquefasciatus* Population to the Insecticide Permethrin in Filariasis Endemic Areas in Central Java

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Abstract

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Resistance among *Culex* mosquitoes to various insecticides has been reported in many countries. However, there have been no studies of the resistance status of *Culex* in Indonesia. There is a need for such studies to develop a database for use in vector control management. This study aimed to investigate the insecticide resistance status of *C. quinquefasciatus*, which is the primary vector of filariasis, to aid the planning of a vector control management program. In the present study, *Culex quinquefasciatus* larvae were collected from five districts/municipalities where filariasis is endemic in Central Java. The larvae were reared to adult stage, and insecticide susceptibility testing was then conducted according to standard bioassay procedures of the World Health Organization (WHO). The results of the bioassays showed that *C. quinquefasciatus* had a high level of resistance against 0.75% permethrin, with mortality rates ranging from 4.8 to 21.6%. The lowest resistance was found among mosquitoes collected from Grobogan district. This may be explained by the district's remote geographical location. The high level of resistance found in the present study may be caused by exposure to local insecticides, which have been applied for many years as part of a dengue vector control program. These insecticides may also have contaminated the breeding sites of *C. quinquefasciatus* mosquitoes. Better vector control management is needed to help prevent the development and spread of resistance. Such management should include routine insecticide surveillance and insecticide alternation.

Abstrak

Tingkat Resistensi Tinggi Pada Populasi *Culex quinquefasciatus* Terhadap Insektisida Permethrin di Daerah Endemis Filariasis, Jawa Tengah Resistensi pada nyamuk *Culex* terhadap berbagai macam insektisida telah dilaporkan di banyak negara. Namun demikian, data terkait status resistensi *Culex* di Indonesia masih sangat terbatas. Oleh karena itu, diperlukan studi terkait resistensi *Culex* sebagai basis data untuk pengembangan manajemen pengendalian vektor. Tujuan penelitian adalah menginvestigasi status resistensi terhadap insektisida pada populasi *Culex quinquefasciatus*, yang merupakan vektor utama filariasis, dan untuk membantu perencanaan program manajemen pengendalian vektor. Pada studi ini, larva *Culex quinquefasciatus* diperoleh dari lima kota/kabupaten yang endemis filariasis di Jawa tengah. Larva dibesarkan sampai menjadi dewasa, dan kemudian diuji menggunakan metode pengujian suseptibilitas sesuai prosedur standar bioassay dari World Health Organization (WHO). Hasil pengujian suseptibilitas menunjukkan bahwa *C. quinquefasciatus* memiliki level resistensi tinggi terhadap permethrin 0.75%, dengan angka kematian berkisar antara 4.8% hingga 21.6%. Resistensi terendah berasal dari populasi nyamuk yang dikoleksi dari Kabupaten Grobogan. Hal ini dapat dijelaskan oleh area geografis Kabupaten Grobogan yang terletak paling terpencil. Tingginya level resistensi mungkin disebabkan oleh paparan insektisida lokal, yang telah diaplikasikan bertahun-tahun sebelumnya sebagai bagian dari program pengendalian vektor dengue. Insektisida tersebut mungkin telah mengkontaminasi tempat perindukan nyamuk *C. quinquefasciatus*. Manajemen pengendalian vektor yang lebih baik dibutuhkan untuk mencegah pengembangan dan persebaran resistensi terhadap insektisida. Manajemen tersebut harus mencakup surveilans insektisida dan pengubahan jenis insektisida secara rutin.

Keywords: *Culex quinquefasciatus*, High resistance status, Central Java, Permethrin

Introduction

Mosquitoes¹ belonging to the genus *Culex* are wide spread in tropical, subtropical, and temperate climates worldwide, especially in urban areas [1]. *Culex pipiens* and *C. quinquefasciatus* are the main vectors of filariasis in many regions of the world [2-4], with the distribution of *Culex* mosquitoes in various countries, including Indonesia, directly associated with the incidence of filariasis [5]. As shown by a survey of vector density, mosquitoes belonging to the genus *Culex* predominate in Malaysia and Indonesia [6-8]. One study reported that the density of *C. quinquefasciatus* was 5.91 mosquitoes/person/hour indoors and 4.75 mosquitoes/person/hour outdoors [9]. Research also showed that the infectivity of *Culex* microfilariae was higher than that of microfilariae from other genera [10]. The existence of habitats suitable for *C. quinquefasciatus* is associated with the incidence of filariasis [11].

¹ Vector control is an important element of any strategy aimed at controlling vector borne diseases, and chemical control is the most widely used approach [12]. Research has demonstrated the effectiveness of insecticides in reducing the incidence of vector borne diseases, including malaria [13]. However, more frequent use of insecticides in the last decade has been directly linked to the development of resistance genes and increased resistance among *Culex*. Data are lacking on vector resistance to insecticides, as many countries do not perform routine susceptibility testing [14].

Many countries have reported that *Culex* mosquitoes are resistant to various insecticides. For example, research conducted in India indicated that *C. quinquefasciatus* was highly resistant to dichloro-diphenyl-trichloroethane, more commonly known as DDT, and malathion [15]. A study conducted in Zambia demonstrated that *Culex* was resistant to pyrethroids, permethrin, and deltamethrin [16]. In Kuala Lumpur, larvae of *C. quinquefasciatus* were resistant to malathion and permethrin [17], and they were resistant to pyrethroids in Zanzibar [18]. The insecticide resistance status of *Culex* has not been reported in Indonesia. Therefore, this study aimed to investigate the insecticide resistance status of *C. quinquefasciatus*, which is the primary vector of filariasis, to aid the planning of a vector control management program.

Materials and Methods

Study sites and mosquito collection. A larval survey of stagnant water, puddles, sewage, and excavation sites was first conducted based on the latest filariasis incidence data for 2014 [unpublished result from authorized provincial health department]. The survey included a

special exhaust deliberately in shape by local people around filariasis patients over which being a breeding

place of the vector. A modified larval collector tool was used to collect the larvae. The survey was conducted during February–April 2015 in five districts/ municipalities in Central Java known to be filariasis endemic areas: Semarang (Sendangguwo), Demak (Wonosari), Jepara (Lebak), Grobogan (2) (Sangri), and Pekalongan (Wiroiditan) (Figure 1). Permission to conduct mosquito and larval collection in households was given by the provincial and district/municipality authorities following recommendations by the relevant district and municipal health departments. *C. quinquefasciatus* larvae were reared in the laboratory to imago stage (3–5 days after hatching). Post-hatching, the mosquitoes were collected in containers and subjected to susceptibility tests, as described below. Additionally, the method to identify the species was based on our previous publication [19].

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Insecticide susceptibility tests. All insecticide susceptibility tests were performed using standard WHO bioassay procedures, using diagnostic 2 test kits and nitrocellulose paper impregnated with one of several pyrethroids or organophosphate compounds [20]. To assess cross-resistance to these compounds, the test papers included 0.75% permethrin.

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In each test group, 125 female mosquitoes 2 from each specified location of district/municipality were divided into five tubes, and each tube contained 25 adults. Four replicates of each treatment compound were established, in conjunction with a matched control. The test was repeated three times for each insecticide, as recommended by WHO protocols [20]. Temperature and humidity during the test were maintained at ranges of 25 ± 2 °C and $80\% \pm 10\%$, respectively. The following day (after 24 h), the numbers of dead mosquitoes were calculated to determine the resistance status. The resistance status of the mosquitoes was defined as follows: resistant if mortality was $< 90\%$, tolerant if mortality was $90\text{--}98\%$, and susceptible if mortality was $> 98\%$ [21–22]. The resistance ratio was calculated using probit analysis, as described previously [23–24].

District (5). All the locations are filariasis endemic areas

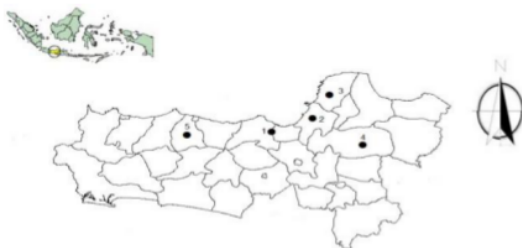


Figure 1. Map Illustrating the Sample Locations in Central Java. Semarang city (1), Demak District (2), Jepara District (3), Grobogan District (4), and Pekalongan

Data analysis. A map of the sample sites was constructed using ArcView 3.3. The coordinates were collected using eTrex® 10 global positioning system (Garmin, USA). Graphical data used to describe the findings were analyzed with R 3.2.1 (Stanford University, CA, USA) and the Microsoft Excel program (Microsoft corporation, USA).

Results and Discussion

In the morphological analysis, all the samples were identified as *C. quinquefasciatus*. Adult *C. quinquefasciatus* mosquitoes have dappled black and white across its abdominal segment, with a black head and white color at the edges. On the thorax, there are two curved white lines. White arches at the back section, characteristics typical of *C. quinquefasciatus*, were also observed.

In the susceptibility tests, after exposure to the insecticide for 24 h, the mortality rates ranged from 4.8% ($n = 6$, Semarang city) to 21.6% ($n = 27$, Grobogan district), with average mortality of 9.6% ($n = 12$) and a standard deviation of 8.515 9 (Table 1). These results indicate that mortality among this *C. quinquefasciatus* population was less than 80%. Figure 2 showed the proportion of mosquitoes that knocked down before 24 h holding time. After holding time, it can be confirmed whether the mosquitoes are truly susceptible or resistance.

The results of the probit analysis indicated that the KDT50 (i.e., time to knock down 50% of the total population) of *C. quinquefasciatus* after exposure to the insecticides ranged from 49.67 to 975 min, whereas the KDT95 (i.e., time to knock down 95% of the population) ranged from 86.3 to 1815 min (Fig. 3). Figure 4 showed the relationship between KDT95 with mortality. Grobogan had the lowest KDT95 and resulted in high mortality.

As shown by the results of the bioassay of resistance in *C. quinquefasciatus* samples collected in Central Java, the mosquito population showed a high level of resistance to 0.75% permethrin, with mortality rates ranging from 4.8% to 21.6%. The KDT50 was also high (range of 49.67 to 975 min) (Fig. 3). These findings differ from those reported in previous studies, which

found that the KDT50 for mosquitoes exposed to 0.75% permethrin ranged from 40 to 50 min [25] in Malaysia and 33.1 min in Thailand [26]. The high resistance of *C. quinquefasciatus* to permethrin in Central Java is in contrast to reports in other countries, which found that *Culex* was vulnerable to 0.75% permethrin [25-27]. In the present study, the mortality rates of *C. quinquefasciatus* in the insecticide susceptibility tests were relatively higher than those reported for isolates sampled in Pathum thani, Nonthaburi, and Bangkok in Thailand, with studies reporting mortality rates following exposure to permethrin of $67.4 \pm 2.4\%$, $72.4 \pm 0.4\%$, and $80.6 \pm 1.7\%$, respectively [28]. Studies conducted in Thailand reported high levels of resistance in *C. quinquefasciatus* to 0.75%

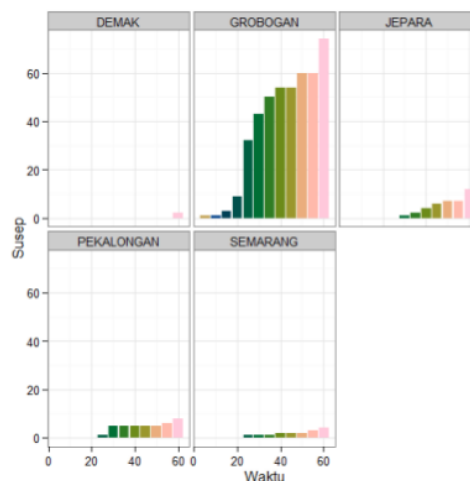


Figure 2. Susceptibility Testing of the Mosquitoes, Showing the Number that Died Before 24 h. “Susep” Indicates the Number of Mosquitoes that Died, and “Waktu” Indicates the Time at which the Number of Dead Mosquitoes was Counted (5–60 min Divided into 5-min Segments, Indicated by Different Colors on the Graph), Based on WHO Standard Procedures for Insecticide Susceptibility Testing.

Table 1. Results of Susceptibility test of *C. quinquefasciatus* to Permethrin Insecticide

Sample sites		Water pH	Number of mosquitoes		Mortality (%)	Resistance status
District/municipality	Location		Tested	Dead		
Semarang city	Sendangguwo	6.5	125	6	4.8	Resistant
Demak district	Worosari	6.6	125	8	6.4	Resistant
Jepara district	Lebak	7.2	125	10	8	Resistant

Grobogan district	Bangsri	6.8	125	27	21.6	Resistant
Pekalongan district	Wiroditan	7.0	125	9	7.2	Resistant

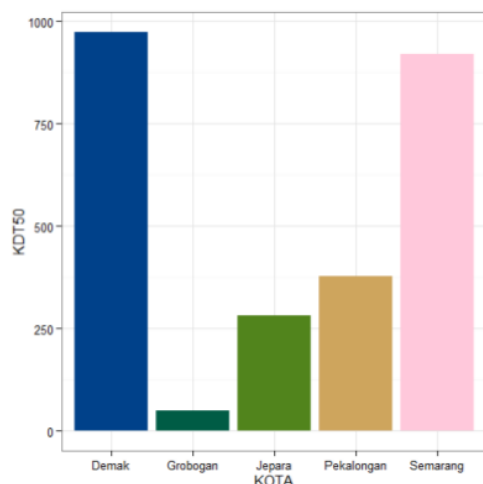
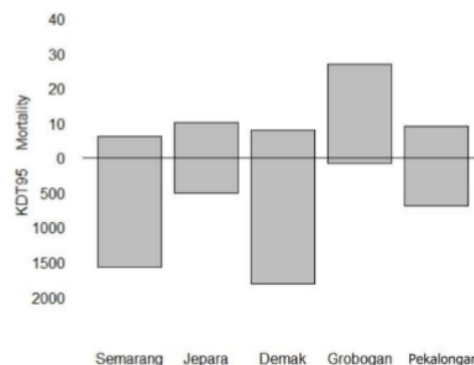


Figure 3. Knock Down Time Data, Showing the Number of Mosquitos that died Following Exposure to Permethrin. KDT50 Refers to the Time it Took to Knock Down 50% of the Total Mosquito Population



cides, which are widely employed in Central Java, household insecticides and repellants containing active compounds (pyrethroids) are increasingly used [33].

permethrin, with resistance of 4–24% found in Benin [29] and resistance of 10.1% found in Baan Suan [30].

Resistance among *C. quinquefasciatus* populations in Indonesia has not been reported previously. The high rate of resistance among *C. quinquefasciatus* in Central Java has important implications for insecticide-based vector control programs. In line with this study, previous studies of the distribution of vector resistance reported increased resistance among *Aedes* mosquitoes in Indonesia [31,32]. High resistance among *Aedes* was also reported in Central Java, with mortality ranging from 1.6 to 15.2% and the KDT50 ranging from 120.2 to 1337.7 min [33].

In the present study, the distribution of insecticide resistance to pyrethroids varied, depending on the region, with a very high level of resistance in four districts (Semarang, Pekalongan, Demak, and Jepara) but lower resistance in Grobogan. Geographically, Grobogan is a much more remote area than the other regions. Nevertheless, the level of resistance was still relatively high. The high level of resistance may be caused by exposure of *C. quinquefasciatus* to local insecticides as part of a dengue vector control program, which has been ongoing for many years, especially in urban areas (Semarang, Demak, Pekalongan and Jepara). Moreover, breeding sites of *C. quinquefasciatus* mosquitoes may have been contaminated with insecticides [34]. Other than insecti-

Figure 4. Susceptibility Distribution and KDT95 of *C. quinquefasciatus* to Permethrin Insecticide in District/municipalities in Central Java. The Mortality Rates in Four Areas (Semarang City, Demak District, Jepara District, and Pekalongan District) were Relatively Lower than in Grobogan District

Interestingly, resistance to insecticides may develop faster in *C. quinquefasciatus* than in other mosquitoes [35]. Given the close interrelatedness between the development of resistance and intensity of insecticide exposure, the use of insecticides in vector control programs needs to be evaluated. Page: 152 In addition, programs are needed to educate the public about the effects of household insecticide use.

Conclusions

In conclusion, long-term and intensive use of insecticides have led to the development of insecticide-resistant phenotypes. Selective pressure resulting from continuous insecticide use favors the development of resistance among mosquitoes [36]. The frequency of these mutants may dramatically increase, and they may become widespread within a population [37], as demonstrated in a recent study. Despite the rapid evolution of resistant mosquitoes, as indicated in recent laboratory studies [38-39], the "resistance" background can rapidly revert back to susceptible when selective pressure is relaxed [40]. Routine surveillance is needed in mosquito vector control management programs based on the application of insecticides to avoid the spread of resistance.

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