ABSTRACT

**Objectives**: Keligesing Subdistrict of Purworejo Regency has been identified as malaria endemic area with a high Annual Parasite Incidence (API) of 2.07%. Yet, intensive vector control in endemic areas can increase vector resistance to insecticides. The aim of this study was to assess the resistance status of *Anopheles spp* to *Lambdacyhalothrin* 0.05% insecticide in Keligesing Subdistrict of Purworejo Regency.

**Methods**: Analytical cross sectional research was conducted by examining in the laboratory the resistance status using susceptibility test method, namely impregnated paper. Respondents of 100 people residing in six malaria endemic villages were interviewed with structured questioner for the insecticide program and community use.

**Results**: The results showed that the most commonly *Anopheles spp* mosquitoes found were *Anopheles maculatus*, *Anopheles aconitus*, and *Anopheles balabacensis*. *Anopheles spp* mosquitoes were resistant to *Lambdacyhalothrin* 0.05% with an average knock down of 60 minutes as many as 20 mosquitoes (80%), and the average mortality of mosquitoes of 24 hours after holding was 17.5 mosquitoes (70%).

**Conclusions**: It concluded that the average knock down of the *Anopheles spp* mosquito against *Lambdacyhalothrin* insecticide being exposed for 60 minutes is 20 (80%). Meanwhile, the average mortality of mosquitoes after 24 hours holding period is 17.5 (70%). *Anopheles spp* mosquitoes in the highland area of Keligesing Subdistrict, Purworejo Regency are resistant to the *Lambdacyhalothrin* insecticide 0.05% with mortality of less than 80%. As an effort to control vector resistance, periodic monitoring and evaluation are needed and rotations in using different insecticides should be applied.

Keywords: Resistance, Anopheles spp, Lambdacyhalothrin 0.05%, Impregnated paper


INTRODUCTION

Malaria is an infectious disease caused by plasmodium and transmitted through the bite of female *Anopheles mosquitoes*[1,2]. In Indonesia, among 80 species of *Anopheles* mosquitoes, 24 of which have been proven to be the transmitters of malaria. Every species of mosquito has different properties, depending on climate, geography, and breeding place [3]. From its breeding sites, the species of *Anopheles* mosquito in Indonesia can often be found in rice fields, hilly/forest areas, and coastal areas/streams river [2].

In Indonesia, malaria is found almost in every region including Central Java Province, where among the 35 regencies/cities, 10 of which have been declared to be malaria endemic areas [4]. The regencies identified of
having high malaria cases are the Regency of Banjarnegara (0.36%), Magelang (0.13%), Purbalingga (0.14%), and Purworejo (1.96%) [5].

Purworejo is one of the malaria endemic regency that is accounted for the highest number of malaria cases in Central Java [2], although the cases fluctuated over the past five years. From 2012 to 2014, the cases increased to be 803, and declined in the following 2 years to be 423 or API 0.59%, the level at which the cases had reached the API target of less than 1% [6-8]. The malaria cases in Purworejo District often occur in highland area [7], where Kaligesing Sub District has the highest malaria cases.

Kaligesing, one of the sub districts of Purworejo District, has the highest number of malaria that belongs to the Middle Case Incidence area [7]. Geographically, Kaligesing Sub District borders on Kulonprogo District; an area having a hilly topography and is located on a plateau of 200 meters above the sea level [9]. In this particular area, many villages have been identified to be endemic malaria that tend to be spread widely since 1996; from 23 to 36 villages including HCl areas [10]. The most common species of mosquitoes causing malaria that often occurs in hilly and upland areas are Anopheles balabacensis and Anopheles maculatus [11].

Meanwhile, in the last five years (2013 – 2017), Kaligesing had always contributed the highest number of cases, as the incidences identified were 215 (API 6.02 ‰), 250 (API 7 ‰), 220 (API 6.16 ‰), 144 (API 4.85 ‰), and 37 (API 1.24 ‰), respectively [11]. Although for three years there had been a decline, there was an increase in cases by 62 (API 2.07 ‰) in January 2018 [12].

In Purworejo Regency, the efforts to do vector control have been conducted using insecticide-treated mosquito nets and Indoor Residual Spraying (IRS) [13]. In particular, in 2014-2016, the insecticide used was ambdacyhalothrin icon 100 CS, whereas Lambdacyhalothrin Icon 25 EC have been used since 2017 [7]. Lambdacyhalothrin is one of the pyrethroid insecticide that is widely used as an active substance in controlling malaria [14].

Previous studies conducted in Central Java and Yogyakarta Province have identified the decline of the vulnerability of malaria vectors to pyrethroid insecticides by 86% -97.5% [15]. Meanwhile, a research conducted in Tojo District of Central Sulawesi Province found the resistance of Anopheles barbirostris mosquito to Lambdacyhalothrin 0.05% with 73.4% death [16]; however, a research conducted in the Bulukumba area found the susceptibility of Anopheles spp mosquitoes to Lambdacyhalothrin insecticide 0.05% [17]. In addition, a research conducted in Republic of Africa testing the vulnerability of Anopheles funnetus concluded that the Lambdacyhalothrin insecticide causing death to An.funetus is only as small as 35% [18]. Several studies suggested that the resistance can occur as a result of cross-crossing, in which the resistance to an insecticide is caused by the exposure to other insecticides [19].

The massive use of insecticides to control malaria in Purworejo and the absence of monitoring regarding the status of vector resistance to insecticides have become a supporting factor in conducting the "Susceptibility Test on Anopheles spp. Mosquito against Lambdacyhalothrin 0.05% in Kaligesing District of Purworejo Regency. The result of the study could be reference by program of the district to control malaria vector, mainly in endemic area of Kaligesing sub district.

METHODS

This research was conducted in Kaligesing Sub-District, Purworejo Regency for 10 months (February-November 2018). The subject of the study were adult female Anopheles mosquito, 0.05% Lambdacyhalothrin insecticide, insecticide impregnated paper, HVS paper, and sugar solution. The tools used were cup, label paper, stereo
microscope, aspirator, susceptibility test kit, maintenance tube, thermometer, hygrometer, stopwatch, adhesive paper, bottles, observation forms, gloves, and stationery.

The method used was quasi experimental with static group comparison design. The sample used was female *Anopheles spp* mosquitoes selected by purposive sampling from the survey. The subject was caught from 06:00 a.m. – 06:00 p.m. in 100 houses.

This study was initiated by collecting larvae samples in 3 replication in order to confirm the existence of mosquito species chosen to be the subject of the study. The collected larvae were kept in the laboratory for 3-5 days until they turn into mosquitoes, and are identified and separated. The female *Anopheles* mosquito species were re-selected for rearing until their F1 were born, and the F1 were selected to be the subjects in the mosquito susceptibility test.

In the process of the mosquitoes vulnerability test, susceptibility test method with impregnated paper following WHO standards was carried out with four repetitions. Each of the repetition consisted of 25 mosquitoes (100 mosquitoes in total), and the other 50 mosquitoes were put in 2 control tubes.

Abbots Formula was used to determine the mosquitoes’ vulnerability status based on the deaths percentage of the tested mosquitoes after 24 hours of observation with the following criteria:

- <90% deaths = resistant
- 90- <98% death = tolerance
- 98-100% deaths = vulnerable

In addition to measuring the resistance status of the vector, interviews were also carried out with 100 respondents living in malaria endemic areas. Respondents were interviewed regarding the use of pesticide bed nets and household insecticides.

The collected data were analyzed descriptively in the form of tabulations.

**RESULTS**

The data of the vulnerable test were collected in two observations; *Lambdacyhalothrin* 0.05% exposure for 60 minutes (knock down) and 24 hours post holding (number of mosquito deaths). After being exposed for 24 hours, identification of the species of the mosquitoes being tested was carried out.

Table 1 exhibits the distribution of knock down of *Anopheles spp* mosquitoes after being exposed to *Lambdacyhalothrin* 0.05% for 60 minutes. In the first 10 minutes of the exposure period, the number of knock-down mosquitoes in average are 7, and it increases following the period of the exposure. The highest number of mosquitoes knock-down, 25 in average, occurs in the 50th and 60th minutes. Meanwhile, the number of control mosquitoes (K1 and K2) being knocked-down in four repetitions is 0.
Table 1. Knock down Effect of *Anopheles* spp mosquitoes after being exposed to *Lambdacyhalothrin* 0.05% for 60 minutes

<table>
<thead>
<tr>
<th>Exposure Time (minutes)</th>
<th>Treatment 1&lt;sup&gt;st&lt;/sup&gt;</th>
<th>Treatment 2&lt;sup&gt;nd&lt;/sup&gt;</th>
<th>Treatment 3&lt;sup&gt;rd&lt;/sup&gt;</th>
<th>Treatment 4&lt;sup&gt;th&lt;/sup&gt;</th>
<th>Control K1</th>
<th>Control K2</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>6</td>
<td>9</td>
<td>5</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>15</td>
<td>20</td>
<td>16</td>
<td>16</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>30</td>
<td>20</td>
<td>23</td>
<td>22</td>
<td>23</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>40</td>
<td>23</td>
<td>24</td>
<td>23</td>
<td>25</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>50</td>
<td>24</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>60</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>average</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

1, 2, 3, 4 = replication of treatment  
K1, K2 = replication of control

The result of the holding after mosquitos being exposed to insecticide was 17.5 deaths mosquitoes (70%); whereas, no control mosquitoes in either K1 or K2 (0.0%) were death for 24 hours after holding. The holding of the tested mosquitoes was carried out at the temperature of 27°C-29°C with air humidity of 75%. This finding suggested that *Anopheles* spp mosquitoes were resistant to *Lambdacyhalothrin* insecticide 0.05% proven by 70% of death (death <80%) as shown in Table 2.

Table 2. Distribution of Deaths of *Anopheles* spp mosquitoes after a holding period for 24 hours

<table>
<thead>
<tr>
<th>Replication</th>
<th>Treatment</th>
<th>Control</th>
<th>Number of mosquitoes tested</th>
<th>Average of deaths</th>
<th>Average of deaths (%)</th>
<th>Number of mosquitoes control</th>
<th>Average of deaths control</th>
<th>% of deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>25</td>
<td>15</td>
<td>60</td>
<td>25</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>25</td>
<td>20</td>
<td>80</td>
<td>25</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>25</td>
<td>17</td>
<td>68</td>
<td>25</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>25</td>
<td>18</td>
<td>72</td>
<td>25</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td></td>
<td></td>
<td>17.5</td>
<td></td>
<td></td>
<td>70</td>
<td>Average</td>
<td>0</td>
</tr>
</tbody>
</table>

1, 2, 3, 4 = replication of treatment  
K1, K2 = replication of control

The highest number of mosquito species obtained during the survey was *Anopheles maculatus*, which was 53 mosquitoes (35.3%), and the least number of mosquitoes obtained is *Anopheles barbirostris*, which is 3 mosquitoes (2%).

Table 3. Types of Mosquitoes Found During Identification

<table>
<thead>
<tr>
<th>Jenis Nyamuk</th>
<th>Amount</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Anopheles maculatus</em></td>
<td>53</td>
<td>35.0</td>
</tr>
<tr>
<td><em>Anopheles aconitus</em></td>
<td>37</td>
<td>24.7</td>
</tr>
<tr>
<td><em>Anopheles balabacensis</em></td>
<td>21</td>
<td>14.0</td>
</tr>
<tr>
<td><em>Anopheles vagus</em></td>
<td>16</td>
<td>10.7</td>
</tr>
<tr>
<td><em>Anopheles barbirostris</em></td>
<td>3</td>
<td>2.0</td>
</tr>
<tr>
<td><em>Anopheles flavirostris</em></td>
<td>7</td>
<td>4.7</td>
</tr>
<tr>
<td><em>Anopheles minimus</em></td>
<td>13</td>
<td>8.7</td>
</tr>
<tr>
<td>Jumlah</td>
<td>100</td>
<td>100.0</td>
</tr>
</tbody>
</table>
Meanwhile, the results of the interviews showed that 61% of the respondents used insecticide-treated bed nets containing insecticides belonging to the same group, Lambdacyhalothrin insecticide, 70% of the respondents claimed to have carried out IRS in their homes, and 30% of the respondents used insect repellent with different formulations.

**DISCUSSION**

**Mortality of Anopheles spp Mosquito to an Exposure of Lambdacyhalotrin 0.05%**

The result of the Anopheles spp mosquitoes exposed to Lambdacyhalothrin insecticide 0.05% for 60 minutes confirmed that the knock down mosquitoes occurred in all repetitions. Furthermore, the average number of the knock down mosquitoes increased following the duration of exposure given. This finding was consistent with the one of the research on vulnerability status of Anopheles sundaicus mosquito conducted by Seniawati and Hakim and in Garut that the longer the mosquito is exposed to an insecticide, the greater the number of knock down time of the mosquito [20].

As for the observations for 24 hours post holding, the result showed that the number of the dead mosquitoes at every repetition varied. The percentage of the dead mosquito after 24 hours of post holding could be identified from the number of knock down mosquitoes during the 60 minute exposure period. The percentage of knock down mosquitoes at 60 minutes exposure will not differ greatly from the number of dead mosquito during the 24-hour holding period [16,17].

The results of the study on the susceptibility test of Anopheles barbirostris mosquitoes to Lambdacyhalothrin and Delthamethryn showed that there is no significant difference between the percentage of the final exposure and the percentage of dead mosquito during 24 hours of observation [17]. This finding was relatively similar to the one of this study that the percentage of the knock down mosquitoes after being exposed for 60 minutes was 100% in all repetitions; however, the number of dead mosquito after being exposed for 24 hours in four repetitions was 60%, 80%, 68%, and 72%.

Furthermore, this research found that not all mosquitoes tested died after 24 hours holding. Several possible factors might become the reasons; such as, genetic, biological, and operational factors [21]. The survival mosquitoes might have had changes the target location in the pyrethroid as well as physiological changes in the body of the mosquitoes. The physiological changes that could happen due to mutation affected by long-term insecticides exposure caused insects capable of overcoming exposed toxin in their body, called Knock down Resistance (KDR). The resistance mechanism triggered KDR mainly occurs in pyrethroid class insecticides [22].

Umar et al 2014 in their research on the susceptibility status of Anopheles spp mosquito to Lambdacyhalothrin in Northeast Nigeria stated that 83.3% of mosquitoes are subjected to knock down; however, 93.3% of mosquitoes after being held for 24 hours do not die [23].

The disproportional of the number of mosquitoes having knock down during the 60-minute exposure period and the number of dead mosquito after the 24-hour holding might be caused by small insecticide dosage given so that it could not penetrate the mosquito's cuticle layer. Wood et al, in a study about the relationship of cuticle thickening on pyrethroid resistance, reported that female Anopheles fenestus mosquitoes which are resistant to insecticides tend to have thicker cuticles compared to vulnerable and tolerant ones [24].
The result of the test using Abbots Formula to calculate the number of dead mosquitoes verified that the number of dead mosquito was 70%. This finding suggested that the Anopheles spp mosquito in Kaligesing Subdistrict was resistant to Lambdacyhalothrin insecticide 0.05%.

**Distribution of Anopheles spp in Purworejo Regency**

The discovery of the Anopheles maculatus mosquito, the most commonly found species in the Purworejo Regency, was in line with the finding of Zumrotus et al regarding the characteristics and risk factors of malaria in Purworejo that the main vector of malaria in Purworejo is the species of Anopheles maculatus[6]. Anopheles maculatus mosquitoes breed in the river bank that are puddle, cloudy, and shallow. In addition, the breeding sites are also found in mountainous and hilly areas, which are mostly found in Kaligesing Sub-District [6,25], an area that has mountainous and hilly topography and is located in the upland with many rivers [10,25].

**Vulnerability Status of Anopheles spp**

As the number of dead mosquitoes was 70%, the Anopheles spp mosquito in Kaligesing District proved to be resistant to Lambdacyhalothrin insecticide 0.05%. The finding of this study was in accordance with the one of Widiarti et al concerning the susceptibility test of mosquitoes to pyrethroid insecticides who identified the decreased susceptibility of the vectors found in Central Java and DIY. The decreased susceptibility was 85% in the Anopheles aconitus mosquitoes and 97% -97.5% in Anopheles maculatus mosquitoes. In other word, the vulnerability of the Anopheles mosquitoes in DIY and Central Java decreased by 86% -97.5% [15].

In line with this finding was the one of Umar et al 2014 on the susceptibility status of Anopheles spp mosquito to Lambdacyhalothrin in Northeast Nigeria who validated that among 60 exposed mosquitoes to insecticide, only 4 died after being held for 24 hours (6.7%) [26]. Meanwhile, Mehdi et al., doing similar studies on the status of Anopheles stephensi resistance to Lambdacyhalothrin in Iran, reported that among 100 mosquitoes tested, the average dead mosquitoes during 4 repetitions are only 8 (8%) and 20 (20%) [27]. Hasrida et al in their study also stated that Anopheles barbirostris mosquitoes in Tojo Una Una District are resistant to Lambdacyhalothrin 0.05%, as the dead mosquitoes are 73.4% [28].

Changes taken place to Anopheles spp mosquitoes from being vulnerable to tolerance and resistance was an evolutionary phenomenon. Mosquitoes being continuously exposed to a particular insecticide for a long time may have improved immunity to be survived from environmental selection [21]. The developed resistance may also occurs as a result of selective suppression both during larvae and adult stages due to continuous spraying (IRS) [15].

In addition, resistance may also have developed due to genetic factors that lately has become the main determinant of resistance to a certain insecticide which are often referred to as KDR [22]. This finding was similar to the one of David and Gilles in relation to the development vector resistance to insecticides. They stated that the resistance is influenced by genetic factors (specific gene frequency), operational factors (type and application of insecticides), and biological factors (vector characteristics) [15].

The genetic factor causing improved resistance caused by differences in the bio-ecological properties of mosquitoes is influenced by metabolic activities of the enzymes of Monooxygenases, Esterases, Acetylcholinesterase (AChE), and Glutathione-S-transferase (GST) in detoxifying insecticides. As a result, differences in the absorption rate of insecticides against target mosquitoes take place. This evidence had also been proven by Surendran et al, conducting research in Sri Lanka, that low activity of the Glutathione-S-transferase (GSTs) enzyme on Anophelessubciptus causes mosquito susceptibility status to Lambdacyhalothrin becomes higher [29].
Moreover, operational factors causing vector resistance was associated with the presence of insecticide formulation resemblance, residual effect duration, and vector species (number of offspring and vector mobility). The presence of the residual exposure caused *Anopheles* spp regeneration vectors to increase so that the rate of the resistance was faster than other interventions. This phenomenon took place, as chances of contact between vector and active substances becomes greater. Widiarti and Musrifah, in their research in Central Java, found the increased rate resistance to heat caused by long time exposure to insecticides [22,15].

Furthermore, *Lambdacyhalothrin* acted in the same way as DDT by attacking sodium channels in nerve fibers to prevent transmission of nerve impulses. Similar working process among insecticides potentially increased vulnerability status due to changes in insect adaptation. This process is called cross resistance; insect resistance to an insecticide due to other insecticides exposure [19]. A study by Barodji et al regarding monitoring resistance concluded that most malaria vectors which are resistance to *pyrethroid* insecticides are also resistant to DDT groups [15].

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