



Difference In The Status Of *Anopheles Sp* Mosquito Resistance. In The Highest Area Of Spraying And The Area That Has Never Been Sprayed In Banjarnegara Regency

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Abstract

The types of insecticides affect the tolerance level of mosquitoes because mosquitoes have high adaptability to their environmental conditions, one of them is the ability to be resistant to the presence of insecticides. The resistance of malaria vectors to insecticides is a global phenomenon that is felt by all stakeholders in developed countries including Indonesia. If most vector populations are resistant, it causes the use of insecticides to be ineffective in their control.

Therefore we are able to do a differences analysis in the status of *Anopheles sp* mosquito resistance. In the highest area of spraying and has never sprayed in Banjarnegara Regency.

The study used the cross-sectional method to test samples of *Anopheles sp* mosquitoes using the CDC Method's insecticide resistance test Bottle Assay with bendiocarb (Ficam 80 WP) 12.5 µg and lamda-cyhalothrin (Icon 100 CS) 12.5 µg. Implementation of resistance status test at the death rate in the 30 minutes in balitbang Banjarnegara entomology laboratory. The status of *Anopheles sp* mosquito resistance to insecticide tests in the highest category of spraying area (IRS) in Gunungjati Village of Banjarnegara Regency resulted in a condition of suspected resistant to lamda-cyhalothrin (Icon 100 CS) 12.5 µg and conditions resistance to bendiocarb (Ficam 80 WP) 12.5 µg. Analysis of differences in resistance status of *Anopheles sp* mosquitoes. In the highest area of spraying and has never sprayed in Banjarnegara Regency shows no significant difference. This condition provides a warning that the type of insecticide must be immediately rotated with other types of insecticides that have different killing properties.

Keywords : resistance, *Anopheles s*

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Introduction

In 2018 who estimated there were about 228 million new cases of malaria and caused the deaths of about 405,000 people worldwide since reported positive cases in 2010(WHO, 2019). In Indonesia in 2019 recorded all cases of malaria with 39% (96,659 cases) in children aged 15 years and 14% (36,293 cases) in toddlers and 2% (3,858 cases) in infants. Malaria cases in Central Java 2019 amounted to 385 cases, out of 35 districts / cities there were 29 districts / cities entered in the maintenance phase of non-endemic malaria elimination and 6 districts entered the release phase. The districts are Purworejo, Banjarnegara, Kebumen, Banyumas, Cilacap and Purbalingga(Dinas Kesehatan Provinsi Jawa Tengah, 2019).

One of the efforts to control malaria vectors is based on recommendations from WHO, namely through breaking the chain of transmission by killing adult mosquitoes with the use of insecticides, both by spraying insecticides at home (indoor residual spraying / IRS) and insecticidal mosquito nets (long lasting insecticed nets/ LLINs) (Permenkes, 2007; Kitau *et al.*, 2014; Glunt *et al.*, 2015). The use of insecticides needs to be controlled, if less controlled can result in resistance in mosquitoes.

The ability of vector populations to survive an insecticid dose that normal circumstances can kill a vector species can be called a resistance(Direktur Jenderal Pengendalian Penyakit dan Penyehatan Lingkungan, 2012). The Vector resistance occurs as a result of environmental pressures in the form of continuous contact of insecticides with mosquitoes. The result of pressure forces mosquitoes to respond with various efforts to neutralize the influence of insecticides. Tolerance to



these insecticides causes the killing power to be lower with doses under normal conditions can kill vulnerable mosquitoes.

The results of a study on malaria vector resistance between 1997-2017 conducted through *systematic review* by Deokary, Anitha, Winifrida and Johnson that insecticides have affected the resistance levels of more than half of population dynamically in Tanzanian and require attention to the handling of malaria vectors going forward.(Matiya *et al.*, 2019). Previous research that conducted by Jamaluddin in Indonesia showed the mortality rate of *Anopheles sp* mosquitoes due to lamda cyhalothrin by 90% or have potential resistance(Agus, 2010).

The number of types of insecticide affects mosquito adaptation because the mosquitoes have high adaptability to their environmental conditions, including the presence of insecticides. The resistance of malaria vectors to insecticides is a global phenomenon that is felt by all stakeholders in developed countries including Indonesia. If most vector populations are already resistance, it causes the use of insecticides to be ineffective in their control. Therefore we can make a difference in the status of *Anopheles sp* mosquito resistance. In the highest area of spraying and the area that has never been sprayed in Banjarnegara Regency

Methods

This study uses *cross sectional methods* by conducting surveys to malaria endemic areas in Banjarnegara Regency and then testing *Anopheles sp* mosquito samples using insecticide resistance tests with the CDC Bottle Assay method. The population and samples were conducted with the inclusion criteria in *Anopheles sp* mosquitoes obtained from malaria endemic areas with the most spraying/ IRS and the area has never sprayed/ Non IRS which numbers 400. The testing is conducted by 10-25 mosquitoes on each test bottle for control and treatment. The active ingredients of resistance



test insecticides are bendiocarb (Ficam 80 WP) with a concentration of 12.5 µg/bottle and lamda cyhalothrin (Icon 100 CS) with a concentration of 12.5 µg/bottle. The implementation of resistance status test at the death rate in the 30 minutes and conducted in the entomology laboratory BALITBANG Banjarnegara.

Results

A. Results of *Anopheles sp* Resistance Status in Banjarnegara Regency

1. Status of mosquito resistance of *Anopheles sp* Gunungjati Village to lamda cyhalothrin insecticide

Table 1. The resistance status *Anopheles sp* mosquito at a diagnostic time of 30 minutes in Gunungjati Village against lamda cyhalothrin insentisida at a concentration of 12.5 µg/bottle

Insecticide	Number of mosquitoes	30-minute observation		Percentage (%)
		Live	Die	
Control of	13	13	0	0
Lamda cyhalothrin	52	11	41	79

Table 1 shows the percentage of mosquito deaths *Anopheles sp* test results with the insecticide lamda cyhalothrin by 79%, thus the status of test mosquitoes has been resistance (death < 80%).

2. Status of mosquito resistance *Anopheles sp* Gunungjati Village to insecticide bendiocarb

Table 2. resistance status of *Anopheles sp* mosquito at a diagnostic time of 30 minutes in Gunungjati Village against 80% bendiocarb insentisida at a concentration of 12.5 µg/bottle

Insecticide	Number of mosquitoes	30-minute observation		Percentage (%)
		Live	Die	
Control of	13	13	0	0
bendiocarb 80%	52	10	42	81



Table shows a mortality percentage is 81% of the total test mosquitoes, indicating that the condition is suspected to be resistant to mosquitoes from the collection from Gunungjati Village.

3. Status of mosquito resistance *Anopheles sp* Lebakwangi Village to insecticide lamda cyhalothrin.

Table 3. resistance status of *Anopheles sp* mosquito at a diagnostic time of 30 minutes in Lebakwangi Village against lamda cyhalothrin insentisida at a concentration of 12.5 µg/bottle

Insecticide	Number of mosquitoes	30-minute observation		Percentage (%)
		Live	Die	
Control Of	15	15	0	0
Lamda cyhalothrin	60	4	56	93

Monitoring the timing of suspected resistance in the first 30 minutes of observations showed the number of *Anopheles sp* mosquito deaths as many as 56. The percentage of death rate amounted to 93% of the total test mosquitoes, this indicates that the condition is suspected resistance (death 80%-<98%) in mosquitoes collected from Lebakwangi Village.

4. The resistance status *Anopheles sp* Lebakwangi Village to bendiocarb insecticide 80%

Table 4. *Anopheles sp* mosquito resistance status at 30 minutes diagnostic time in Lebakwangi Village against 80% bendiocarb insentisida at a concentration of 12.5 µg/bottle

Insecticide	Number of mosquitoes	30-minute observation		Percentage (%)
		Live	Die	
Control Of	15	15	0	0
Bendiocarb 80%	60	4	56	93

Monitoring the timing of suspected resistance in the first 30 minutes of observations showed the number of *Anopheles sp* mosquito deaths as many as 56 (Table 4). The percentage



of death rate amounted to 93% of the total test mosquitoes, this indicates that the condition is suspected resistance (death 80%-<98%) in mosquitoes collected from Lebakwangi Village.

B. Differences in the status of *Anopheles sp* mosquito resistance in Banjarnegara Regency

The results of the test of the difference in the status of *Anopheles sp* mosquito resistance in Gunungjati Village as the highest IRS category area with Lebakwangi Village as a category area have not been done by the IRS, presented in table 5 for testing with the type of insecticide lamda cyhalothrin 12.5 µg (Icon 100 CS) and table 6 or testing with insecticide type 12.5 µg (Ficam 80 WP).

Table 5. Test results differ the status of *Anopheles sp* mosquito resistance in Gunungjati Village and Lebakwangi Village against lamda cyhalothrin insecticide 12.5 µg (Icon 100 CS)

Test mosquito deaths		
Test village group	Average (%)	p Value
IRS (Gunungjati Village)	76,45	0,386
Non IRS (Lebakwangi Village)	87,18	

The results of the analysis of the difference in the resistance status of *Anopheles sp* mosquito based on table 5 showed p value of $0.386 > 0.05$, so H_0 was accepted and H_a was rejected. This means that there is no difference in the status of *Anopheles sp* mosquito resistance to the insecticide lamda cyhalothrin 12.5 µg (Icon 100 CS) in Gunungjati Village (the highest IRS area) with Lebakwangi Village (area not yet done IRS/ Non IRS) Banjarnegara Regency.

The average death of *Anopheles sp* mosquitoes in Gunungjati Village is 76.45% and 87.18% in Lebakwangi Village. The average difference in deaths between the two villages



was -10.727%. This condition indicates that the death rate of *Anopheles sp* mosquitoes in Gunungjati Village is lower because mosquitoes have been resistant.

Table 6. Test results differ the status of *Anopheles sp* mosquito resistance in Gunungjati Village and Lebakwangi Village against insecticide bendiocarb 12.5 µg (Ficam 80 WP)

Test mosquito deaths		
Test village group	Average (%)	p Value
IRS (Gunungjati Village)	81,00	0,639
Non IRS (Lebakwangi Village)	86,73	

The table 6 is the result of difference analysis in the resistance status of *Anopheles sp* mosquitoes against the type of insecticide bendiocarb 12.5 µg (Ficam 80 WP) with a p value of $0.639 > 0.05$ (H_0 accepted, H_a rejected). This means that it can be concluded that the resistance status condition does not differ in the area of the highest IRS application category with the area that has not been done IRS application.

The difference in the percentage of mosquito deaths *Anopheles sp* Gunungjati Village with Lebakwangi village -5,727. This means that the death rate of *Anopheles sp* mosquitoes in Gunungjati Village is less because mosquitoes have experienced conditions resistant to insecticides of the type bendiocarb 12.5 µg (Ficam 80 WP).

Discussion

1. *Anopheles sp* mosquito resistance status in Bajarnegara Regency

Test results of *Anopheles sp* mosquito resistance status in Gunungjati Village against insecticides of bendiocarb type 12.5 µg and lamda-cyhalothrin 12.5 µg showed large results of mosquito deaths of 81% (suspected resistance) and 79% (resistant). The results of testing the status of resistance to the insecticide indicate that mosquitoes have been tolerant to insecticide



tests. Insecticide type bendiocarb 12.5 μg which is carried out as a malaria vector controller by IRS method in Gunungjati Village, Banjarnegara Regency in the category of suspected resistance. This condition is due to the reduction of vulnerable mosquito individuals in the population in an area (Ningtyas *et al.*, 2020).

The results of testing the resistance status of *Anopheles sp* mosquitoes in Lebakwangi Village to insecticides of bendiocarb type 12.5 μg and lamda-cyhalothrin 12.5 μg in the category of resistant terguda in both types of insecticides (mosquito death 93%). Lebakwangi village as an area category has not been done IRS application should the mosquito population *Anopheles sp* not experience conditions of increased tolerance to insecticides. This case could be caused by excessive or continuous use of household insecticides (Almet *et al.*, 2018).

2. The differences in the resistance status of *Anopheles sp* mosquitoes

Testing of the difference in the resistance status of *Anopheles sp* mosquitoes against the insecticides bendiocarb 12.5 μg and lamda-cyhalothrin 12.5 μg in Gunungjati and Lebakwangi Villages, Banjarnegara Regency showed that there is no difference in resistance status between IRS and Non IRS areas. Control of malaria vectors by IRS in Banjarnegara Regency, over the past 10 years using several insecticides derived from pyrethroid and carbamat groups with rotation 2-3 years after application. The occurrence of resistance conditions in *Anopheles sp* mosquitoes from collections in Banjarnegara Regency is influenced by several factors comprehensively, so as to reduce the efficacy of insecticide use.

Biological and operational factors trigger more resistance in mosquitoes. The vector control activities using pesticides will kill vulnerable individuals in vector populations and leave individuals resistant.⁹ The application of insecticides by residual will lead to a faster rate



of resistance. This is due to the presence of residues that allow contact of the active ingredient of insecticides with mosquitoes *Anopheles sp* (malaria vector) more than with other application methods(Ahmad *et al.*, 2016).

The previous research by *Anopheles* mosquito resistance showed a medium tolerant (suspected resistance) to the insecticide lambda cyhalothrin in the city of Manhica, Southern Mozambique in an An mosquito sample *An. arabiensis*(Glunt *et al.*, 2015) . The resistance testing in Misserete City and Seme City, Benin against insecticides bendiocarb and deltamethrin applied to *Indoor Residual Sprays (IRS)* and *Long Lasting Insecticidal Nets (LLINs)* showed that *An. gambiae* has been resistant(Ossè *et al.*, 2012).

Gunungjati and Lebakwangi villages, Banjarnegara regency is included in the malaria receptive area and is included in the active area of monitoring and control of malaria vectors, thus allowing *Anopheles sp* mosquitoes to breed well(Mahdalena, Suryaningtyas and Ni'mah, 2015). Around this village in this research indicates the presence of rice fields, the use of pesticides commonly used in insect control / pest crops. The use of agricultural pesticides can be one of the triggers for resistance in *Anopheles* mosquitoes(Permenkes RI, 2017). The previous research mentioned that in some districts in Africa *anopheles* mosquito test experienced potential resistance that is closely related to the use of agricultural pesticides(Reid and McKenzie, 2016).

CDC testing of bioassay bottles coated with a single insecticide provides information about the status of resistance to certain insecticides in adult mosquitoes. The areas with the status of suspected resistance to an insecticide could be continued in the control program with a maximum application of 6 times (2-3 years). Then the solution that could be taken from the



problem is to replace it with other chemicals that are more toxic or increase the dose or increase the amount of spraying.

Conclusion

The status of *Anopheles sp* mosquito resistance to insecticide tests in the highest category of spraying areas (IRS) in Gunungjati Village of Banjarnegara Regency resulted in conditions suspected of resistance to bendiocarb 12.5 µg (Ficam 80 WP) and conditions resistant to lamda-cyhalothrin 12.5 µg (Icon 100 CS).

While in Lebakwangi Village which has never been sprayed / Non IRS, the status of *Anopheles sp* mosquito resistance in the category of suspected resistance to two types of test insecticides.

The difference analysis in resistance status of *Anopheles sp* mosquitoes. In the highest area of spraying and it has never sprayed in Banjarnegara Regency shows no significant difference.

Suggestion

Periodic monitoring of the status of resistance to insecticides used in active areas of malaria transmission early is necessary to ensure effective control. Use of insecticides lamda cyhalothrin (100 CS) and bendiocarb (80 WP) in mosquito resistance status test *Anopheles sp*. The results of a single test have been subjected to resistance, for that insecticide needs to be rotated with other insecticides that have different killing properties.



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