International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

Relationship between the use of cypermethrin and zeta-cypermethrin insecticides of resistance status of Aedes sp.

Sitti Aisyah Jamaluddin[1], Anwar Mallongi[2], Agus Bintara Birawida[3], Hasnawati Amqam[4], Atjo Wahyu[5], Muhammad Alwy Arifin[6]

[1,2,3,4]Environmental Health Department, Faculty of Public Health, Hasanuddin University, Makassar, Indonesia.
[5]Occupational Safety and Health Department, Faculty of Public Health, Hasanuddin University, Makassar, Indonesia.
[6]Health Administration and Policy Department, Faculty of Public Health, Hasanuddin University, Makassar. Indonesia.

Abstract

Dengue fever is a disease that has become endemic in many areas whose transmission is influenced by rainfall, temperature, and especially the distribution of mosquito vectors. Then, the most effective and efficient control to break the chain of transmission is the use of insecticides. Insecticides are chosen because they are highly toxic to insects. However, various determinants affect the use of insecticides in controlling the spread of mosquitoes, including using insecticides with inappropriate doses and methods. This study aimed to determine the resistance status of Aedes sp. mosquitoes to cypermethrin and zeta-cypermethrin insecticides. The samples of this study were mosquitoes collected from the field and then examined by the CDC Bottle Bioassay method. The results showed that Aedes sp. mosquitoes were resistant to cypermethrin with a mortality rate of <80% but still tolerant to zeta-cypermethrin with a mortality rate of 80-98%. There was a significant relationship between the use of cypermethrin and zeta-cypermethrin insecticides and the resistance status of Aedes sp. mosquitoes ($\rho=0.000<0.05$). Resistance management is important to maintain effective vector control and focuses on determining appropriate strategies to reduce selective pressure on vectors and assists in informing program policy.

Keywords: CDC bottle bioassay; cypermethrin; insecticide; resistance; zeta-cypermethrin

Full-length article
**Corresponding Author*, e-mail: jamaluddinsa19k@student.unhas.ac.id

1. Introduction

Dengue fever is a disease transmitted by Aedes mosquitoes often found in tropical and subtropical regions [1]. This disease has become endemic in various world regions, especially in more than 100 cities, Southeast Asia, the Western Pacific, and the Americas [2]. Dengue virus transmission can be widespread because it is influenced by rainfall, temperature, urbanization, and especially the distribution of Aedes sp. mosquito vectors [3]. DHF cases have an endemic status consisting of high endemic, medium endemic, low endemic, and non-endemic [4]. This endemicity status is mentioned if, in the last three years in an area every year, there are cases of DHF. Based on the National Long- and Medium-Term Plan (RPJMN) and the Strategic Plan (RENSTRA) of the Indonesian Ministry of Health 2015-2019, the DHF disease control program is an effort to prevent and manage the incidence of DHF, including limiting the spread of DHF disease [5]. The RPJMN and RENSTRA focus on early and continuous eradication of DHF. The development of a national program to actively promote dengue elimination has been initiated over the past few years through community-based activities such as vector control strategies and viral vaccination program trials [6]. So far, the most effective and efficient vector control is to break the chain of transmission by killing the vector either mechanically, biologically, or chemically, such as using insecticides. However, seeing the need for results from the program based on not achieving the desired goals in its implementation, several things need to be reviewed, such as the use of insecticides. Insecticides were chosen because they are highly toxic to insects. Insecticide control is also widely used due to its effectiveness in regulating larval and adult mosquito populations [7]. Therefore, control using large-scale fogging and insecticide spraying still prevails today. Various determinants affect the use of insecticides in controlling the spread of mosquitoes, including using insecticides with inappropriate doses and methods over a long period. This can lead to vector resistance and reduce the insecticide's effectiveness [8]. Several studies have subsequently shown the development of resistance in target mosquitoes due to insecticides over a long period [9]. Resistance occurs due to innate immunity, where resistant insects have inherited immunity and produce new immune populations, and gene changes cause mutations to have insecticide immunity. In addition, immunity is also obtained due to the adjustment of these insects to insecticides so that there is no death and new immune populations form [10]. Monitoring vector resistance to insecticides with early detection can indicate the susceptibility status of vector species and assist in providing input to program policies, such as determining the type of insecticide and strategy to be used and understanding the mechanisms by which vector susceptibility is reduced. This study aims to determine Aedes sp. mosquito resistance against cypermethrin and zeta-cypermethrin insecticides. Include the correlation of resistance to cypermethrin and zeta-cypermethrin insecticide.

2. Materials and Methods

Samples used in this research were 230 *Aedes* sp. mosquitoes collected in two locations in Turikale sub-district, Maros district: Adatongeng Village and Alliritengae Village. The independent variable in this research was insecticide with cypermethrin and zeta-cypermethrin as its active compound, while the dependent variable in this research was *Aedes* sp. mosquitoes.

2.1. Preparation of test samples

Mosquitoes were collected by purposive sampling using ovitrap and brought to the laboratory for breeding into F0 and F1 before further testing. Collected mosquitoes were stored in paper cups labeled by their collection location and would then be used for bottle bioassay test resistance test. Coordinate points were taken using the Global Positioning System (GPS) to mark mosquito collection tests. This research used cypermethrin insecticide in 10µg/bottle and zeta-cypermethrin in 15µg/bottle dosage. Cypermethrin and zeta-cypermethrin insecticide, which would then be used for this research's resistance test. Each 1 ml cypermethrin and zeta-cypermethrin was added to the bottle and then sealed. One ethanol drip was used for the control bottle, which was then sealed. The test bottles were placed horizontally and then flipped, and then the bottle cap was slightly opened to let ethanol evaporate. The bottles were then covered by fabric to shield them from light.

2.2. Dead mosquito criteria

Mosquitoes are considered dead when they are unable to stand anymore. Test bottles were slowly rolled to determine whether the mosquitoes were dead. If the mosquitoes could not move and fell, they would be considered dead. CDC bottle bioassay test interpretation was interpreted according to the protocol used. The vulnerability hold was calculated on 30 minutes of diagnostic time for all tested insecticides with the following criteria: a) The tested mosquito's death rate would be considered vulnerable to 98-100%, b) If the tested mosquito's death rate were 80-97%, it would be categorized as suspected resistance, and c). If the tested mosquito's death was <80%, it would be categorized as resistant.

3. Results and Discussion

3.1. Aedes sp. mosquito mortality after resistance test

Adatongeng and Alliritengae villages are among the Turikale sub-district of Maros with many dengue cases, so fogging is applied more frequently than in other villages. The high application rate often comes from self-funded communities that try to take control measures independently without coordination by using insecticides that can overcome mosquito bites. This causes many types of insecticides that may be used for control and cause resistance [11]. One method that can be used to determine the resistance status of disease vectors such as mosquitoes to insecticides is using the CDC bottle bioassay method. CDC bottle bioassays detect and characterize resistance to insecticide active ingredients in certain vector species. The test measures the time required for an insecticide to produce mosquito mortality. The results then indicate the proportion of the resistant population [12]. Table 1 and Table 2 showed the result of Aedes sp. mosquito resistance against cypermethrin and zeta-cypermethrin insecticide in two test sites. Based on table 1, the results of the resistance test with a diagnosis time of 30 minutes using the CDC bottle bioassay method in Adatongeng Village (62%) and Alliritengae Village (70.24%), where the death of Aedes sp. mosquitoes in the area against the use of cypermethrin insecticide is included in the criteria for death that occurs ≤ 80%. This means that the sensitivity of Aedes sp. mosquitoes to cypermethrin insecticide is no longer sensitive or resistant. Adatongeng Village and Alliritengae Village are endemic areas with the highest frequency of exposure to insecticides for almost five years and have been resistant to cypermethrin insecticide. This resistance can occur in areas often exposed to insecticides with prolonged doses and time [13]. These results align with the research of Irawati and Putri [14], which said that the continuous use of cypermethrin insecticide has impacted resistance in dengue vectors with mortality of 89% and 97% after 30 minutes. Based on the table 2, the results of the resistance test with a diagnosis time of 30 using the CDC bottle bioassay method in Adatongeng Village (88%) and Alliritengae Village (88.10%), where the death of Aedes sp. mosquitoes in the area against the use of zeta-cypermethrin insecticide is included in the criteria for death that occurs 80%-98%. This means that the sensitivity of Aedes sp. mosquitoes to zeta-cypermethrin insecticide is still tolerant. Zeta-cypermethrin is a type of insecticide belonging to synthetic pyrethroids, a particular variant of cypermethrin synthesized to improve stability and effectiveness as an insecticide. Based on Figure 1, the resistance of Aedes sp. mosquitoes to the use of cypermethrin and zeta-cypermethrin insecticides in Adatongeng Village and Alliritengae Village does not have a big difference. Two main mechanisms are thought to be associated with resistance to insecticides: changes in target sites and increased rates of insecticide detoxification [15]. Adaptability to insecticide exposure in the form of changes in behavior to avoid exposure, increased rates of excretion of active ingredients from the body, and reduced sensitivity to biochemical targets and operational factors, which

Table 1. Percentage mortality of Aedes sp. mosquitoes against cypermethrin insecticide

Sampling site	Number of tested mosquitoes	RR (30 minutes)	Status
Adatongeng	100	62%	Resistant
Alliritengae	84	70.24%	Tolerant

Table 2. Percentage mortality of Aedes sp. mosquitoes against zeta-cypermethrin insecticide

Sampling site	Number of tested mosquitoes	RR (30 minutes)	Status
Adatongeng	100	62%	Resistant
Alliritengae	84	70.24%	Tolerant

Table 3. Normality Test for the Use of Cypermethrin and Zeta-cypermethrin Insecticides with the Resistance Status of Aedes sp. Mosquitoes.

Data		Sig.	Description
Cypermethrin	Adatongeng	0.048	The data is not normally distributed
	Alliritengae	0.105	The data is normally distributed
Zeta-cypermethrin	Adatongeng	0.008	The data is not normally distributed
	Alliritengae	0.003	The data is not normally distributed

Table 4. Relationship between cypermethrin and zeta-cypermethrin insecticides on the resistance status of Aedes sp. mosquitoes with T-Independent Test

		Levene's Test for Equality of Variances		t-test for Equality of Means		
		F	**Sig.**	**t**	**df**	**Sig. (2-tailed)**
Status resistance	Equal variances assumed	22.062	0.000	-6.600	18	0.00
	Equal variances not assumed			-6.600	9.000	0.000

Table 5. Relationship between cypermethrin and zeta-cypermethrin insecticides on the resistance status of Aedes sp. mosquitoes with Mann-Whitney U Test

	Insecticide	**Mann-Whitney U**	**Z**	**Asymp. Sig. (2-tailed)**
Adatongeng	Cypermethrin	5.000	-3.730	0.000
	Zeta-cypermethrin	5.000	-3.758	0.000
Alliritengae	Zeta-Cypermethrin	5.000	-3.784	0.000

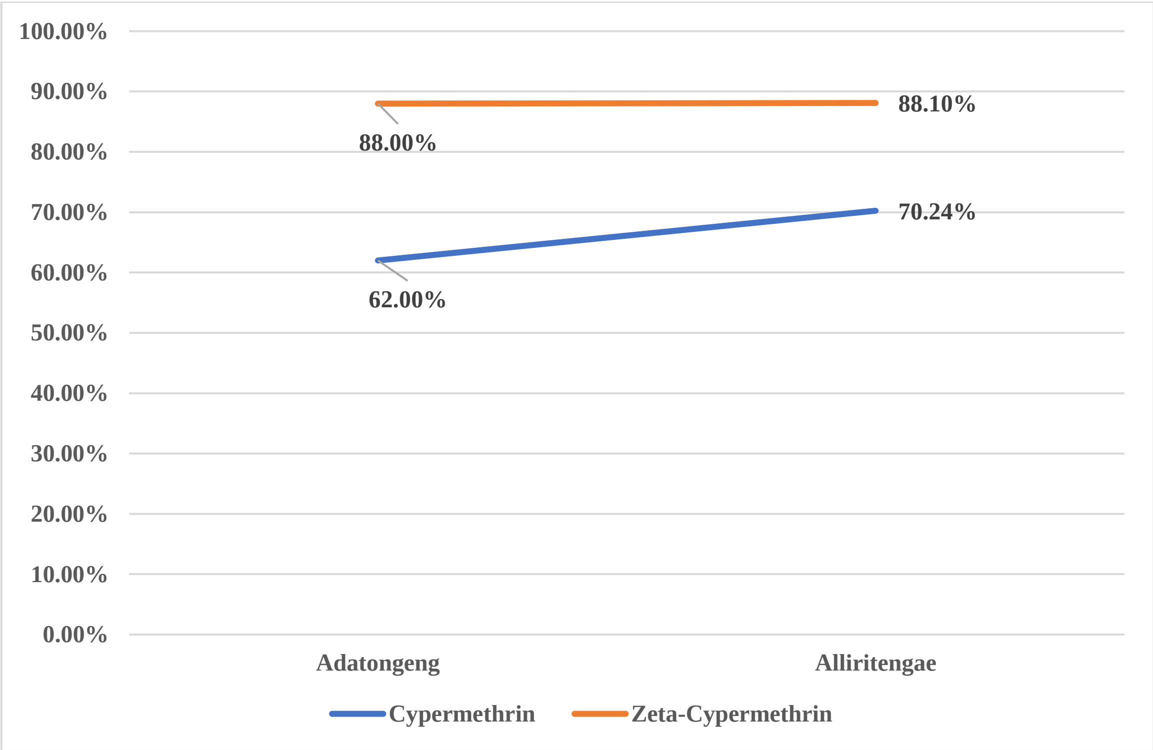

Figure 1. Aedes sp. mosquito resistance against insecticide

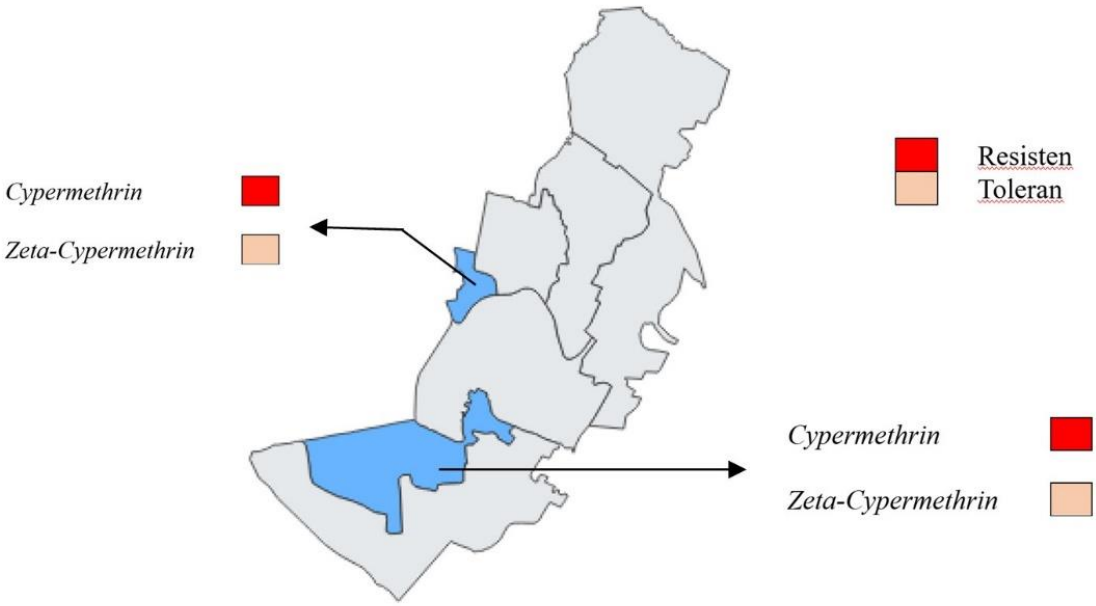

Figure 2. Resistance Status of Aedes sp. Mosquitoes Based on Insecticide Use

include the type of insecticide, application technique, dose, and frequency of application are also triggered for resistance [16-17]. One type of insecticide that the Ministry of Health generally uses is the synthetic pyrethroid group, such as cypermethrin and zeta-cypermethrin, whose use has been going on for a long time and is applied by thermal fogging. In addition, synthetic pyrethroid insecticides with the active ingredients cypermethrin and zeta-cypermethrin themselves are also widely used in vector control activities originating from household use [18].

3.2. Relationship between cypermethrin and zeta-cypermethrin insecticides on the resistance status of Aedes sp. mosquitoes

Based on the results of the normality test in Table 3, data with a ρ value <0.05 were obtained, so it can be said that the data were not normally distributed, so the Mann-Whitney test was carried out to see the existing relationship. For data with a value of ρ> 0.05, then the data is normally distributed, so an independent t-test is performed to see the existing relationship. The results of the relationship test between the use of cypermethrin and zeta-cypermethrin insecticides and mosquito resistance status then showed that there was a significant relationship as shown in Table 4 and Table 5 below. Based on figure 2, Aedes sp. mosquitoes in Adatongeng and Alliritengae villages are resistant to cypermethrin insecticide and tolerant to zeta-cypermethrin insecticide. There is a relationship between the use of cypermethrin and zeta-cypermethrin insecticides and the resistance status of Aedes sp. mosquitoes in Adatongeng and Alliritengae villages. This statement is supported by the results of Cahyati & Fitriani's research [19], which said that fogging conducted by the Semarang Regency Health Office from 2016 - 2019 as many as 47 using insecticides containing the active ingredient zeta-cypermethrin 15 g/l did not make dengue cases in the sub-district well controlled. Several studies have reported that the susceptibility status of Aedes aegypti to synthetic pyrethroid insecticides (cypermethrin, deltamethrin, alpha-cypermethrin) has primarily been reported to be resistant in Indonesia and in various countries [20]. Pyrethroid synthetic insecticides act on the insect nervous system by inhibiting axons in ion channels, resulting in a continuous action potential. Where synthetic pyrethroids bind to voltage-gated sodium channel (VGSC) proteins that regulate nerve impulse pulses, this causes the nerve impulse to be stimulated continuously and causes the insect to experience hyperexcitation (anxiety) and convulsions (spasticity) [19]). Continuous exposure can cause immunity in the vector's body by thickening the cuticula, increasing cholinesterase enzymes, and genetic mutations [11]. This was proven in the research of Ikawati B. et al. [21], which said that the resistance of the Aedes aegypti population to deltamethrin in Cuba was proven to be passed down from one generation to the next. However, the results of research that state the resistance or tolerance of a population of Aedes sp. mosquitoes to insecticides do not indicate that all areas are in the same condition. This is because resistance can be localized, especially if there is no application of the same mosquito control or no movement of resistant mosquitoes, which can contribute to the inheritance of resistant mosquitoes [18]. Zeta-cypermethrin is one type of insecticide that is also included in the synthetic pyrethroid group in the form of a special variant synthesized to increase stability and effectiveness as an insecticide. Based on the results of the research conducted, there is a relationship between the use of cypermethrin and zeta-cypermethrin insecticides and the resistance status of Aedes sp. mosquitoes in the Adatongeng Village and Alliritengae Village areas. Some research results say the susceptibility status of Aedes aegypti to synthetic pyrethroid insecticides (cypermethrin, deltamethrin, alpha-cypermethrin) has mostly been reported to be resistant in Indonesia and in various countries [20]. This is because cypermethrin and zeta-cypermethrin are from the same class and are related. Where toxicokinetic and physicochemical parameters between cypermethrin and zeta-cypermethrin were found to be comparable (low solubility in water, lipophilicity, partition coefficient, oral absorption, and excretion route) [22]. Differences in resistance status are usually related to people's habits in using insecticides to eradicate insects and mosquitoes. The habits include different concentrations, periods, and frequency of use. Consistent monitoring of resistance can help determine changes in the susceptibility of mosquito populations over time [12]. Monitoring insecticide susceptibility in Aedes sp populations is essential for decision-making in the use of insecticides [23]. The purpose of susceptibility testing is to know the status of susceptibility. It can also be used as an information map of the susceptibility of vector species to insecticides that have been and will be used for vector control [18]. So, the importance of resistance management is to maintain the effectiveness of vector control and focus on determining the right strategy to reduce selective pressure on vectors [24].

4. Conclusions

In this study, insecticide resistance detection was based on the CDC Bottle Bioassay testing method principle as a form of integrated vector control development. The results showed that Aedes sp. mosquitoes in Adatongeng Village and Alliritengae Village were resistant to cypermethrin with a mortality rate of < 80% and were still tolerant to zeta-cypermethrin with a mortality rate of 80 - 98%. There is a significant relationship between the use of cypermethrin and zeta-cypermethrin insecticides and the resistance status of Aedes sp. mosquitoes in Adatongeng Village and Alliritengae Village ($\rho = 0.000 < 0.05$). This is due to the resistance that occurs due to inappropriate insecticide application. Therefore, resistance management is essential to maintain effective vector control and focuses on determining appropriate strategies to reduce selective pressure on vectors. It is suggested that it is necessary to conduct early detection of vector susceptibility to insecticides by conducting periodic testing using several different methods, such as CDC bottle bioassay, biochemical tests, and molecular tests; rotate the types of insecticides used. Especially those that are resistant to vectors; conduct supervision and regulation related to the use of insecticides both at the government and community levels; and the need for health counseling to the public about the use of household insecticides, starting from the selection of active ingredients, doses, application methods and periodic replacement.

References

[1] M.U. Kraemer, M.E. Sinka, K.A. Duda, A.Q. Mylne, F.M. Shearer, C.M. Barker, S.I. Hay. (2015). The global distribution of the arbovirus vectors Aedes aegypti and Ae. albopictus. elife. 4 e08347.
[2] S.A. Kularatne. (2015). Dengue fever. Bmj. 351.
[3] M.G. Guzman, D.J. Gubler, A. Izquierdo, E. Martinez, S.B. Halstead. (2016). Dengue infection. Nature reviews Disease primers. 2 (1) 1-25.
[4] T. Iswidaty, M. Martini, D. Widiastuti. (2016). Status Resistensi Nyamuk Aedes Aegypti terhadap Malathion 0, 8% di Area Perimeter dan Buffer Pelabuhan Tanjung Emas Semarang (Pengujian Berdasarkan Teknik Bioassay dan Biokimia). Jurnal Kesehatan Masyarakat Universitas Diponegoro. 4 (1) 18432.
[5] O. Mote (2017). Evaluasi Pelaksanaan Program Pencegahan Dan Penanggulangan Penyakit Demam Berdarah Dengue (DBD) Di Wilayah Kerja Puskesmas Padang Selasa Kota Palembang.
[6] P.H. Hamid, J. Prastowo, A. Widyasari, A. Taubert, C. Hermosilla. (2017). Knockdown resistance (kdr) of the voltage-gated sodium channel gene of Aedes aegypti population in Denpasar, Bali, Indonesia. Parasites & vectors. 10 1-9.
[7] A. Manjarres-Suarez, J. Olivero-Verbel. (2013). Chemical control of Aedes aegypti: a historical perspective. Revista Costarricense de Salud Pública. 22 (1) 68-75.
[8] S. Riyadi, T.B.T. Satoto. (2017). Penggunaan insektisida dan status kerentanan nyamuk Aedes aegypti di daerah endemis di Kabupaten Purbalingga. Berita Kedokteran Masyarakat. 33 (10) 459-66.
[9] I.R. Yulianti. (2016). Status Resistensi Aedes Aegypti Terhadap Insektisida Golongan Organokarmabat Dan Pola Kejadian Demam Berdarah Dengue Di Kota Bogor.
[10] S. Sutarto, A.Y. Syani. (2018). Resistensi Insektisida pada Aedes aegypti. Jurnal Agromedicine Unila. 5 (02) 582-586.
[11] I. Anggraini, L.A. Sinaga, A. Kurniawan. (2022). Uji Resistensi Vektor Demam Berdarah Dengue (DBD) Terhadap Insektisida Permethrin di Kota Binjai Provinsi Sumatera Utara Tahun 2022. Balaba: Jurnal Litbang Pengendalian Penyakit Bersumber Binatang Banjarnegara. 177-182.
[12] World Health Organization. (2022). Manual for monitoring insecticide resistance in mosquito vectors and selecting appropriate interventions. World Health Organization.
[13] I.G. Karauwan, J.B. Bernadus, G.P. Wahongan. (2017). Uji resistensi nyamuk aedes aegypty dewasa terhadap cypermethrin di daerah pasar tua bitung 2016. JKK (Jurnal Kedokteran Klinik). 1 (3) 42-46.
[14] N.B.U. Irawati, N.E. Putri. (2021). Resistensi Nyamuk Aedes aegypti terhadap Cypermethrin di Kabupaten Klaten, Jawa Tengah. Ruwa Jurai: Jurnal Kesehatan Lingkungan. 15 (1) 1-7.
[15] G.A. Yahouédo, F. Chandre, M. Rossignol, C. Ginibre, V. Balabanidou, N.G.A. Mendez, S. Cornelie. (2017). Contributions of cuticle permeability and enzyme detoxification to pyrethroid resistance in the major malaria vector Anopheles gambiae. Scientific reports. 7 (1) 11091.
[16] K. Dang, S.L. Doggett, G. Veera Singham, C.Y. Lee. (2017). Insecticide resistance and resistance mechanisms in bed bugs, Cimex spp.(Hemiptera: Cimicidae). Parasites & vectors. 10 1-31.
[17] M. Musfirah. (2017). Pengendalian Kimia dan Resistensi Vektor Anopheles Dewasa pada Kawasan Endemis Malaria di Dunia. Kes Mas: Jurnal Fakultas Kesehatan Masyarakat Universitas Ahmad Daulan. 11 (1) 46-51.
[18] S. Sunaryo, D. Widiastuti. (2018). Resistensi Aedes aegypti terhadap insektisida kelompok organopospat dan sintetik piretroid di Provinsi Sumatera Utara dan Provinsi Jambi. Balaba: Jurnal Litbang Pengendalian Penyakit Bersumber Binatang Banjarnegara. 95-106.
[19] W.H. Cahyati, L. Fitriani. (2020). Detection of Aedes aegypti Resistance towards Cypermethrin using Polymerase Chain Reaction (PCR) Techniques in Ambarawa Semarang Regency. Unnes Journal of Public Health. 9 (1) 71-77.
[20] J. Ariati, D. Perwitasari, R. Marina, S. Shinta, D. Lasut, R. Nusa. (2018). Status kerentanan Aedes aegypti terhadap insektisida golongan organofosfat dan piretroid di Indonesia. Jurnal Ekologi Kesehatan. 17 (3) 135-145.
[21] B. Ikawati, S. Sunaryo, D. Widiastuti. (2015). Peta status kerentanan Aedes aegypti (Linn.) terhadap insektisida cypermethrin dan malathion di Jawa Tengah. ASPIRATOR-Journal of Vector-Borne Disease Studies. 7 (1) 23-28.
[22] E.F.S. Authority, G. Bellisai, G. Bernasconi, M. Binaglia, A. Brancato, L.C. Cabrera, A. Verani. (2023). Review of the existing maximum residue levels for cypermethrins according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal. 21 (3).
[23] B. Mulyaningsih, S.R. Umniyati, T.B.T. Satoto, A. Diptyanusa, D.A.A. Nugrahaningsih, Y. Selian. (2018). Insecticide resistance and mechanisms of aedes aegypti (Diptera: Culicidae) in Yogyakarta, Indonesia. Journal of the Medical Sciences (Berkala Ilmu Kedokteran). 50 (1).
[24] J. Hemingway. (2018). Resistance: A problem without an easy solution. Pesticide biochemistry and physiology. 151 73-75.