Kesmas

Volume 19
Issue 3 *August 2024*

Article 6

7-23-2024

Differences in Active Ingredients of White Chicory Leaves (Brassica pekinensis L) as a Bio-Larvicidal Against Aedes aegypti larvae

Marlik Marlik
Poltekkes Kemenkes Surabaya, Surabaya, marlik@poltekkesdepkes-sby.ac.id

Dhea Stya Okta
Poltekkes Kemenkes Surabaya, Surabaya, dheaoktavv@gmail.com

Ngadino Ngadino
Poltekkes Kemenkes Surabaya, Surabaya, bungdino1960@gmail.com

Demes Nurmayanti
Poltekkes Kemenkes Surabaya, Surabaya, demes@poltekkesdepkes-sby.ac.id

Irwan Sulistio
Poltekkes Kemenkes Surabaya, Surabaya, irwan.sulistio@poltekkesdepkes-sby.ac.id

See next page for additional authors

Recommended Citation

Marlik M , Okta DS , Ngadino N , et al. Differences in Active Ingredients of White Chicory Leaves (Brassica pekinensis L) as a Bio-Larvicidal Against Aedes aegypti larvae. *Kesmas*. 2024; 19(3): 193-198
DOI: 10.21109/kesmas.v19i3.1393
Available at: https://scholarhub.ui.ac.id/kesmas/vol19/iss3/6

Differences in Active Ingredients of White Chicory Leaves (Brassica pekinensis L) as a Bio-Larvicidal Against Aedes aegypti larvae

Authors

Marlik Marlik, Dhea Stya Okta, Ngadino Ngadino, Demes Nurmayanti, Irwan Sulistio, and Aries Prasetyo

Marlik *et al. Kesmas: Jurnal Kesehatan Masyarakat Nasional (National Public Health Journal)*. 2024; 19 (3): 193-198
DOI: 10.21109/kesmas.v19i3.1393

Kesmas: Jurnal Kesehatan Masyarakat Nasional (National Public Health Journal)

Differences in Active Ingredients of White Chicory Leaves *(Brassica pekinensis L)* as a Bio-Larvicidal Against *Aedes aegypti larvae*

Marlik*, Dhea Stya Okta Avianti, Ngadino, Demes Nurmayanti, Irwan Sulistio, Aries Prasetyo

Department of Environmental Health, Poltekkes Kemenkes Surabaya, Surabaya, Indonesia

Abstract

Continuous chemical dengue control can cause vector resistance and environmental pollution. Developing natural larvicides (bio-larvicides) from plant toxins like white chicory, which can poison *Aedes aegypti larvae,* is necessary for a sustainable alternative. This study aimed to analyze differences in active ingredients in white chicory leaves *(Brassica pekinensis L*) as bio-larvicide against *Aedes aegypti larvae.* This study was a pure experiment using a post-test-only control design using 1,225 *Aedes aegypti* instar III *larvae* with extracts of alkaloid active ingredients, flavonoids, and concentrations of 0%, 24%, 34%, and 40% with 3 times replication. Data were analyzed using a probit test, one-way ANOVA, and Post Hoc LSD. The results showed the potential of active ingredients in white chicory leaves against the death of *Aedes aegypti larvae* (p-value = 0.000). The average percentage of larval mortality concentration was 24%, 34%, 40%, in alkaloids was 41%, 60%, 66%, and in flavonoids was 45%, 64%, 68%. The active ingredient of white chicory leaves can kill *Aedes aegypti larvae* with LC_{50} in 29% alkaloids and 27% flavonoids.

Keywords: *Aedes aegypti larvae,* bio-larvicidal, *Brassica pekinensis L, pest control, vector-borne diseases*

Introduction

Dengue hemorrhagic fever (DHF) is an infectious disease caused by the dengue virus of the genus *Flavivirus*, family *Flaviviridae,* and transmitted through the bite of *the Aedes aegypti* mosquito[1] leading to death in a short period if no proper treatment is performed.[2] DHF remains a public health problem in tropical countries, specifically Indonesia.[3] The 2020 Indonesia Health Profile data showed that DHF cases in the country reached a total of 108,303 cases, and the death rate was 747 people, even though it decreased in 2021, dengue cases reached 73,518, and the death rate was 705 people.[4] The total number of cases and deaths only decreased by 32% from 2020 to 2021; thus, some control efforts are critically needed.

The DHF control efforts made in general are through the elimination of breeding sites, protective measures, and biological and chemical means.[5,6] General control often taken by the community is by chemical methods.[7] Over time, the *Aedes aegypti* mosquito population can develop resistance to commonly used larvicides, occurring through natural selection, where the surviving mosquitoes pass on their resistant traits to their offspring so that subsequent control efforts become less effective.[8,9] Judging from the side effects caused, it is necessary to make innovation by making natural larvicide or bio-larvicide derived from plants having a toxic effect on insects,[10] but not causing side effects on environment. The effect of bio-larvicide can be found in active metabolites in the forms of saponins, tannins, alkaloids, and flavonoids.[11,12] These substances can be found in Indonesia in various types and parts of plants, such as roots, stems, leaves, flowers, fruits, and green seeds.[13] As for plants containing bio-larvicide, one of them is white chicory (*Brassica pekinensis L*).[14]

Based on the preliminary study test results from the phytochemical laboratory, white chicory leaves (*Brassica pekinensis L*) contained flavonoid active ingredients with a percentage of 2.61% and alkaloids of 3.32%, which can be used as natural larvicides. Flavonoids and alkaloids contained in white chicory leaves (*Brassica pekinensis L*) have the potential

***Correspondence**:* *Marlik, Department of Environmental Health, Poltekkes Kemenkes Surabaya, Surabaya, Indonesia.*
Email: marlik@poltekkesdepkes-sby.ac.id, *Phone: +62 812-1727-831*

Received: March 17, 2024
Accepted: July 9, 2024
Published: August 23, 2024

to be bio-larvicides against *Aedes aegypti larvae*. Flavonoids work by disrupting larval metabolic processes, increasing the production of free radicals, resulting in oxidative damage to cells, and damaging larval cell membranes, resulting in ion leakage and dehydration. Alkaloids act as neurotoxins, disrupting the larval nervous system, inhibiting the enzyme cholinesterase, causing muscle overstimulation, and disrupting the hormonal balance of larval development. These mechanisms lead to paralysis, molting failure, and larval death, making white chicory leaves extract an environmentally friendly alternative for mosquito population control.[15]

A study on betel leaf extract (*Piper betle L*) containing active ingredients of saponins, tannins, alkaloids, flavonoids, phenols, chavicol, eugenol, and essential oils at concentrations of 5%, 10%, 15%, 20%, and 25% was declared capable of killing *Aedes aegypti larvae* with a mortality rate above 50% within 24 hours of each treatment.[16] Within one hour, 5% concentration can kill 60% of *larvae*, 10% and 15% concentrations can kill 60% of *larvae*, and 20% and 25% concentrations show a 100% effective killing of *larvae* because they can kill 100% of tested *larvae* within 24 hours.[16] A study on tobacco extract bio-larvicide emulsions against *Aedes aegypti larvae* stated that LC_{50} nanoemulsions for 24 hours and 48 hours were effectively used as bio-larvicides.[17] Extraction using the reflux method has the advantage of being able to extract samples that are relatively heat resistant. However, reflux extraction has several disadvantages; it requires a relatively long time and a lot of solvents, and it also allows certain compounds not to be extracted for their low solubility at room temperature.[17]

Based on this description, the development of bio-larvicides that are harmless and environmentally friendly needs to be advanced, and the killing power derived from toxic substances in plants can act as stomach poison and contact poisons for mosquitoes. Leaves are parts not widely utilized compared to other parts of the plant. Hence, the use of plant leaves provides added value to a plant. With this phenomenon, this study intended to conduct an evidence-based practice study to determine differences in active ingredients, alkaloids, and flavonoids in white chicory leaves (*Brassica pekinensis L*) as bio-larvicide against *Aedes aegypti larvae* for 24 hours with variations in concentrations of 0%, 23%, 34%, and 40%.

The purpose of this study was to evaluate the difference in active flavonoids and alkaloids in white chicory leaves (*Brassica pekinensis L*) as a bio-larvicide against *Aedes aegypti larvae*. This study compared the effectiveness of flavonoids and alkaloids in killing *Aedes aegypti larvae*. In addition, this study also aimed to identify specific compounds in flavonoids and alkaloids with the highest larvicidal activity.

Method

This study utilized a purely experimental approach with a post-test-only control design to investigate the efficacy of active ingredients extracted from white chicory leaves (*Brassica pekinensis L*) as bio-larvicides against *Aedes aegypti larvae*. The research tools included analytical scales, a stopwatch, a porcelain dish, an extractor, a blender, a rotary evaporator, a drip pipette, a thermometer, a spatula, a room thermometer, a measuring cup, a pH meter, and filter cloth. Materials used encompass white chicory leaves, 2N sulfate, 96% ethanol, hydrochloric acid, $FeCl_3$ 5%, *Aedes aegypti larvae*, 2N HCl, paper labels, Aquades, and plastic cups.

The experiment involved exposing 1,225 *Aedes aegypti* instar III *larvae* to extracts containing varying concentrations (0%, 24%, 34%, and 40%) of alkaloids and flavonoids, replicated three times. Mortality data of *larvae* were collected and analyzed using probit analysis, one-way ANOVA, and Post Hoc LSD tests to determine the effectiveness of different extract concentrations. This study was conducted with sufficient repetition to ensure the validity and reliability of the results. Each extract concentration and control was repeated three times. Each replicate involved the same number of *larvae*, 20 *larvae* per container. This repetition was done for each concentration of flavonoid and alkaloid extracts, as well as the control group.

The time-based method of measuring *Aedes aegypti larvae* mortality in this study was designed to evaluate the effectiveness of flavonoids and alkaloids extracts from white chicory leaves (*Brassica pekinensis L*) as bio-larvicides. *Larvae* that had been bred in the laboratory were placed in Petri dishes filled with hygienic water and treated with extracts in various concentrations, as well as a control group without extracts. Mortality observations were made at 24-hour intervals for a period of 72 hours after treatment. *Larvae* were considered dead if they showed no movement when touched. Mortality data were recorded and analyzed to determine the percentage of mortality at each time interval and extract concentration. These analyses would provide information on the potential effectiveness of white chicory leaf extract as an alternative control for *Aedes aegypti larvae*, with potential applications in environmentally friendly vector control.

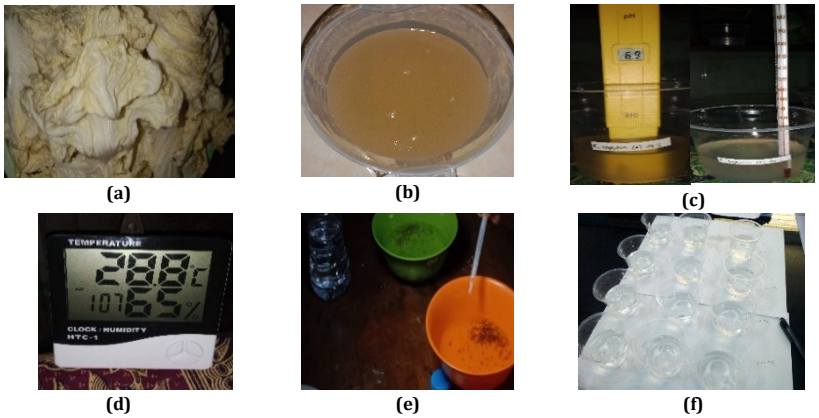

Figure 1. (a) white chicory leaves drying process; (b) extract results; (c) pH and water temperature measurement; (d) room temperature and humidity measurement; (e) transfer *of Aedes aegypti larvae;* (f) process of administering white chicory leaves active ingredient extract

Results

The room temperature results for 24 hours obtained an average of 28.5°C and a room humidity of 64%. The results of water temperature and water pH measurements at a concentration of 0% or control treatment of the death of *Aedes aegypti* instar III *larvae* for 24 hours obtained an average water temperature of 29 and a water pH of 0.68. The active ingredient of alkaloids was obtained at an average water temperature of 29°C and water pH of 6.9, while the active ingredient of flavonoids was obtained at an average water temperature of 28°C and water pH of 6.8.

Table 1 . Average Results and Standard Deviations of Water Temperature, Water pH, Room Temperature, and Room Humidity of White Chicory Leaves (*Brassica pekinensis L*) Active Ingredients for 24 Hours

Active Ingredient	Concentration	Temperature Water (°C)		Water pH		Room Temperature (°C)		Room Humidity	
		$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Control	0%	29	0.68	6.2	0.25	28.5	0.79	64%	1.29
Alkaloid	24%	29	0.61	7.0	0.10	28.5	0.79	64%	1.29
	34%	28	0.49	6.9	0.07	28.5	0.79	64%	1.29
	40%	29	0.60	6.9	0.05	28.5	0.79	64%	1.29
Flavonoid	24%	28	0.27	6.8	0.04	28.5	0.79	64%	1.29
	34%	29	0.64	6.7	0.05	28.5	0.79	64%	1.29
	40%	28	0.49	6.9	0.04	28.5	0.79	64%	1.29

Note: SD = standard deviation

Table 2. Average and Standard Deviation of Percentage of Mortality of *Aedes aegypti Larvae* After 24-hour Exposure to White Chicory Leaves (*Brassica pekinensis L*) Active Ingredient Extract

Active Ingredients	Concentration	Average	SD
Control	0%	0%	0%
Alkaloid	24%	41%	3.31
	34%	60%	4.89
	40%	66%	6
Flavonoid	24%	45%	3.31
	34%	64%	2.82
	40%	68%	4.89

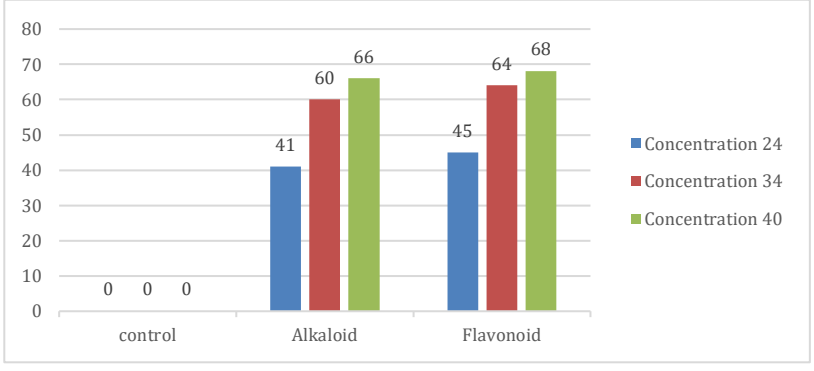

Figure 1. Average Percentage of *Aedes aegypti larvae* Mortality Over 24 Hours

Based on Table 2 and Figure 1, the death of *Aedes aegypti* instar III *larvae* at a control or concentration of 0% for 24 hours obtained an average larval mortality of 0% and a standard deviation of 0%. Extracts of alkaloid active ingredient concentrations of 24%, 34%, and 40% for 24 hours with four-fold replication obtained the average mortality of *Aedes aegypti larvae* of 41%, 60%, and 66%, with a standard deviation of 3.31-6. The active ingredients of flavonoids obtained the average mortality of *Aedes aegypti larvae* amounted to 45%, 64%, and 68% with a standard deviation of 2.82-4.89. Based on Table 3, the results of probit analysis of the extract of the active ingredient of chicory (*Brassica pekinensis L*) against *Aedes aegypti larvae* in treatment for 24 hours found that the LC50 value of the alkaloid active ingredient was located at a concentration of 29.084%. Meanwhile, flavonoids were located at a concentration of 26.580%.

Table 3. Results of LC_{50} Value of Chicory (*Brassica pekinensis L*) Active Ingredient Extract Against the Mortality of *Aedes aegypti larvae* for 24 Hours

Active Ingredients	Lower Limit	Middle Border	Upper Limit
Alkaloid	22.780%	29.084%	32.610%
Flavonoid	16.985%	26.580%	30.509%

Discussion

Water temperature measurement of *Aedes aegypti larvae* breeding media at control (0%) and at extract media of active ingredients of alkaloids and flavonoids in white chicory leaves (*Brassica pekinensis L*) was 28°-29°C. The standard water temperature is at 25°C-30°C;[18] therefore, the temperature of the test media at the time of the study was the optimum temperature for larval breeding. The pH measurement of the test media in the control group and alkaloid extracts, white chicory leaves (*Brassica pekinensis L*) flavonoids was 6.2–7.0 and in the control group the average pH was 6.2. The standard of water pH is 5.8–8;[19,20] therefore, the water pH of the test media at the time of the study, both treatment and control groups, was the optimum pH for larval breeding.

The measures of room temperature were obtained at an average of 28°C. The standard room temperature was 25°C-30°C;[21,22] Therefore, the room temperature used at the time of the study was the optimum temperature for larval development. The measure of room humidity obtained an average result of 64%. The optimum humidity for the life of *Aedes aegypti larvae* ranges from 60% to 80%;[18,23] therefore, room humidity during the study was the optimum humidity for larval breeding.

The results of using alkaloid active ingredient extracts at concentrations of 24%, 34%, and 40% with treatment for 24 hours obtained an average percentage of larval death of 41%, 60%, and 66%. These results proved that there was a larvicide effect on the administration of the alkaloid extract as an active ingredient of white chicory leaves (*Brassica pekinensis L*). The mechanism of alkaloid compounds is inhibiting the feeding power of *larvae* and acting as stomach poison.[24]

Meanwhile, the results of using flavonoid active ingredient extracts at concentrations of 24%, 34%, and 40% with treatment for 24 hours obtained an average percentage of larval death of 45%, 64%, and 68%. These results proved that there was a larvicide effect on the administration of flavonoid extract as the active ingredient of white chicory leaves (*Brassica pekinensis L*). Flavonoid compounds work as powerful respiratory inhibitors or as respiratory toxins.[25–27] The results of the Post Hoc LSD test of the active ingredients of alkaloids and flavonoids in general found that the higher the concentration, the higher the larvicide effect that could cause the death of test *larvae*. This was evidenced by the advantage of 40% extract concentration compared to 24% and 34% concentrations.

According to a study on the potential of fragrant pandan leaf (*Pandanus amaryllifolius Roxb*), larvicide from ethanol extract is effective as a natural larvicide for *Aedes aegypti* with six effective concentrations compared to controls. The concentrations are at 0.125%, 0.5%, 1%, 2%, and 4%, with an ineffective concentration of 0.05% and LC values of 2.113% and 3.497%.[24] LC_{50} value in the extract of alkaloid and flavonoid active ingredients contained in white chicory leaves (*Brassica pekinensis L*) probit test results showed that the LC_{50} value was a concentration that could kill 50% of *larvae* test for 24 hours at 29.084% alkaloid and 26.580% flavonoids active ingredients. World Health Organization (WHO) also stated that the minimum percentage of larvicide studies used is 1%,[28] meaning that the LC_{50} test results met the WHO standards and effectively killed 50% of *larvae* test.

The weakness of this study was that it used white chicory leaves from traditional markets. It also explored only alkaloids and flavonoids as the active ingredients of white chicory leaves, with a duration of exposure of 24 hours. The concentrations used were 24%, 34%, and 40%, obtained from the results of probit analysis. The *larvae* imposed were *Aedes aegypti* instar III *larvae* from the rearing results of the Entomology Laboratory of Poltekkes Kemenkes Surabaya, and water temperature, water pH, room temperature, and room humidity measured were rooms used as research sites.

Furthermore, the implication of this study was to provide considerable alternatives for the prevention and control of *Aedes aegypti* vectors that were environmentally friendly, affordable, and natural. Also, it was hoped to broaden horizons and knowledge of the *Aedes aegypti larvae* control through alkaloid and flavonoid extracts as active ingredients of white chicory leaves (*Brassica pekinensis L*) and can be used as a reference for the control of *Aedes aegypti larvae* with the use of bio-larvicide.

Conclusion

This study finds that higher concentrations of both alkaloid and flavonoid extracts significantly increased the mortality rate of *Aedes aegypti larvae*. A 40% concentration of both extracts shows the most effective results compared to lower concentrations. Further research is needed to understand the mechanism of action of the alkaloid and flavonoid extracts on *Aedes aegypti larvae*. In addition, more extensive field studies are needed to ensure the effectiveness and safety of using these extracts on a large scale in natural environments.

Abbreviations

DHF: Dengue hemorrhagic fever; SD: standard deviation; WHO: World Health Organization.

Ethics Approval and Consent to Participate

Ethical clearance has been obtained for the study from Poltekkes Kemenkes Surabaya (No.EA/1398/KEPK-Poltekkes_Sby/V.2023).

Competing Interest

All authors declare no conflicts of interest.

Availability of Data and Materials

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request. Any additional materials and data used in this research are also accessible upon request, ensuring transparency and reproducibility of the findings.

Authors' Contribution

M, DSOA, and N developed the research concept and design. DN and IS carried out laboratory experiments and collected data. M and AP were responsible for data analysis and interpretation of results. All authors, including M, DSOA, N, DN, IS, and AP, contributed to the writing and revision of the article and approved the final version for publication.

Acknowledgment

The authors' gratitude is conveyed to the Poltekkes Kemenkes Surabaya, especially to the Entomology Laboratory, which has facilitated and supported the implementation of the research.

References

1. Hestiningsih R, Syahputra GR, Martini M, et al. Aktivitas nokturnal Aedes spp. vektor demam berdarah dengue di Kota Semarang. Vektora J Vektor Reserv Penyakit. 2021; 13 (1): 27-34. DOI: 10.22435/vk.v13i1.3916.
2. Ali H, Mulyati S. Efektivitas ekstrak daun salam (Syzygium polyanthum) sebagai larvasida nyamuk Aedes sp. JNPH. 2021; 9 (1): 27-34. DOI: 10.37676/jnph.v9i1.1433.
3. Widiarti W, Setiyaningsih R, Pratamawati DA. Implementasi pengendalian vektor DBD di Provinsi Jawa Tengah. J Ekol Kesehat. 2018; 17 (1): 20-30. DOI: 10.22435/jek.17.1.116.20-30.
4. Kementerian Kesehatan Republik Indonesia. Profil Kesehatan Indonesia Tahun 2021. Jakarta: Kementerian Kesehatan Republik Indonesia; 2022.
5. Sukaningtyas R, Udijono A, Martini M. Status kerentanan nyamuk Aedes aegypti terhadap insektisida sipermetrin di area perimeter dan buffer pelabuhan Tanjung Emas Kota Semarang. Vektora J Vektor Reserv Penyakit. 2021; 13 (1): 11-18.
6. Dhenge NF, Pakan PD, Lidia K. Uji efektivitas larvasida ekstrak daun pepaya (Carica papaya) terhadap mortalitas larva vektor demam berdarah dengue Aedes aegypti. CMJ. 2021; 9 (1): 156-163. DOI: 10.35508/cmj.v9i1.4950.
7. Hidayati A. Densitas vektor dengue dan metode pengendalian pilihan keluarga. JKMI J Kesehat Masy Indones. 2018; 13 (2): 17-22.
8. Farich A, Perdana AA, Yunita D. Efektivitas tanaman sereh wangi sebagai larvasida nyamuk Aedes aegypti. Vektora J Vektor Reserv Penyakit. 2021; 13 (1): 19-26. DOI: 10.22435/vk.v13i1.3767.
9. Sutarto S, Syani AY. Resistensi insektisida pada Aedes aegypti. J Agromedicine Unila. 2018; 5 (2): 582-586.
10. Maulana S. Pengaruh biolarvasida daun tanaman sebagai kontrol vektor nyamuk Aedes aegypti penyebab demam berdarah: A literature review. JMH J Med Hutama. 2021; 2 (3): 978-989.
11. Putra FIE. Peran tanaman sebagai insektisida nabati terhadap gigitan nyamuk Aedes aegypti vektor demam berdarah dengue. Essent Essence Sci Med J. 2020; 18 (2): 1-4.
12. Priya SS, Vasantha-Srinivasan P, Altemimi AB, et al. Bioactive molecules derived from plants in managing dengue vector Aedes aegypti (Linn.). Molecules. 2023; 28 (5): 2386. DOI: 10.3390/molecules28052386.
13. Putri MA, Respatijarti R. Uji daya hasil tanaman sawi putih (Brassica rapa L. ssp. pekinensis) di dataran tinggi. Protan. 2019; 7 (3): 474-479.
14. Khusniyah N, Rohmah J. Efektivitas sabun transparan antibakteri ekstrak etanol sawi putih (Brassica rapa subsp. pekinensis) dengan kombinasi coconut oil dan castor oil. J Med Lab Sci Technol. 2019; 2 (1): 10-17. DOI: 10.21070/medicra.v2i1.1488.

15. Rahmaningtyas D, Pakan PD, Setianingrum ELS. Uji efektivitas larvasida ekstrak daun kelor (Moringa oleifera) terhadap mortalitas larva vektor demam berdarah dengue Aedes aegypti. Cendana Med J. 2022; 10 (2): 234-240. DOI: 10.35508/cmj.v10i2.8569.
16. Ningrum ASC. Uji ekstrak daun sirih (Piper betle Linn) sebagai larvasida alami larva Aedes aegypti (Studi di Desa Sambirejo Kecamatan Jogoroto Kabupaten Jombang). J Insan Cendekia. 2019; 6 (2): 73-77. DOI: 10.35874/jic.v6i2.540.
17. Ekapratiwi Y, Rachmadiva R, Virgine KA, et al. The effect of tobacco extracts based biolarvicide emulsion formulation against Aedes aegypti larvae. In: AIP Conference Proceedings. AIP Publishing; 2019. DOI: 10.1063/1.5096713.
18. Suryaningtyas NH, Margarethy I, Asyati D. Karakteristik habitat dan kualitas air terhadap keberadaan jentik Aedes spp di Kelurahan Sukarami Palembang. Spirakel. 2017; 9 (2): 53-59. DOI: 10.22435/spirakel.v8i2.8057.
19. Krissanti O, Setiawan S, Koerniasari K. Efektivitas air perasan daun alpukat (Persea americana Mill.) terhadap kematian larva nyamuk Aedes aegypti [Undergraduate Thesis]. Surabaya: Politeknik Kesehatan Kementerian Kesehatan Surabaya; 2018.
20. Janah M, Pawenang ET. Karakteristik sumur gali dengan keberadaan jentik nyamuk Aedes aegypti. HIGEIA J Public Health Res Dev. 2017; 1 (1): 8-14.
21. Lahdji A, Putra BB. Hubungan curah hujan, suhu, kelembaban dengan kasus demam berdarah dengue di Kota Semarang. Syifa Med J Kedokt Kesehat. 2017; 8 (1): 46-53. DOI: 10.32502/sm.v8i1.1359.
22. Anggraini TS, Cahyati WH. Perkembangan Aedes aegypti pada berbagai kondisi pH air dan salinitas air. HIGEIA J Public Health Res Dev. 2017; 1 (3): 1-10.
23. Fitriana BR, Yudhastuti R. Hubungan faktor suhu dengan kasus demam berdarah dengue (DBD) di Kecamatan Sawahan Surabaya. IJPH. 2018; 13 (1): 85-97. DOI: 10.20473/ijph.v13i1.2018.85-97.
24. Purnamasari MR, Sudarmaja IM, Swastika IK. Potensi ekstrak etanol daun pandan wangi (Pandanus amaryllifoliusRoxb.) sebagai larvasida alami bagi Aedes aegypti. E J Med. 2017; 6 (3): 1-8.
25. Sogandi S, Gunarto F. Efek larvasida fraksi etil asetat daun bangun-bangun (Plectranthus amboinicus) terhadap mortalitas larva Aedes aegypti. ASPIRATOR J Vector Borne Dis Stud. 2020; 12 (1): 27-36. DOI: 10.22435/asp.v12i1.1288.
26. Wahyudi R, Indriani H, Abror Y. The effect of corn silk extract (Zea mays) as biolarvicides of Aedes aegyptimosquito larvae in efforts to control spread of dengue hemorrhagic fever. IJPH. 2021; 16 (1): 23-31. DOI: 10.20473/ijph.v16i1.2021.23-31.
27. Nurdin N, Yasril AI, Anggraini DP. Faktor yang berhubungan dengan keberadaan jentik nyamuk Aedes aegypti di Kelurahan Garegeh. J Vektor Penyakit. 2022; 16 (2): 89-96. DOI: 10.22435/vektorp.v16i2.5440.
28. World Health Organization. Guidelines for laboratory and field testing of mosquito larvicides. Geneva: World Health Organization; 2005.