1. Submission received for Makara Journal of Science (21 Desember 2021)

2. Revision of Manuscript (27 Mei 2022)

Dokumen: a. Reference Number : SS21-078

b. Reference Number : SS21-078 Review

c. SS21-078 Trackcanges AB.doc

3. First revision: received with major revision (24-Juni-2022)

Dokumen: a. Response Letter Reviewer 1

b. Response Letter Reviewer 2

c. Revised Result Mayor

4. Ubdate Submitted Revised Result Minor (12 Juli 2022)

Dokumen: a. Response Letter Reviewer 1&2

b. Revised Result Minor

5. Proofreading (4 Juli 2022)

Dokumen: a. Tracked proofreading

b. Respon letter-Proofreading

6. Paper accepted (12 Juli 2024)

7. Published (12 Juli 2024)

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1. Submission received for Makara Journal of Science
   (21 Desember 2021)

Denai Wahyuni <denaiwahyuni69@htp.ac.id>

MS #1303: Submission received for Makara Journal of Science

Editors of Makara Journal of Science <editors-science-1303@dcuischolarhub.bepress.com> 21 Desember 2021 pukul 14.15
Kepada: =?UTF-8?Q?=22denaiwahyuni69=40htp=2Eac=2Eid=22?= <denaiwahyuni69@htp.ac.id>
Cc: The Authors <authors-science-1303@dcuischolarhub.bepress.com>, The Editors <editors-science-1303@dcuischolarhub.bepress.com>

A new submission for Makara Journal of Science has been uploaded by "denaiwahyuni69@htp.ac.id" <denaiwahyuni69@htp.ac.id>.

The authors are:
"denaiwahyuni69@htp.ac.id" <denaiwahyuni69@htp.ac.id>
"Nila Puspita Sari" <n.hyuk2704@gmail.com>
"Jasril Jasril" <jasril_k@yahoo.com>
"Jufrizal Syahri" <jsyachri@umri.ac.id>
The title is:
"VEGETABLE INSECTICIDE EXTRACT OF SCENTED ROOT (Polygala paniculata) IN CONTROLLING Aedes aegypti MOSQUITO"

The keywords are:
extract P. paniculata, Aedes aegypti mosquito, vegetable insecticide
The disciplines are:
Life Sciences | Organisms | Public Health

The submission has been assigned #1303. Please refer to this number in any correspondence related to the submission.

Authors may check the status of the submission, submit revisions, and contact editors via the following link:

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Best regards,
The Editors
Makara Journal of Science

2. Revision of Manuscript (27 Mei 2022)

Dokumen: a. Reference Number : SS21-078

b. Reference Number : SS21-078 Review

c. SS21-078 Trackcanges AB.doc

Denai Wahyuni <denaiwahyuni69@htp.ac.id>

SS21-078 Revision of Manuscript

Makara Journal of Science <editor_mss@ui.ac.id> 27 Mei 2022 pukul 13.47
Kepada: Denai Wahyuni <denaiwahyuni69@htp.ac.id>
Cc: "Dr. Ivandini Tribidasari A." <ivandini.tri@sci.ui.ac.id>

Dear Author,

We would like to inform you that your article titled “Vegetable Insecticide Extract of Scented Root (Polygala paniculata) in Controlling Aedes aegypti Mosquito” (SS21-078) has been reviewed by reviewers.

Based on the comments from two reviewers, this manuscript will be acceptable with major revision. Please indicate the changes that have been made with point-by-point responses to the reviewer's comments (response letter). If you are unable to revise according to the reviewer's recommendation, unfortunately, your manuscript can not be published in our journal.

Please respond to the comments of reviewers and submit a revised manuscript within 15 days of this notification. Thank you for submitting your manuscript to Makara Journal of Science.

Sincerely yours,

Chief Editor
Makara Journal of Science
Prof. Dr. Ivandini Tribidasari A.

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SS21-078 AB.rtf
108K

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475K

Response letter.doc
34K

2.a. KOREKSI…. SS21-078 AB

Referee's Report

Reference Number : SS21-078
Title : Vegetable Insecticide Extract of Scented Root (Polygala paniculata) in Controlling Aedes aegypti Mosquito

Please provide an overall assessment of the paper and provide answers to the following questions. Detailed comments and recommendations should not be written in the text, but should be numerically listed on separate sheets of paper that do not reveal your identity or affiliation. Minor wording or grammatical changes can be indicated in red pen or pencil in the text.

A. Please give your *appreciation of the scientific interest and novelty* of results described
(in Indonesia or in English)

This manuscript has novelty since Scented Root (Polygala paniculata) which has potential as an insecticide has never been tested on *Aedes aegypty* mosquitoes.

Unfortunately, this paper is written in poor English and does not meet the rules of academic English. many expressions and translations that do not conform to academic rules are found in this manuscript. the author should use a language consultant whenever this manuscript is accepted.

B. Style and Organization (Please check as appropriate) **Double Click**

1.	Is it clearly presented, well organized, and clearly written?	☒ Yes ☐ No
2.	Does it contain superfluous material?	☐ Yes ☒ No
3.	Is the title appropriate?	☒ Yes ☐ No
4.	Does the abstract include the important points of the paper	☒ Yes ☐ No
5.	If applicable, is the experiment section sufficiently detailed?	☐ Yes ☒ No
6.	Is sufficient information included or cited to support the assertions made and conclusions drawn?	☒ Yes ☐ No
7.	Are references to related work adequate, up to date and readily available?	☒ Yes ☐ No
8.	Are the illustrations and tables all necessary and adequate?	☐ Yes ☒ No
9.	Are the figure and table captions complete and accurate?	☒ Yes ☐ No
10.	Are the conclusions satisfactory, sound and justified	☒ Yes ☐ No
11.	Do you agree with the "Prime Novelty" as indicated (by the author)? Why or why not? It is first time try on Aedes aegypty …………………………………………………………………………………	☒ Yes ☐ No
12.	Does the manuscript contain original and self-consistent ideas? Please comment ………………………………………………………………… …………………………………………………………………………………	☒ Yes ☐ No
13.	Does the manuscript contain subject matter that might/should be omitted? If so, what? ……………………………………………………………………. …………………………………………………………………………………	☐ Yes ☒ No

14. For manuscript in English, Is the English satisfactory? ☐ Yes ☒ No

C. Quality and Assessment
(Please check as appropriate)

- ☐ Makes major contributions to the advancement of the subject
- ☒ Sound, original, and of interest
- ☐ Sound, original, but not suitable for Makara Seri Sains
- ☐ Does not add to knowledge of the subject
- ☐ Contains fundamental errors or faulty judgements

D. Referee's Recommendation
(Please check all boxes that apply)

- ☐ Acceptable in present form
- ☐ Acceptable with grammatical revision
- ☐ Acceptable with minor technical revision
- ☒ Acceptable with major technical revision
- ☐ Submit to further refereeing :
  (Suggest name of referee) ....
- ☐ Reject

Check here if you want to see the Revised Version ☐

Check here if you made annotation on the manuscript ☐

E. Timeliness of Research	☐ New	☒ Somewhat New	☐ Old
F. Scientific Impact	☐ High	☒ Moderate	☐ No Impact
G. Match between title, abstract, data and conclusions	☐ Excellent	☒ Good	☐ Poor
H. Rate scientific novelty to the community	☐ High	☒ Medium	☐ Low
I. Rate the interest to the community	☐ High	☒ Medium	☐ Low

J. Remarks/Additonal Comments (if any)
(in Indonesia or in English)

Please see/read the comments on the manuscript

2.b. KOREKSI 2…..SS21-078 AMR

Referee's Report

Reference Number : SS21-078 Review

Title : Vegetable Insecticide Extract of Scented Root (Polygala paniculata) in Controlling Aedes aegypti Mosquito

Please provide an overall assessment of the paper and provide answers to the following questions. Detailed comments and recommendations should not be written in the text, but should be numerically listed on separate sheets of paper that do not reveal your identity or affiliation. Minor wording or grammatical changes can be indicated in red pen or pencil in the text.

A. Please give your *appreciation of the scientific interest and novelty* of results described
(in Indonesia or in English)

The manuscript presents an investigation of the insecticidal effect of *Polygala paniculata* for the control of *Aedes aegypti*. It is an original and relevant work for research with insecticides obtained from natural products, however, the work should include more statistical analysis to accurately estimate the time needed to kill 50% of mosquitoes.

B. Style and Organization (Please check as appropriate) **Double Click**

0	Is it clearly presented, well organized, and clearly written?	☐ Yes ☒ No
1	Does it contain superfluous material?	☐ Yes ☒ No
2	Is the title appropriate?	☒ Yes ☐ No
3	Does the abstract include the important points of the paper	☒ Yes ☐ No
4	If applicable, is the experiment section sufficiently detailed?	☒ Yes ☐ No
5	Is sufficient information included or cited to support the assertions made and conclusions drawn?	☒ Yes ☐ No
7.	Are references to related work adequate, up to date and readily available?	☒ Yes ☐ No
8.	Are the illustrations and tables all necessary and adequate?	☒ Yes ☐ No
9.	Are the figure and table captions complete and accurate?	☐ Yes ☒ No
10.	Are the conclusions satisfactory, sound and justified	☒ Yes ☐ No
11.	Do you agree with the "Prime Novelty" as indicated (by the author)? Why or why not? …………………………………………………………. ………………………………………………………………………………	☒ Yes ☐ No
12.	Does the manuscript contain original and self-consistent ideas? Please comment …………………………………………………………… ………………………………………………………………………………	☒ Yes ☐ No
13.	Does the manuscript contain subject matter that might/should be omitted? If so, what? …………………………………………………………………. ………………………………………………………………………………	☐ Yes ☒ No
14.	For manuscript in English, Is the English satisfactory?	☐ Yes ☒ No

C. Quality and Assessment
(Please check as appropriate)

- [ ] Makes major contributions to the advancement of the subject
- [x] Sound, original, and of interest
- [ ] Sound, original, but not suitable for Makara Seri Sains
- [ ] Does not add to knowledge of the subject
- [ ] Contains fundamental errors or faulty judgements

D. Referee's Recommendation
E. (Please check all boxes that apply)

- [ ] Acceptable in present form
- [ ] Acceptable with grammatical revision
- [x] Acceptable with minor technical revision
- [ ] Acceptable with major technical revision
- [ ] Submit to further refereeing : (Suggest name of referee) ....
- [ ] Reject

Check here if you want to see the Revised Version ☐
Check here if you made annotation on the manuscript ☐

E. Timeliness of Research	☐ New	☒ Somewhat New	☐ Old
F. Scientific Impact	☐ High	☒ Moderate	☐ No Impact
G. Match between title, abstract, data and conclusions	☒ Excellent	☐ Good	☐ Poor
H. Rate scientific novelty to the community	☐ High	☒ Medium	☐ Low
I. Rate the interest to the community	☐ High	☒ Medium	☐ Low

J. Remarks/Additonal Comments (if any)
(in Indonesia or in English)

1. Consider using *Ae. aegypti* as an abbreviation for *Aedes aegypti.*

2. The abbreviations YFV, ZIKV, CHIKV and DENV are used to refer to viruses (etiological agents) and not to diseases. Consider tweaking your text (page 01)

3. There is redundancy between the Materials and Methods topic presented in the subtopics "Preparation of Materials and Tools" and "Extract Making". The text needs to be adjusted to avoid such redundancies (page 2-3).

4. In Figure 1, only one caption should be kept for an image. The descriptions that are in the figure must be placed in the legend (page 4).

5. When presenting the data in Figure 01, it is interesting to consider the average number of deaths by concentration and not the sum of deaths (Line 135).

6. Note the writing of the scientific name "*Aedes aegypti*" on pages 5 and 6.

7. In table 01 the date presented in the table (0.000) are different from those discussed in the text (0.001) (line

167).

8. The value 0.08 is greater than 0.05, not less. (line 185)

9. The Time required for 50% knockdown of mosquitoes (KT_{50}) could be accurately estimated using probit analysis (table 4).

10. In obtaining table 05, were the mortality averages considered?

11. In line 196 refers to table 04 and in line 207 refers to table 05.

12. According to the presentation of the text, I suggest that the Results section be presented separately from the Discussion section.

2.c. SS21-078 Trackhanges AB (2)

Vegetable Insecticide Extract of Scented Root (*Polygala paniculata*) in Controlling *Aedes aegypti* Mosquito

Comment [AB1]: Please revise the title with appropriate english, vegetable insectiside is not comon to use in this title

ABSTRACT

Controlling *Aedes aegypti* mosquitoes with chemical insecticides causes resistance effects on humans, environmental residues, as well as contaminates food and water. *Polygala paniculata* has potential as an alternative of insecticide in controlling *A. aegypti* mosquitoes. This study further aimed to determine the effect of the Insecticide Score of *P. paniculata* extract on *A. aegypti* mosquitoes' mortality based on KT_{50}. The 20 mosquitoes were used to examine the effectiveness of 10%, 15%, 20%, 25% of *P. paniculata* concentration with positive and negative controls in four repetitions every five minutes for one hour. The results showed the effect of extract *P. paniculata* on *A aegypti* mosquitoes' mortality. The Kruskal-Wallis test resulted in a p-value of 0.001 < 0.05, while the Spearman Correlation test gave a p-value of 0.008 < 0.05. Furthermore, the correlation strength was + 0.312 or 31.2%, with 10% concentration, while 15% had KT_{50}, with Insecticide Scores of 1 and 2, implying no knockdown effect. A 20% concentration had KT_{50} with an Insecticide Score of 3, indicating a weak knockdown effect, while a 25% concentration had KT_{50}, with an Insecticide Score of 5, signifying a quick knockdown effect. This shows that a 25% concentration has a quick knockdown time in *A. aegypti* mosquitoes. Therefore, extract *P. paniculata* may have the potential as a vegetable insecticide in controlling *A aegypti* mosquitoes.

Comment [AB2]: It is wrong way to abbrevaiate Aedes. Please follow the rule!!!

Keywords: extract *P. paniculata*, *Aedes aegypti* mosquito, vegetable insecticide

1. Introduction

The *Aedes aegypti* mosquito (L) belongs to the Order Diptera and family Culicidae, the main vector often neglected as a transmitter of diseases in humans. The diseases include yellow fever (YFV), Zika virus (ZIKV), Chikungunya (CHIKV), dengue virus (DENV), and other arboviruses [1]–[4]. The human arboviral disease transmitted by *A. aegypti* is a global public health threat [5]. This disease causes significant morbidity and mortality in developing countries [2]. The transmission of dengue fever is increasing in urban and semi-urban areas in tropical countries worldwide [6]. It is estimated that 40% or 50–528 million people worldwide are at risk of becoming infected with dengue fever and around 10,000–20,000 people die yearly [7]. According to WHO, about 390 million cases of dengue virus infection occur every year, of which 96 million manifest clinically with high severity. Furthermore, WHO reports that about 3.9 billion people are at risk of being infected with the dengue virus [8].

Indonesia is one of the developing countries where dengue hemorrhagic fever (DHF) is an infectious disease and an unresolved health problem. In 2020, there were 15,132 DHF cases in Indonesia, with a death rate of 145 people and a DHF incident rate of 31.23 [9]. This implies problems in efforts to control the disease.

Comment [AB3]: DHF not only problem in developing conutry. Please rewrite the paragraph

There is no specific vaccine for dengue fever, hence its control is conducted by eradicating the mosquito vector using synthetic insecticides [2]–[4], [10], [11]. However, long-term application and extensive use of synthetic insecticides cause the accumulation of residues in

Comment [AB4]: It is not relate between vaccine, insectiside, vector. Author can delete this paragraph. Focus, please...

food, water, soil and give adverse health effects on humans and ecosystems [12], [13]. This leaves residues that pollute the environment [4], [14] increasing population resistance of *A. aegypti* [3], [5], [15]–[19].

Controlling *A. aegypti* mosquitoes using synthetic insecticides involves fumigation with pyrethroids and larvacides containing temephos [11]. Pyrethroid resistance causes *A. aegypti* to keep growing, increasing the incidence of dengue fever (DF) worldwide. This increases the risk of almost half of the world's population being infected with the disease [5]. Similarly, using temephos to kill larvae increases the resistance of mosquitoes larvae, *A. aegypti*. This has been reported in several countries, such as Brazil [3], Mexico [20] and Southeast Asia countries, including Indonesia [5], Malaysia, Philippines, Thailand, Singapore, Laos, and Myanmar [21]

There is a need to obtain alternative insecticides effective in controlling the mosquito population *A. aegypti* [22]. This could be achieved using natural plant chemical compounds with the potential to control the mosquito population effectively. The environmentally friendly method would guarantee plant protection and avoid the side effects of synthetic insecticides [13], [23], [24]. Bioactive plant compounds are biodegradable, environmentally friendly, and non-toxic to other insects [1]. This is because bio-insecticides only affect the target insect without destroying beneficial natural enemies. Additionally, they are a safe, economical, target-specific, biodegradable, and residue-free environmental food source [23], [25].

Polygala paniculata, also known as vetiver in Riau, Indonesia, is a good-smelling annual herbaceous plant belonging to the family Polygalaceae and the genus Polygala. The plant is often used as traditional medicine, a tonic, and in inflammation cases of asthma, bronchitis, arthritis, and other pathologies, as well as kidney disorders [26]. Moreover, *P. paniculata* is used for in-vivo protection against the neurotoxic effects of Methylmercury (Hg) [27]. bronchitis, neurahenia, inflammation, amnesia, topical anesthetic, and expectorant drugs [28]. The Polygala extract produces secondary metabolites, including alkaloids, saponins, flavonoids, phenols, tannins, steroids, and terpenoids [28]. Similarly, *P. paniculata* contains alkaloids, flavonoids, tannins, saponins, and steroids [29]. Therefore, these bioactive compounds make *P. paniculata* potentially useful as a vegetable insecticide. This becomes an interesting study topic because the bioactive compounds extracted from *P. paniculata* have never been explored as vegetable insecticides in controlling the *A. aegypti* mosquito. Therefore, it is important to examine the insecticide score of the toxin contained in the bioactive compounds of *P. paniculata* in killing the *A. aegypti* mosquito.

Comment [AB5]: Explain the dsitribution this plant, in the world and in Indonesia.

2. Materials and Methods

Preparation of Materials and Tools

This study used 2500 grams of plant *P. paniculata* obtained in Kubang Region Siak Hulu sub-district, Kampar, Riau. This plant was proven by a laboratory certification test at the Botanical Laboratory, Faculty of Mathematics and Natural Sciences, Riau University. The test used 96% ethanol to macerate the *P. paniculata* plant powder. Furthermore, distilled water was used as a solvent and as a negative control in the extraction process, while synthetic insecticide Baygon cypermethrin was used as a positive control. The *A. aegypti* mosquito was obtained from breeding kept at the Parasitology Laboratory of Abdurrab University Pekanbaru. The equipment used in this study includes a blender, analytical scales, Rotary Vacuum Evaporator, stopwatch, shaker water bath, thermometer, hygrometer, Buchner funnel, stick, basin, test box, syringe, and a spray bottle.

Test Animal Preparation

Test animals were bred using media containing clean water in a cool place and protected from direct sunlight for the *A. aegypti* mosquitoes to lay their eggs. The larvae were reared in an aquarium at 24.2^0C - 24.4^0C, with a relative humidity of 67-70%. The larvae were fed with coconut water [30] to become larvae instar III and IV before becoming mosquitoes. The adult *A. aegypti* mosquitoes were then used as test animals.

Extract Making

Comment [AB6]: Use good english please

2500 grams of *P. paniculata* plants were washed and air-dried at room temperature and blended them to form 400 grams of powder. The powder was macerated with 96% ethanol until completely submerged for three days. The resulting solution was filtered using a Buchner funnel and placed in a dark bottle. Furthermore, the dregs from the first filtering were then soaked again for one day, filtered, and the process repeated in the third immersion. The results from the three maceration processes were combined and concentrated using a Vacuum Rotary Evaporator to evaporate 96% ethanol and obtain an extract. The extract obtained was stored in the refrigerator to be used later [31].

Testing

Comment [AB7]: Explain the kind of testing

The test was conducted by transferring 20 *A. aegypti* mosquitoes to each test boxes and spraying them with various *P. paniculata* plant extract, K (+), and K(-) concentrations four times. The effect of the concentrations on the *A. aegypti* mosquito was observed by looking at the changes in behavior, movement, and physical condition until death. The dead of *A. aegypti* mosquitoes were counted every five minutes for one hour. Moreover, the insecticide score of *P. paniculata* was determined from the number of *A. aegypti* mosquitoes considered dead at 5-minute intervals. At the end of each treatment, *A. aegypti* mosquitoes that remained alive were left to die or killed with Baygon.

Data analysis

Data were analyzed using the Statistical Test of Analysis of Variance with RAL, followed by the One Way ANOVA test. However, when the One Way ANOVA test does not meet the requirements, the Kruskal-Wallis Non-Parametric Test and the Test Spearman Analysis are conducted to examine the relationship between the independent and dependent variables.

3. Results and Discussion

The results of observing the *A. aegypti* mosquitoes' death after spraying the *P. paniculata* plant extract

The *A. aegypti* mosquitoes died after spraying the *P. paniculata* plant extract at 10% and 15% concentrations. They died slowly by flying irregularly and actively and falling in a tilted body position. The mosquitoes appeared weak, with some legs still moving, before becoming paralyzed, dying, and their bodies remaining stiff. The death was faster at 20% and 25% concentrations. In the positive control (K+), death occurred in less than five minutes, while in the negative control (K-), the *A. aegypti* mosquitoes tried to avoid the spray during the four experiment repetitions.

Total mortality of *A. aegypti* mosquitoes at each concentration with four repetitions

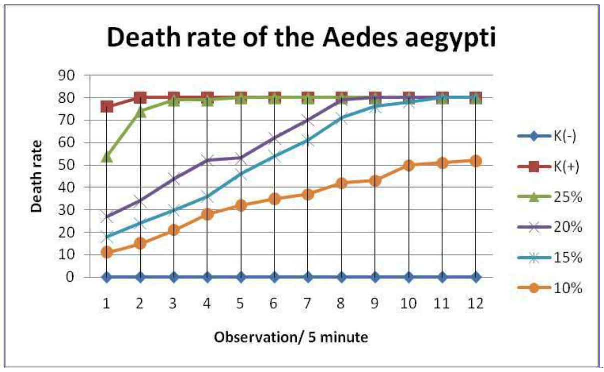

Figure 1. Total mortality of *A. aegypti* mosquitoes at each concentration with four repetitions (Primary Data. 2020)

Figure 1 shows that 10%. 15%, 20%, and 25% concentrations of the *P. paniculata* plant extract were sprayed to the treatment group. In the first five minutes of observation, the four repetitions of spraying caused the mortality of 11, 18, 27, and 54 *A. aegypti* mosquitoes. The mortality increased to 52, 80, 80, and 80 in the 60^{th} minute. In positive control (K+) using synthetic insecticide Baygon (cypermethrin), the four repetitions of spraying caused the death of 76 *A. aegypti* mosquitoes in less than five minutes. The test in the negative control (K-) using distilled water did not cause death. The highest total mortality of 80 *A. aegypti* mosquitoes at 15% concentration occurred at 51-55 minutes. Furthermore, a 20% concentration caused 80 deaths at 41-45 minutes, while a 25% concentration caused 80 deaths at 21-25 minutes.

The death percentage of *A. aegypti* mosquitoes at each concentration after every five minutes of observation

In Figure 2, the treatment group was sprayed with 10%, 15%, 20%, and 25% concentrations of the *P. paniculata* plant extract. The mortality percentage of *A. aegypti* mosquitoes in four repetitions was 13.75%, 22.5%, 33.75%, and 67.5% in five minutes of the observation. The total mortality increased to 65%, 100%, 100%, and 100% in the 60^{th} minute. The positive control (K+) test using synthetic insecticide Baygon (cypermethrin) caused a 95% mortality, where the first *A. aegypti* mosquito died in less than five minutes during the four test repetitions. In contrast, the negative control (K-) test using distilled water did not cause death. The highest total mortality of 100% *A. aegypti* mosquitoes at 15% concentration occurred at 51-55 minutes. The 20% and 25% concentrations caused 80 deaths at 41-45 and 21-25 minutes, respectively.

Comment [AB8]: Aedes aegypti italic

Comment [AB9]: Use good English please

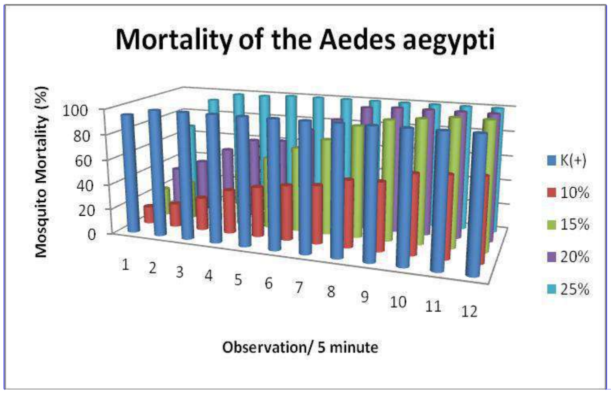

Figure 2: Percentage of *A. aegypti* mosquito mortality at each concentration every five minutes of observation (Primary Data, 2020)

Comment [AB10]: Not clear, please make in 2 dimension graph

Kruskal Wallis Non-Parametric Test

a. Concentration

Table 1. Non-Parametric Test Results Kruskal Wallis Concentration

	Knockdown
Chi-Square	30.324
Df	5
Asymp. Sig.	.000

Primary Data, 2020

Comment [AB11]: No need to put the statistical anaylisis in here. Write as result please

In the Non-Parametric Kruskal-Wallis concentration, the significant value was 0.001 < 0.05. This indicates a significant difference in mosquito mortality between the treatment groups with different mean rank values. Therefore, the different concentrations of *P. paniculata* plant extract significantly affect mosquito mortality because the significant value is <0.05.

b. Time

Table 2. Non-Parametric Test Results *Kruskal Wallis* (Time)

	Knockdown
Chi-Square	19.512
Df	11
Asymp. Sig.	.052

Primary Data, 2020

Comment [AB12]: No need to put the statistical anaylisis in here. Write as result please

In the Non-Parametric Kruskal Wallis, the significant value is 0.052 > 0.05. This implies a significant difference in the *A. aegipty* mosquito mortality between the treatment groups with different mean rank values. Therefore, the contact time of *P. paniculata* plant extracts significantly affects the mortality of *A. aegipty* mosquitoes because the significant value is > 0.05.

Spearman Analysis

Table 3. Spearman Analysis Test Results

			Concentration	Time	Knockdown
Spearman's rho	Concentration	Koefisien korelasi	1.000	.000	.312**
		Sig. (2-tailed)	.	1.000	.008
		N	72	72	72

Primary Data, 2020

Comment [AB13]: No need to put the statistical anaylisis in here. Write as result please

The Spearman Analysis test results show a significant value of 0.08 <0.05. This indicates a correlation between the different concentrations of *P. paniculata* plant extracts and the *mortality of A. aegipty* mosquitoes. The correlation strength is denoted by the coefficient of 0.312 or 31.2%. Moreover, the positive correlation means that the higher concentration of *P. paniculata* plant extract increased the number of deaths of *A. aegipty* mosquitoes.

Insecticide Score of *P. paniculata Plants*

Table 4. The average percentage of death of *A. aegypti* mosquitoes at various concentrations of treatment with *Knockdown Time* (KT_{50})

Time	10%	15%	20%	25%	K (+)	K(-)
5	2,75	4,5	6,75	13,5	19	0
10	3,75	6	8,5	18,5	20	0
15	5,25	7,5	11	19,75	20	0
20	7	9	13	19,75	20	0
25	8	11,5	13,25	20	20	0
30	8,75	13,5	15,5	20	20	0
35	9,25	15,25	17,5	20	20	0
40	10,5	17,75	19,75	20	20	0
45	10,75	19	20	20	20	0
50	12,5	19,5	20	20	20	0
55	12,75	20	20	20	20	0
60	13	20	20	20	20	0

Primary Data, 2020

Comment [AB14]: Please make the table more clear. Explain the highlighting data!

In Table 1, the test repetition using a 10% concentration of the *P. paniculata* plant extract produced KT_{50} between 36-40 minutes. The test using 15%, 20%, and 25% concentrations produced KT_{50} between 21-25, 11-15, and less than five minutes, respectively. On average, no *A. aegypti* mosquitoes fell in the negative control, while KT_{50} occurred in less than five minutes in the positive control. This means that the different concentrations of *P. paniculata* plant extracts affected the number of *A. aegypti* differently during each treatment and repetition.

Table 5: Insecticide Score of *P. paniculata* Plant Extract Based on KT_{50}

Group Control	**KT50 (Time)**	***Knockdown Effect***	**Insecticide Score**	**Interpretation**
Concentration 10%	36-40	-	1	-
Concentration 15%	21-25	-	2	-
Concentration 20%	11-15	+	3	Weak Knockdown
Concentration 25%	< 5	+++	5	Quick Knockdown
Positive Control	< 5	+++	5	Quick Knockdown

Primary Data, 2020

Comment [AB15]: What mean +?

Table 2 shows the effectiveness level of the four *P. paniculata* plant extract concentrations and positive control based on Insecticide Knockdown Time$_{50}$ (KT_{50}). A 10% concentration of the *P. paniculata* plant extract had a KT of$_{50,}$ between 36-40 minutes. This implies an Insecticide Score of 1 or no knockdown effect. Similarly, a 15% concentration of the plant extracts had a KT of$_{50,}$ between 21-25 minutes, indicating an Insecticide Score of 2 or no knockdown. A 20% concentration of the plant extract had a KT of$_{50}$ between 11-15 minutes, implying an Insecticide Score of 3 or a weak knockdown effect. Furthermore, a 25% concentration of *P. paniculata* plant extract had a KT_{50} in less than 5 minutes. This indicates an Insecticide Score of 5 or a quick knockdown effect. The 25% concentration had a KT_{50} in less than 5 minutes, similar to the positive control, with an Insecticide Score of 5 or a quick knockdown effect. Therefore, the 25% concentration of *P. paniculata* plant extract was most effective in knocking out *A. aegypti* mosquitoes, with an Insecticide Score of 5 or a quick knockdown effect.

The results in Figures 1 and 2 show that the number and percentage of *A. aegypti* mosquito deaths increased with the concentration of *P. paniculata* plant extract. Higher concentrations increase the accumulation of *P. paniculata* plant extract's toxic bioactive compounds in *A. aegypti* mosquitoes' bodies, increasing their mortality.

Kosini examined the effect of the *Gnidia kaussiana (Thymeleaceae)* extract on *Callosobruchus maculatus.* The study explained that increased absorption of toxic compounds in the *Gnidia kaussiana* extract accelerates the mortality process of larvae *Callosobruchus maculatus* by melanizing the cuticle. This disrupts the endocrine system due to the presence of secondary metabolites such as terpenoids, alkaloids, and flavonoids [32]. Furthermore, another study examined the effect of *Ocimmum basilicum*, vegetable insecticides, on the death of *A. aegypti* mosquitoes. The results showed that the mortality of *A. aegypti* mosquitoes increases with an increase in the absorption of toxic compounds in the *O. basilicum* extract. Also, longer exposure to the extract increases the toxicity [33]. A previous study examined the effect of vegetable larvicides of *Carbera manghas* leaves on *A. aegypti* mosquito larvae. The findings showed the larvicide toxicity increased with the *C. manghas* extract's concentration and exposure time. This is because the absorption of more toxic compounds affects the body's metabolism and increases the mortality of *A. aegypti* larvae [34].

Many previous studies stated that the bioactive plant compounds have insecticidal, larvicidal, repellent, and environmentally-friendly effects useful for insect control. According to Hikal, essential oils, flavonoids, alkaloids, glycosides, esters, and fatty acids have anti-insect effects. Therefore, they could be used as an alternative to chemical compounds in insect control as repellents, feeding deterrents or antifeedants, toxicants, inhibitors, growth factors, chemosterilants, and attractants [23].

Previous studies explained that *Polygala* plants contain bioactive compounds with various biological activities, such as alkaloids, saponins, flavonoids, phenols, tannins, steroids, and terpenoids [28], [29]. Tannins and flavonoids are phenolic plant compounds that act as primary antioxidants or free radical scavengers [35]. The bioactive compounds of *P. paniculata* able to affect the mortality of *A. aegipty* mosquitoes are flavonoids, saponins, tannins, alkaloids, steroids, and terpenoids. In this study, the *A. aegypti* mosquitoes death was caused by compounds in the *P. paniculata* plant extract. The compounds entered the mosquito's body through contact or respiratory poisoning and the mouth and digestive tract, causing stomach poisoning.

The analysis shows that the number of *A. aegypti* mosquitoes that died when exposed to the *P. paniculata* plant extract varied with the extract's concentration. Variations in the concentrations affected the mortality of *A. aegypti* mosquitoes differently in each treatment and repetition. The results in Figures 1 and 2 show that spraying a 25% concentration of the *P. paniculata* plant extract for 60 minutes kills 80 *A. aegypti* mosquitoes in less than the first five minutes. This is the largest number of deaths that occurred faster than other concentrations. Therefore, the higher concentration of the plant extract increased its effectiveness as a vegetable insecticide against mosquitoes.

This study compared the effectiveness level of the four *P. paniculata* plant extract concentrations with positive controls based on Insecticide Knockdown $Time_{50}$ (KT_{50}). A 25% concentration of the plant extract had a KT_{50} of less than five minutes, implying an Insecticide Score of 5 or a quick knockdown effect. This is in line with the 2006 WHO standard, which stated that an insecticide is has a knockdown time required to drop a vector when the median knockdown ranges between 3-5. Furthermore, it has a quick knockdown effect when it has a KT_{50} of less than five minutes. According to Norris, a good insecticide requirement for controlling disease vector insect species must cause a rapid knockdown of the target species, especially active pathogens. Additionally, it must quickly intervene and kill adult mosquitoes [16].

The 25% concentration had a KT_{50} of less than five minutes, similar to a positive control Baygon containing cypermethrin. They both had an Insecticide Score 5, implying a quick knockdown effect. Therefore, the 25% concentration of the *P. paniculata* plant extract was the most effective in knocking down *A. aegypti* mosquitoes. This is because it had an Insecticide Score of 5 or a quick knockdown effect. The positive control treatment was intended to compare the quality of *P. paniculata* plant extract concentration. In contrast, the negative control treatment was used to compare its effectiveness with the plant extract. The results showed that no mosquito died after 60 minutes of observation.

Chang highlighted the need to use vegetable insecticides as an alternative insect controller. Using inappropriate insecticides causes insects to adapt easily by metabolic detoxification quickly and survival. This causes synchronization of insect immunity passed on to their offspring. Therefore, it is necessary to develop safe alternative insecticides, larvicides, and repellents effective for humans, animals, the environment, and the ecosystem. Natural insecticides are needed to suppress vector resistance and slow down their genetic adaptation [36]. According to Hikal et al., botanical insecticides only affect target insects, do not destroy beneficial natural enemies, and are a safe and residue-free food source. Hikal et al. recommended using plant-based insecticides as an integrated insect management program that greatly reduces the use of synthetic insecticides [23]. It is more environmentally friendly, effective, cheap, and naturally available [14].

Conclusion

The active substance contained in the *P. paniculata* plant extract has the ability to drop and kill *A. aegypti* mosquitoes. This is because more compounds in the plant extracts exposed to the mosquitoes increase the knockdown effect time. Therefore, the plant extract is a potential alternative insecticide for controlling *A. aegypti* mosquitoes. It does not leave residues in the environment and is safe for other living beings. Also, the extract's compounds do not cause resistance against *A. aegypti* mosquitoes, and the plant has economic value and is beneficial to cultivate.

Acknowledgments

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REFERENCE

Comment [AB16]: 1.Please change the scopus non-indexed reference to the scopus indexed reference

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3. First revision: received with major revision (24-Juni-2022)

Dokumen: a. Response Letter Reviewer 1

b. Response Letter Reviewer 2

c. Revised Result Mayor

Denai Wahyuni <denaiwahyuni69@htp.ac.id>

SS21-078 Revision of Manuscript

Makara Journal of Science <editor_mss@ui.ac.id> 24 Juni 2022 pukul 10.07
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Dear Author,

Your revision has been received by us nicely and needs to be checked from our editor.
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Best Regards,

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Editorial Assistant
Makara Journal of Science

[Kutipan teks disembunyikan]

3. a. Response letter Reviewer 1

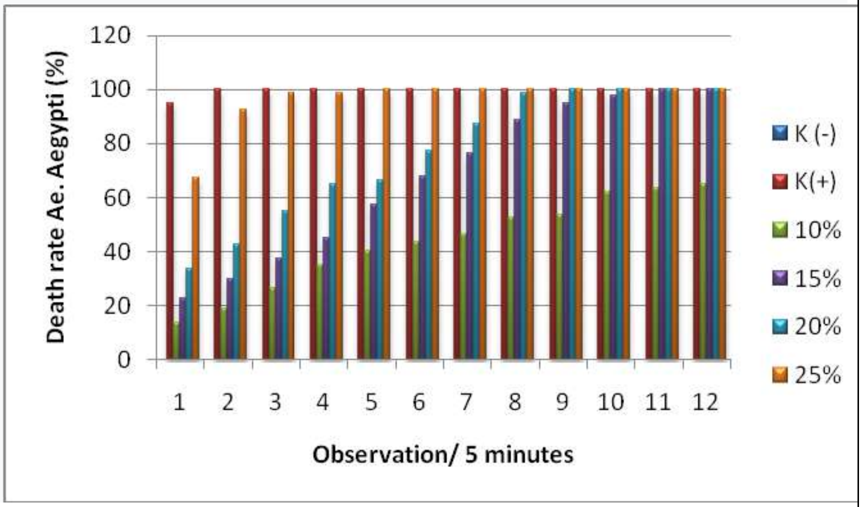
Journal : Makara Journal of Science
Manuscript ID :
Title: **Bio-insecticides Extract of Scented Root (*Polygala paniculata*) in Controlling *Aedes aegypti* Mosquito**

There are some following issues that needs to be addressed:

Reviewer 1

Reviewer's Comments	Author's Comments
Reference Number : SS21-078; Comment (AB1). Please revise the title with appropriate english, vegetable insectiside is not comon to use in this title	Thank you, we have corrected the suggestion regarding the title, here are the improvements: Bio-insecticide's Extract of Scented Root (*Polygala paniculata*) in Controlling *Aedes aegypti* Mosquito
Comment (AB2): It is wrong way to abbrevaiate Aedes. Please follow the rule!!!	Thank you for the suggestion regarding the correct abbreviation regarding *Aedes* to *Ae. aegypti* we have corrected everything in the manuscript regarding the title we have corrected, here are the corrections:
Comment (AB3): DHF not only problem in developing conutry. Please rewrite the paragraph	Thank you for your input regarding this paragraph, we have corrected it in the text on lines 34-37 as follows: Indonesia is one of where dengue hemorrhagic fever (DHF) is an infectious disease and an unresolved health problem. In 2020, there were 15,132 DHF cases in Indonesia, with a death rate of 145 people and a DHF incident rate of 31.23[9].. This implies problems in efforts to control the disease.
Comment (AB4): It is not relate between vaccine, insectiside, vector. Author can delete this paragraph. Focus, please...	Thank you for your suggestion regarding there is no relationship between vaccines , insecticides, vectors we have corrected in the text on lines 38-41 as follows: However, long-term application and extensive use of synthetic insecticides cause the accumulation of residues in food, water, soil and give adverse health effects on humans and ecosystems[10], [11]. This leaves residues that pollute the environment [3], [12]. increasing population resistance of *Ae. aegypti*[4], [5], [13]–[17].
Comment (AB5): Explain the dsitribution this plant, in the world and in Indonesia	Thank you for your suggestion to explain the distribution of this plant in the world and Indonesia, we have added it to the text on lines 57-63 as follows:

	Polygala L. is one of the largest genera belonging to the *Polygalaceae* tribe. This genus consists of 500 species and can be found in tropical, sub-tropical, temperate and mountainous areas throughout the world except New Zealand. Most of these species grow in Central and South Tropical America. Several types of *Polygala L*. that can be used as medicine such as :*Polygala chinensis L., Polygala paniculata L., Polygala polifoliaPresl., and Polygala sibirica L.*[24].*Polygala paniculata*, also known as vetiver in Riau, Indonesia, is a good-smelling annual herbaceous plant belonging to the family Polygalaceae and the genus Polygala.
Comment (AB6): Use good english please	Thank you for We have corrected the correction regarding the subtitles to use good English in the text on line 91 as follows Extraction Process
Comment (AB7): Explain the kind of testing	Thank you for the correction regarding the type of test we have corrected and added to the text on line 100-109 as follows: **Bio-insecticide's Extract of *P. paniculata* Test against *Ae. Aegypti* Mosquitos** Bio-insecticide's extract of *P. paniculata* tests against *Ae. aegypti* was carried out by transferring 20 *Ae. aegypti* in each test boxes, then sprayed with various concentrationsof *P*. paniculata plant extract with four repetitions, as well as for K (+) and K(-).The effect of the concentrations on the *A. aegypti* mosquito was observed by looking at the changes in behavior, movement, and physical condition until death. *Ae. Aegypti* mosquitoes death were counted every five minutes for one hour. Moreover, the insecticide score of *P. paniculata* was determined from the number of *Ae. Aegypti* mosquitoes considered deadat 5-minute intervals. *Ae. Aegypti* mosquitoes that remained alive were left to die or killed with Baygon.
Comment (AB9): Use good English please	Thank you for the correction to use correct English, we have corrected the text on line 134-143 as follows: Based on Figure 1 In the treatment group, the *P. paniculata* plant extract was sprayed with 10%, 15%, 20% and 25% concentration in the first 5 minutes of observation. *Ae.aegypti* mosquitoes death rate with 4 repetitions in a row was 11, 18, 27 and 54 individuals. In the 60 minutes, the total mortality of *Ae. aegypti* mosquitoes in 4 repetitions was 52, 80, 80 and 80. In positive control (K+) using synthetic insecticide baygon (cypermethrin) in less than 5 minutes *Ae. aegypti* mosquitoes fell and died in 4 repetitions, namely 76 individuals.While in the negative control (+) using distilled water did not cause death.There was 80 *Ae. aegypti*

	mosquitoes were death at a concentration of 15% occurred at 51-55 minutes, a 20% concentration of 80 total deaths occurred at 41-45 minutes and a 25% concentration of 80 total deaths occurred at 21-25 minutes.
Comment (AB10): Not clear, please make in 2 dimension graph	Thank you for the correction regarding the three (3) dimensional graph we have corrected to a two (2) dimensional graph in the text on lines 162-168 as follows:  Figure 2: The Average Mortality of *Ae. aegypti* at each Concentration of Five Minutes Observation
Comment (AB11); (AB12); (AB13): No need to put the statistical anaylisis in here. Write as result please	Thank you for the correction to not include statistical analysis, we have changed it to include only the results in the text on lines 169-188 as follows: Based on the results of study in figure 1 and 2, explained that the different concentrations of *P. paniculata* plant extracts gave different effects on number of *Ae. aegypti* mosquitoes death in each treatment and repetition. The number of *Ae.aegypti* mosquitoes death tends to increase along with the increase in the concentration of *P. paniculata* plant extracts. It's means that the higher used of the concentration, the higher of potency of the *P. paniculata* plant extract as a bioinsecticide against the *Ae. Aegypti* mosquito.During one hour observations showed the increasing number of *Ae. Aegypti* mosquitos's death. It's explained the longer an observation time, the greater potential as a bioinsecticide. This is supported by the results of the Kruskall-Wallis test (p-value of 0.001 <0.05), which means that there is a significant difference between the death rate of the *Ae. aegypti* mosquito and the difference in the concentration of the *P. paniculata* plant extract (10%, 15%, 20%, 25 %) of Knockdown Time acceleration. The results of the Kruskall-Wallis test in this study were correct and continued with the Spearman correlation test with the results of p-value 0.008 <0.05 there was a significant correlation between the increase in the concentration

of the extract (treatment group) and the knockdown time of the *P. paniculata* plant extract. From the results of the Kruskall-Wallis test and the Spearman correlation test, it can be concluded that the greater of concentration, the faster the time of death of *Ae. aegypti* mosquito, the strength of the correlation is denoted by a correlation coefficient 0.312 (31.2%).Correlation coefficient occurred with positive value means that correlation is moderate. The higher concentrations of *P. paniculata* plants extract then the faster of knockdown time

Comment (AB14): Please make the table more clear. Explain the highlighting data!

Thanks for the suggestion we have corrected the explanation on lines 194-198 in the manuscript

Table 1 Average of *Ae. Aegypti* Falling Down At Various Treatment Concentrations With Knockdown Time$_{50}$ (KT$_{50}$)

Time	10%	15%	20%	25%	K (+)	K(-)
5	2,75	4,5	6,75	13,5	19	0
10	3,75	6	8,5	18,5	20	0
15	5,25	7,5	11	19,75	20	0
20	7	9	13	19,75	20	0
25	8	11,5	13,25	20	20	0
30	8,75	13,5	15,5	20	20	0
35	9,25	15,25	17,5	20	20	0
40	10,5	17,75	19,75	20	20	0
45	10,75	19	20	20	20	0
50	12,5	19,5	20	20	20	0
55	12,75	20	20	20	20	0
60	13	20	20	20	20	0

: *Ae. Aegypti* Knockdown Time(KT$_{50}$);

Comment (AB15) untuk tabel 5. What mean +?

Thank you we tried to explain Regarding the meaning of +
In table 5 : Insecticide Score of *P. paniculata* Plant Extract Based on KT$_{50}$.

The knockdown effect has been defined by WHO 2006 in the book Guidelines for Testing Mosquitos Adulticides for Indoor Residual Spraying and Treatment of Mosquitos Nets
That: Insectiside Score Based on KT 50

KT50 (minute)	Score	Knockdown effect	Interpretation
>50	0	-	-
31-49	1	-	-
16-30	2	-	-
11-15	3	+	Weak Knockdown
5-10	4	++	Strong Knockdown
<5	5	+++	Quick Knockdown

source: WHO, 2006

A value of 3 means the median knockdown is in the range of 11-15 minutes which is interpreted to have a knockdown

	effect (+) but is weak. A value of 4 means the median knockdown is in the range of 5-10 minutes which is interpreted to have a strong knockdown effect (++). A value of 5 means the median knockdown is in the range of less than 5 minutes which is interpreted to have a knockdown effect (+++) that the insecticide has a "Quick Knockdown Effect" (WHO, 2006). So the + sign means explaining the Knockdown effect status of Bioinsecticide
Comment (AB16): Please change the scopus non-indexed reference to the scopus indexed reference	Thank you for the correction to change all non-Scopus references to Scopus-indexed references, all of which we have changed to Scopus-indexed references in the script as follows **References** [1] L. H. Guimarães de Oliveira *et al.*, "Agave sisalana extract induces cell death in Aedes aegypti hemocytes increasing nitric oxide production," *Asian Pac. J. Trop. Biomed.*, vol. 6, no. 5, pp. 396–399, May 2016. [2] D. A. Fernandes *et al.*, "Larvicidal compounds extracted from helicteres velutina K. Schum (Sterculiaceae) evaluated against aedes aegypti L.," *Molecules*, vol. 24, no. 12, p. 2315, 2019. [3] L. Scalvenzi *et al.*, "Larvicidal activity of Ocimum campechianum, Ocotea quixos and Piper aduncum essential oils against Aedes aegypti," *Parasite*, vol. 26, no. 23, pp. 1–8, 2019. [4] D. Valle, D. F. Bellinato, P. F. Viana-Medeiros, J. B. P. Lima, and A. D. J. Martins Junior, "Resistance to temephos and deltamethrin in aedes aegypti from Brazil between 1985 and 2017," *Mem. Inst. Oswaldo Cruz*, vol. 114, no. 3, pp. 1–17, Mar. 2019. [5] Z. H. Amelia-yap, C. D. Chen, M. Sofian-azirun, and V. L. Low, "Pyrethroid resistance in the dengue vector Aedes aegypti in Southeast Asia : present situation and prospects for management," *Parasit. Vectors*, vol. 11, no. 332, pp. 1–17, 2018. [6] S. Leta, T. Jibat, E. M. De Clercq, K. Amenu, M. U. G. Kraemer, and C. W. Revie, "International Journal of Infectious Diseases Global risk mapping for major diseases transmitted by Aedes aegypti and Aedes albopictus," *Int. J. Infect. Dis.*, vol. 67, pp. 25–35, 2018. [7] M. Y. Lee, "Essential Oils as Repellents against

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3.b. Response letter Reviewer 2

<table>
<tr><th>Reviewer’s Comments</th><th>Author’s Comments</th></tr>
<tr><td>1. Consider using Ae. aegypti as an abbreviation for Aedes aegypti.</td><td>Thank you for providing corrections to change the abbreviation Aedes aegypti to Ae. Aegypti, we have corrected this suggestion in the manuscript</td></tr>
<tr><td>2. The abbreviations YFV, ZIKV, CHIKV and DENV are used to refer to viruses (etiological agents) and not to diseases. Consider tweaking your text (page 01)</td><td>Thank you for the suggestion to consider changing the text regarding the abbreviations YHF, ZIKV, CHIKV and DENV in paragraph 1. We have changed this in the text on lines 23-25 as follows:

The Aedes aegypti mosquito (L) belongs to the Order Diptera and family Culicidae, the main vector often neglected as a transmitter of diseases in humans. The diseases include Yellow Fever, Zica, Chikungunya, Dengue Haemorhaegic Fever and other arbo viruses[1]–[4].</td></tr>
<tr><td>3. There is redundancy between the Materials and Methods topic presented in the subtopics "Preparation of Materials and Tools" and "Extract Making". The text needs to be adjusted to avoid such redundancies (page 2-3).</td><td>Thank you for the correction

Regarding the redundancy between the topic of Materials and Methods presented in the subtopic Preparation of Materials and Tools and Preparation of Extracts in the text, we have corrected it on lines 78-83 in the text as follows:

Preparation of Materials and Tools
This study used 2500 gram P. paniculata, 5 litre of 96% ethanol, 5 litre of distilled water, 1 bottle of synthetic insecticide Baygon (cypermethrin), and 480 Ae. aegypti mosquito. The equipment used in this study includes a blender, analytical scales, Rotary Vacuum Evaporator, stopwatch, shaker water bath, thermometer, hygrometer, Buchner funnel, stick, basin, test box, syringe, and a spray bottle.</td></tr>
<tr><td>4. In Figure 1, only one caption should be kept for an image. The descriptions that are in the figure must be placed in the legend (page 4).</td><td>Thank you for the correction regarding the information in Figure 1, we have corrected it on line 144-147 as follows:</td></tr>
</table>

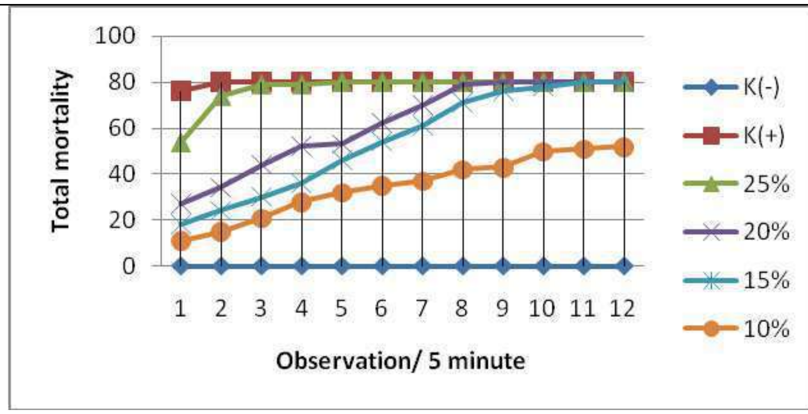
<table>
<tr>
<td></td>
<td>


Figure 1 Ae. Aegypti mosquitoes total mortality at each concentration with four repetitions
</td>
</tr>
<tr>
<td>5. When presenting the data in Figure 01, it is interesting to consider the average number of deaths by concentration and not the sum of deaths (Line 135).</td>
<td>
Thank you for the advice. Figure 01 is retained but the average mortality is made in Figure 02 as follows: on lines 144-147 and lines 164-168

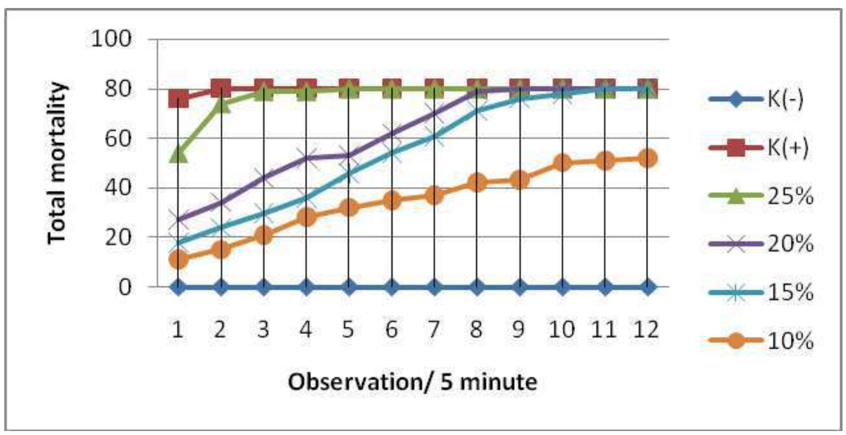
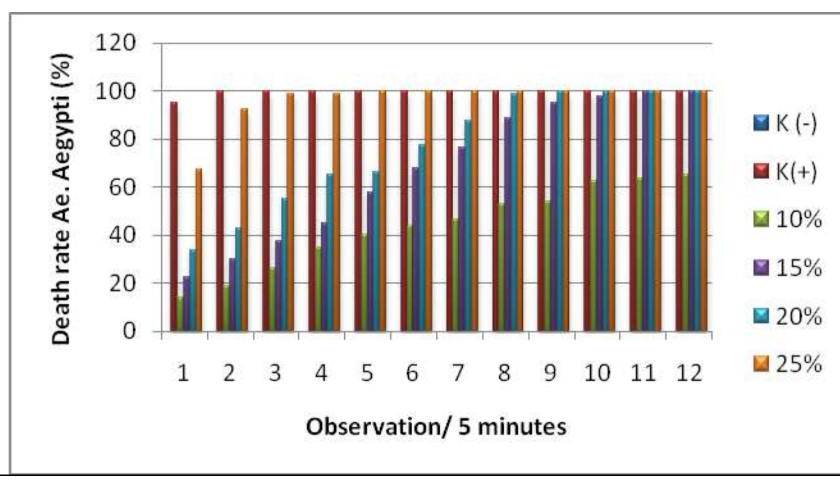


Figure 1 Ae. Aegypti mosquitoes total mortality at each concentration with four repetitions



</td>
</tr>
</table>

	Figure 2: The Average Mortality of *Ae. aegypti* at each Concentration of Five Minutes Observation
6. Note the writing of the scientific name "*Aedes aegypti*" on pages 5 and 6.	Thank you for the advice regarding scientific writing Aedes aegypti " on pages 5 and 6 we have corrected it as suggested
7. In table 01 the date presented in the table (0.000) are different from those discussed in the text (0.001) (line 167)	Thank you for the advice. Based on input from other reviewers that this table is not needed but the results from the table. This is conveyed in the script narrative only on lines 169-188 as follows: Based on the results of study in figure 1 and 2, explained that the different concentrations of *P. paniculata* plant extracts gave different effects on number of *Ae. aegypti* mosquitoes death in each treatment and repetition. The number of *Ae.aegypti* mosquitoes death tends to increase along with the increase in the concentration of *P. paniculata* plant extracts. It's means that the higher used of the concentration, the higher of potency of the *P. paniculata* plant extract as a bioinsecticide against the *Ae. Aegypti* mosquito.During one hour observations showed the increasing number of *Ae. Aegypti* mosquitos's death. It's explained the longer an observation time, the greater potential as a bioinsecticide. This is supported by the results of the Kruskall-Wallis test (p-value of 0.001 <0.05), which means that there is a significant difference between the death rate of the *Ae. aegypti* mosquito and the difference in the concentration of the *P. paniculata* plant extract (10%, 15%, 20%, 25 %) of Knockdown Time acceleration. The results of the Kruskall-Wallis test in this study were correct and continued with the Spearman correlation test with the results of p-value 0.008 <0.05 there was a significant correlation between the increase in the concentration of the extract (treatment group) and the knockdown time of the *P. paniculata* plant extract. From the results of the Kruskall-Wallis test and the Spearman correlation test, it can be concluded that the greater of concentration, the faster the time of death of *Ae. aegypti* mosquito, the strength of the correlation is denoted by a correlation coefficient 0.312 (31.2%).Correlation coefficient occurred with positive value means that correlation is moderate. The higher concentrations of *P. paniculata* plants extract then the faster of knockdown time
8. The value 0.08 is greater than 0.05, not less. (line 185)	Thanks for the correction. We have deleted this information because the related table has been changed to a new narrative
9. The Time required	Thank you for the suggestion, to use probit analysis to determine the

for 50% knockdown of mosquitoes (KT50) could be accurately estimated using probit analysis (table 4).	time required for 50% mosquito knockdown (KT50) in Table 4 We try to explain Knockdown time (KT50) means the time it takes for each concentration of P. paniculata Bioinsecticide to drop 50% of the average Ae. aegypti.We did not use the probit analysis in Table 4, because here we only looked at how many minutes the average number of mosquitoes fell by 50% for each concentration of P. paniculata Bioinsecticide.
10. In obtaining table 05, were the mortality averages considered?	Thank you for the correction. We try to explain, We have changed Table 05 to Table 02 because Tables 01, 02, 03 have been omitted according to input from other reviewers. For Table 02, we do not use the average mortality, but we use KT_{50} (Time) which is the time it takes to drop 50% of Ae. aegypti after being sprayed with P. paniculata Bioinsecticide at each test concentration.
11. In line 196 refers to table 04 and in line 207 refers to table 05.	Thank you for the correction. We have corrected the script on line 201-202 as follows: Knockdown $Time_{50}$ (KT_{50}) is the times to knockdown of *Ae*, *aegypti* mosquitoes. In Table 1, the average repetition of the study show during the 10% concentration of *P. paniculata* plant
12. According to the presentation of the text, I suggest that the Results section be presented separately from the Discussion section.	Thank you for the advice. We have corrected the manuscript according to the following suggestions: A. Results pada line 117 B. Discussion pada line 229

3.c. Revised result mayor

Bio-insecticide's Extract of Scented Root (*Polygala paniculata*) in Controlling *Aedes aegypti* Mosquito

ABSTRACT

Controlling *Aedes aegypti* mosquitoes with chemical insecticides causes resistance effects on humans, environmental residues, as well as contaminates food and water. *Polygala paniculata* has potential as an alternative of insecticide in controlling *Ae. Aegypti* mosquitoes. This study further aimed to determine the effect of the Insecticide Score of *P. paniculata* extract on *Ae. Aegypti* mosquitoes' mortality based on KT_{50}. The 20 mosquitoes were used to examine the effectiveness of 10%, 15%, 20%, 25% of *P. paniculata* concentration with positive and negative controls in four repetitions every five minutes for one hour. The results showed the effect of extract *P. paniculata* on *Aeaegypti* mosquitoes' mortality. The Kruskal-Wallis test resulted in a p-value of 0.001 < 0.05, while the Spearman Correlation test gave a p-value of 0.008 < 0.05. Furthermore, the correlation strength was + 0.312 or 31.2%, with 10% concentration, while 15% had KT_{50}, with Insecticide Scores of 1 and 2, implying no knockdown effect. A 20% concentration had $KT_{50}$with an Insecticide Score of 3, indicating a weak knockdown effect, while a 25% concentration had KT_{50}, with an Insecticide Score of 5, signifying a quick knockdown effect. This shows that a 25% concentration has a quick knockdown timein*Ae. Aegypti* mosquitoes. Therefore, extract *P. paniculata* may have the potential as a bio-insecticidesin controlling *Ae. aegypti* mosquitoes.

Keywords: extract *P. paniculata*, *Aedes aegypti* mosquito, bio-insecticides

1. Introduction

The *Aedes aegypti* mosquito (L) belongs to the Order Diptera and family Culicidae, the main vector often neglected as a transmitter of diseases in humans. The diseases include yellow fever (YFV), Zika virus (ZIKV), Chikungunya (CHIKV), dengue virus (DENV), and other arboviruses[1]–[4]. The human arboviral disease transmitted by *Ae.aegypti* is a global public health threat[5]. This disease causes significant morbidity and mortality in developing countries [1]. The transmission of dengue fever is increasing in urban and semi-urban areas in tropical countries worldwide[6].. It is estimated that 40% or 50–528 million people worldwide are at risk of becoming infected with dengue fever and around 10,000–20,000 people die yearly[7]. According to WHO, about 390 million cases of dengue virus infection occur every year, of which 96 million manifest clinically with high severity. Furthermore, WHO reports that about 3.9 billion people are at risk of being infected with the dengue virus[8]

Indonesia is one of where dengue hemorrhagic fever (DHF) is an infectious disease and an unresolved health problem. In 2020, there were 15,132 DHF cases in Indonesia, with a death rate of 145 people and a DHF incident rate of 31.23[9].. This implies problems in efforts to control the disease.However, long-term application and extensive use of synthetic insecticides cause the accumulation of residues in food, water, soil and give adverse health effects on humans and ecosystems[10], [11]. This leaves residues that pollute the environment [3], [12]. increasing population resistance of *Ae. aegypti*[4], [5], [13]–[17].

Controlling *Ae.aegypti* mosquitoes using synthetic insecticides involves fumigation with pyrethroids and larvacides containing temephos[18].Pyrethroid resistance causes *Ae. aegypti* to keep growing, increasing the incidence of dengue fever (DF) worldwide. This increases the risk of almost half of the world's population being infected with the disease[5]. Similarly, using temephos to kill larvae increases the resistance of mosquitoes larvae, *A. aegypti*. This has been reported in several countries, such as Brazil[4], Mexico[19]and Southeast Asia countries, including Indonesia Malaysia, Philippines, Thailand, Singapore, Laos, and Myanmar[5].

There is a need to obtain alternative insecticides effective in controlling the mosquito population *A. aegypti*[20]. This could be achieved using natural plant chemical compounds with the potential to control the mosquito population effectively. The environmentally friendly method would guarantee plant protection and avoid the side effects of synthetic insecticides,[10], [21], [22].Bioactive plant compounds are biodegradable, environmentally friendly, and non-toxic to other insects[2].This is because bio-insecticides only affect the target insect without destroying beneficial natural enemies. Additionally, they are a safe, economical, target-specific, biodegradable, and residue-free environmental food source[23].

Polygala L. is one of the largest genera belonging to the *Polygalaceae* tribe. This genus consists of 500 species and can be found in tropical, sub-tropical, temperate and mountainous areas throughout the world except New Zealand. Most of these species grow in Central and South Tropical America. Several types of *Polygala L*. that can be used as medicine such as :*Polygala chinensis L., Polygala paniculata L., Polygala polifoliaPresl., and Polygala sibirica L*.[24].*Polygala paniculata*, also known as vetiver in Riau, Indonesia, is a good-smelling annual herbaceous plant belonging to the family Polygalaceae and the genus Polygala. The plant is often used as traditional medicine, a tonic, and in inflammation cases of asthma, bronchitis, arthritis, and other pathologies, as well as kidney disorders[25]. Moreover, *P. paniculata* is used for in-vivo protection against the neurotoxic effects of Methylmercury (Hg)[26], bronchitis, neurahenia, inflammation, amnesia, topical anesthetic, and expectorant drugs[27]. The Polygala extract produces secondary metabolites, including alkaloids, saponins, flavonoids, phenols, tannins, steroids, and terpenoids[27].Therefore, these bioactive compounds make *P. paniculata* potentially useful as a bio-insecticides. This becomes an interesting study topic because the bioactive compounds extracted from *P. paniculata* have never been explored as bio-insecticides in controlling the *Ae. aegypti* mosquito. Therefore, it is important to examine the insecticide score of the toxin contained in the bioactive compounds of *P. paniculata* in killing the *Ae. aegypti* mosquito.

2. Materials and Methods

Preparation of Materials and Tools

This study used 2500 gram sofplant *P. paniculata* obtained in Kubang Region Siak Hulu sub-district, Kampar, Riau. This plant was proven by a laboratory certification test at the Botanical Laboratory, Faculty of Mathematics and Natural Sciences, Riau University. The test used 96% ethanol to macerate the *P. paniculata* plant powder. Furthermore,distilled water was used as a solvent and as a negative control in the extraction process, while synthetic insecticide Baygon (cypermethrin) was used as a positive control. The *A. aegypti* mosquito was obtained from breeding kept at the Parasitology Laboratory of Abdurrab University Pekanbaru. The equipment used in this study includes a blender, analytical scales, Rotary Vacuum Evaporator, stopwatch, shaker water bath, thermometer, hygrometer, Buchner funnel, stick, basin, test box, syringe, and a spray bottle.

Test Animal Preparation

Test animals were bred using media containing clean water in a cool place and protected from direct sunlight for the *Ae*. *Aegypti* mosquitoes to lay their eggs. The larvae were reared in an aquarium at 24.2^0C - 24.4^0C, with a relative humidity of 67-70%. The larvae werefed with coconut water[28] to become larvae instar III and IV before becoming mosquitoes. The adult *Ae.aegypti* mosquitoes were then used as test animals.

Extraction Process

2500 grams of *P*. *paniculata* plants were washed and air-dried at room temperature and blended them to form 400 grams of powder. The powder was macerated with 96% ethanol until completely submerged for three days. The resulting solution was filtered using a Buchner funnel and placed in a dark bottle. Furthermore, the dregs from the first filtering were then soaked again for one day, filtered, and the process repeated in the third immersion. The results from the three maceration processes were combined and concentrated using a Vacuum Rotary Evaporator to evaporate 96% ethanol and obtain an extract. The extract obtained was stored in the refrigerator to be used later[29].

Bio-insecticide's Extract of *P. paniculata* Test against *Ae. Aegypti* Mosquitos

Bio-insecticide's extract of *P*. *paniculata* tests against *Ae*. *aegypti* was carried out by transferring 20 *Ae*. *aegypti* in each test boxes, then sprayed with various concentrationsof *P*. paniculata plant extract with four repetitions, as well as for K (+) and K(-).The effect of the concentrations on the *A*. *aegypti* mosquito was observed by looking at the changes in behavior, movement, and physical condition until death. *Ae*. *Aegypti* mosquitoes death were counted every five minutes for one hour. Moreover, the insecticide score of *P*. *paniculata* was determined from the number of *Ae*. *Aegypti* mosquitoes considered deadat 5-minute intervals. *Ae*. *Aegypti* mosquitoes that remained alive were left to die or killed with Baygon.

Data analysis

Data were analyzed using the Statistical Test of Analysis of Variance with RAL, followed by the One Way ANOVA test. However, when the One Way ANOVA test does not meet the requirements, the Kruskal-Wallis Non-Parametric Test and the Test Spearman Analysis are conducted to examine the relationship between the independent and dependent variables.

3. Results and Discussion

A. Results

The Results of Observing *Ae.aegypti* Mosquitoes' Death After Spraying the *P. paniculata* Plant Extract

The *Ae*. *aegypti* mosquitoes died after spraying the *P*. *paniculata* plant extractat 10% and 15% concentrations. They died slowly by flying irregularly and actively and falling in a tilted body position. The mosquitoes appeared weak, with some legs still moving, before becoming paralyzed, dying, and their bodies remaining stiff. The death was faster at 20% and 25%

concentrations. In the positive control (K+), death occurred in less than five minutes, while in the negative control (K-), the *Ae. Aegypti* mosquitoes tried to avoid the spray during the four experiment repetitions.

Ae. aegypti Mosquitoes Death Rate at Each Concentration With Four Repetitions

Based on Figure 1 In the treatment group, the *P. paniculata* plant extract was sprayed with 10%, 15%, 20% and 25% concentration in the first 5 minutes of observation. *Ae.aegypti* mosquitoes death rate with 4 repetitions in a row was 11, 18, 27 and 54 individuals. In the 60 minutes, the total mortality of *Ae. aegypti* mosquitoes in 4 repetitions was 52, 80, 80 and 80. In positive control (K+) using synthetic insecticide baygon (cypermethrin) in less than 5 minutes *Ae. aegypti* mosquitoes fell and died in 4 repetitions, namely 76 individuals.While in the negative control (+) using distilled water did not cause death.There was 80 *Ae. aegypti* mosquitoes were death at a concentration of 15% occurred at 51-55 minutes, a 20% concentration of 80 total deaths occurred at 41-45 minutes and a 25% concentration of 80 total deaths occurred at 21-25 minutes.

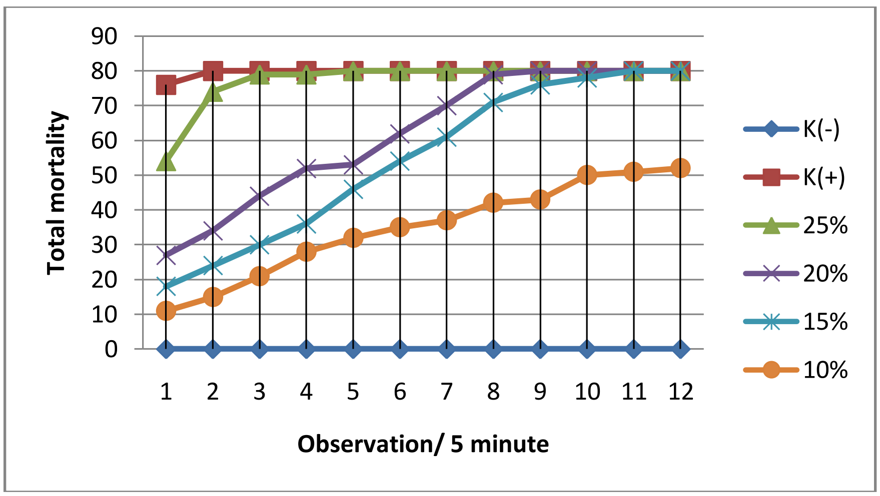


Figure 1 *Ae. Aegypti* mosquitoes death rate at each concentration with four repetitions
(Primary Data. 2020)

The Average of *Ae. aegypti* Death Rate at Each Concentration Every Five Minutes Observation

Based on Figure 2, the treatment group was sprayed with *P. paniculata* plant extract with a concentration of 10%, 15%, 20% and 25% in the first 5 minutes of observation, the average mortality of *Ae. aegypti* mosquitoes with 4 repetitions in a row was 2.75 tails (13.75%), 4.5 tails (22.5%), 6.75 tails (33.75%) and 13 tails (67.5%).*Ae.aegypti* mosquitoes death rate in 60 minutes with 4 repetitions at a concentration of 10% was 13 tails (65%), while the concentrations of 15%, 20% and 25% were 20 tails (100%). 95% Ae. aegypti mosquito fell and died in positive control (K+) using synthetic insecticide Baygon (cypermethrin) in less than 5 minutes with 4 repetitions.While the negative control (-) using distilled water did not cause death.*Ae.Aegypti mosquitos* totally died at 15% concentration in 51 until 55 minutes, 20% concentration of 80 total deaths occurred at 41-45 minutes and 25% concentration of 80 total deaths occurred at 21-25 minutes.

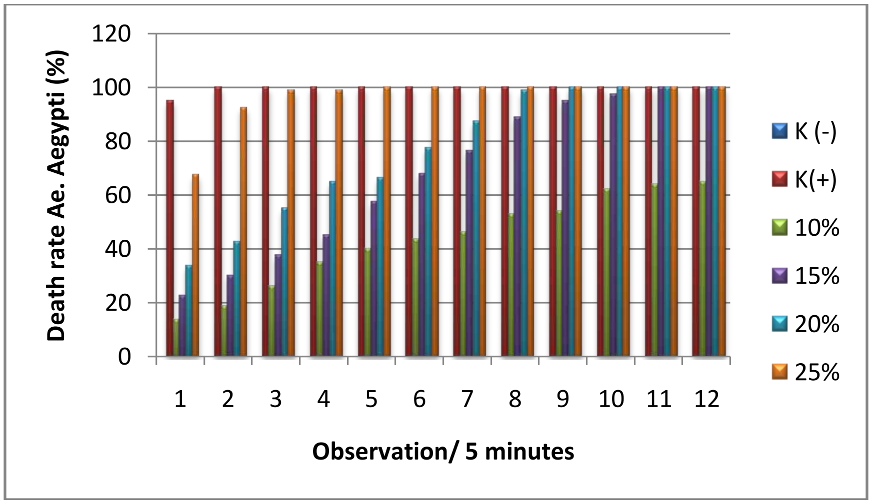


Figure 2: The Average Mortality of *Ae. aegypti* at each Concentration of Five Minutes Observation

Based on the results of study in figure 1 and 2, explained that the different concentrations of *P. paniculata* plant extracts gave different effects on number of *Ae. aegypti* mosquitoes death in each treatment and repetition. The number of *Ae.aegypti* mosquitoes death tends to increase along with the increase in the concentration of *P. paniculata* plant extracts. It's means that the higher used of the concentration, the higher of potency of the *P. paniculata* plant extract as a bioinsecticide against the *Ae. Aegypti* mosquito.During one hour observations showed the increasing number of *Ae. Aegypti* mosquitos's death. It's explained the longer an observation time, the greater potential as a bioinsecticide. This is supported by the results of the Kruskall-Wallis test (p-value of 0.001 <0.05), which means that there is a significant difference between the death rate of the *Ae. aegypti* mosquito and the difference in the concentration of the *P. paniculata* plant extract (10%, 15%, 20%, 25 %) of Knockdown Time acceleration. The results of the Kruskall-Wallis test in this study were correct and continued with the Spearman correlation test with the results of p-value 0.008 <0.05 there was a significant correlation between the increase in the concentration of the extract (treatment group) and the knockdown time of the *P. paniculata* plant extract. From the results of the Kruskall-Wallis test and the Spearman correlation test, it can be concluded that the greater of concentration, the faster the time of death of *Ae. aegypti* mosquito, the strength of the correlation is denoted by a correlation coefficient 0.312 (31.2%).Correlation coefficient occurred with positive value means that correlation is moderate. The higher concentrations of *P. paniculata* plants extract then the faster of knockdown time.

Insecticide Score of *P. paniculata* Plants

Table 1 Average of *Ae. Aegypti* Falling Down At Various Treatment Concentrations With Knockdown Time$_{50}$ (KT$_{50}$)

Time	10%	15%	20%	25%	K (+)	K(-)
5	2,75	4,5	6,75	13,5	19	0
10	3,75	6	8,5	18,5	20	0
15	5,25	7,5	11	19,75	20	0
20	7	9	13	19,75	20	0
25	8	11,5	13,25	20	20	0
30	8,75	13,5	15,5	20	20	0
35	9,25	15,25	17,5	20	20	0
40	10,5	17,75	19,75	20	20	0
45	10,75	19	20	20	20	0
50	12,5	19,5	20	20	20	0
55	12,75	20	20	20	20	0
60	13	20	20	20	20	0

: *Ae. Aegypti* Knockdown Time(KT$_{50}$);

Source :(Primary Data, 2020)

Knockdown Time$_{50}$ (KT$_{50}$) is the times to knockdown of *Ae, aegypti* mosquitoes. In Table 1,the average repetition of the study show during the 10% concentration of *P. paniculata* plant extract had a KT$_{50}$ between 36 until 40 minutes, 15% concentration had a KT$_{50}$ between 21 until 25 minutes, in the *P. paniculata* plant extract with 20% concentration the KT$_{50}$ was obtained between 11 and 15 minutes and in the *P. paniculata* plant extract with a concentration of 25%, the KT$_{50}$ was less than 5 minutes. In the average iteration of the study, there were no *Ae. aegypti* mosquitoes that fell on the negative control and the Knockdown Time$_{50}$ (KT$_{50}$) was less than 5 minutes in the positive control. This means that the different concentrations of *P. paniculata* plant extracts gave different effects on the number of *Ae. aegypti* that fell, as well as on each treatment and repetition.

Table 2: Bio Insecticide Extract *P. paniculata* based on Knockdown Time$_{50}$

Group Control	KT50 (Time)	*Knockdown Effect*	Insecticide Score	Interpretation
Concentration 10%	36-40	-	1	-
Concentration 15%	21-25	-	2	-
Concentration 20%	11-15	+	3	Weak Knockdown
Concentration 25%	< 5	+++	5	Quick Knockdown
Positive Control	< 5	+++	5	Quick Knockdown

Interpretation Data(+ *weak Knockdown* +++ *Quick Knockdown*)[30]
(Primary Data, 2020)

Table 2 shows the effectiveness level of the four *P. paniculata* plant extract concentrations and positive control based on Insecticide Knockdown Time$_{50}$ (KT_{50}). A 10% concentration of the *P. paniculata* plant extract had a KT of$_{50,}$ between 36-40 minutes. This implies an Insecticide Score of 1 or no knockdown effect. Similarly, a 15% concentration of the plant extracts had a KT of$_{50,}$ between 21-25 minutes, indicating an Insecticide Score of 2 or no knockdown. A 20% concentration of the plant extract had a KT of$_{50}$between 11-15 minutes, implying an Insecticide Score of 3 or a weak knockdown effect. Furthermore, a 25% concentration of *P. paniculata* plant extract had a KT_{50} in less than 5 minutes. This indicates an Insecticide Score of 5 or a quick knockdown effect. The 25% concentration had a KT_{50} in less than 5 minutes, similar to the positive control, with an Insecticide Score of 5 or a quick knockdown effect. Therefore, the 25% concentration of *P. paniculata* plant extract was most effective in knocking out *Ae. Aegypti* mosquitoes, with an Insecticide Score of 5 or a quick knockdown effect.

B. Discussion

The results in Figures 1 and 2 show that the number and percentage of *Ae. aegypti* mosquito deaths increased with the concentration of *P. paniculata* plant extract. Higher concentrations increase the accumulation of *P. paniculata* plant extract's toxic bioactive compounds in *A. aegypti* mosquitoes' bodies, increasing their mortality.

Kosini examined the effect of the*Gnidia kaussiana (Thymeleaceae)* extracton *Callosobruchus maculatus.* The study explained that increased absorption of toxic compounds in the *Gnidia kaussiana* extract accelerates the mortality process of larvae *Callosobruchus maculatus*by melanizing the cuticle. This disrupts the endocrine system due to the presence of secondary metabolites such as terpenoids, alkaloids, and flavonoids[31].The study of da Botas et al explained that essential oil *Baccharisreticularia* DC and *limonene* as a larvacide agent on Controlling *Ae. Aegypti* (Diptera: Culicidae),it's also able to inhibit the formation of acetylcholinesterase enzyme by blocking the nerve signal of transduction which can cause the death and paralysis in *Ae. aegypti*larvae. The more larvae of *Ae.aegypti* absorbs the toxic compounds in *B. reticularia* essential oil, the more *Ae. aegypti* death. Also, the longer exposure to *B. reticularia* essential oil compounds will increase the level of toxicity.[32].

Many previous studies stated that the bioactive plant compounds have insecticidal, larvicidal, repellent, and environmentally-friendly effects useful for insect control. According to Suluvoy, essential oils, flavonoids, alkaloids, glycosides, esters, and fatty acids have anti-insect effects. Therefore, they could be used as an alternative to chemical compounds in insect control as repellents, feeding deterrents or antifeedants, toxicants, inhibitors, growth factors, chemosterilants, and attractants[21].

Previous studies explained that *Polygala* plants contain bioactive compounds with various biological activities, such as alkaloids, saponins, flavonoids, phenols, tannins, steroids, and terpenoids[27]. Tannins and flavonoids are phenolic plant compounds that act as primary antioxidants or free radical scavengers[33].The bioactive compounds of *P. paniculata* able to affect the mortality of *Ae. aegipty* mosquitoes are flavonoids, saponins, tannins, alkaloids, steroids, and terpenoids. In this study,the *Ae.aegypti* mosquitoes death was caused by compounds in the *P. paniculata* plant extract. The compounds entered the mosquito's body through contact or respiratory poisoning and the mouth and digestive tract, causing stomach poisoning.

The analysis showsthat the number of *Ae.aegypti* mosquitoes that died when exposed to the *P. paniculata* plant extract varied with the extract's concentration. Variations in the concentrations affected the mortality of *Ae.aegypti* mosquitoes differently in each treatment and repetition. The results in Figures 1 and 2 show that spraying a 25% concentration of the *P. paniculata* plant extract for 60 minutes kills 80 *Ae. Aegypti* mosquitoes in less than the first five minutes. This is the largest number of deaths that occurred faster than other concentrations. Therefore, the higher concentration of the plant extract increased its effectiveness as a vegetable insecticide against mosquitoes.

This study compared the effectiveness level of the four *P. paniculata* plant extract concentrations with positive controls based on Insecticide Knockdown Time$_{50}$ (KT_{50}). A 25% concentration of the plant extract had a KT_{50} of less than five minutes, implying an Insecticide Score of 5 or a quick knockdown effect. This is in line with the 2006 WHO standard, which stated that an insecticide is has a knockdown time required to drop a vector when the median knockdown ranges between 3-5[30]. Furthermore, it has a quick knockdown effect when it has a KT_{50} of less than five minutes. According to Norris, a good insecticide requirement for controlling disease vector insect species must cause a rapid knockdown of the target species, especially active pathogens. Additionally, it must quickly intervene and kill adult mosquitoes [17].

The 25% concentration had a KT_{50} of less than five minutes, similar to a positive control Baygon containing cypermethrin. They both had an Insecticide Score 5, implying a quick knockdown effect. Therefore, the 25% concentration of the *P. paniculata* plant extract was the most effective in knocking down *Ae. aegypti* mosquitoes. This is because it had an Insecticide Score of 5 or a quick knockdown effect. The positive control treatment was intended to compare the quality of *P. paniculata* plant extract concentration. In contrast, the negative control treatment was used to compare its effectiveness with the plant extract. The results showed that no mosquito died after 60 minutes of observation.

Chang highlighted the need to use bio-insecticides as an alternative insect controller. Using inappropriate insecticides causes insects to adapt easily by metabolic detoxification quickly and survival. This causes synchronization of insect immunity passed on to their offspring. Therefore, it is necessary to develop safe alternative insecticides, larvicides, and repellents effective for humans, animals, the environment, and the ecosystem. Natural insecticides are needed to suppress vector resistance and slow down their genetic adaptation[34].According to Sulovoy and Grace., botanical insecticides only affect target insects, do not destroy beneficial natural enemies, and are a safe and residue-free food source. [21].It is more environmentally friendly, effective, cheap, and naturally available[12].

Conclusion

The active substance contained in the *P. paniculata* plant extract has the ability to drop and kill *Ae. aegypti* mosquitoes. This is because more compounds in the plant extracts exposed to the mosquitoes increase the knockdown effect time. Therefore, the plant extract is a potential alternative insecticide for controlling *Ae. aegypti* mosquitoes. It does not leave residues in the environment and is safe for other living beings. Also, the extract's compounds do not cause resistance against *Ae. aegypti* mosquitoes, and the plant has economic value and is beneficial to cultivate.

Acknowledgments

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4. Ubdate Submitted Revised Result Minor (12 Juli 2022)

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b. Revised Result Minor

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MS #1303: Update submitted for "Bio-insecticide's Extract of Scented Root (Polygala paniculata) in Controlling the Mosquito Aedes aegypti (L.)"

Eksternal Kotak Masuk ×

Editors of Makara Journal of Science <editors-science-1303@dcuischolarhub.bepress.... 12 Jul 2022, 12.48

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This is an automatically-generated note to inform you that "Makara Journal of Science" <editor_mss@ui.ac.id> has submitted an update to MS #1303, "Bio-insecticide's Extract of Scented Root (Polygala paniculata) in Controlling the Mosquito Aedes aegypti (L.)," in Makara Journal of Science.

4.a. Response letter Review 1 dan 2

Journal : Makara Journal of Science
Manuscript ID :
Title: **Bio-insecticides Extract of Scented Root (*Polygala paniculata*) in Controlling *Aedes aegypti* Mosquito**

There are some following issues that needs to be addressed:

Reviewer 1

Reviewer's Comments	Author's Comments
Reference Number : SS21-078; Comment (AB1). Please revise the title with appropriate english, vegetable insectiside is not comon to use in this title	Thank you, we have corrected the suggestion regarding the title, here are the improvements: Bio-insecticide's Extract of Scented Root (*Polygala paniculata*) in Controlling *Aedes aegypti* Mosquito
Comment (AB2): It is wrong way to abbrevaiate Aedes. Please follow the rule!!!	Thank you for the suggestion regarding the correct abbreviation regarding *Aedes* to *Ae. aegypti* we have corrected everything in the manuscript regarding the title we have corrected, here are the corrections:
Comment (AB3): DHF not only problem in developing conutry. Please rewrite the paragraph	Thank you for your input regarding this paragraph, we have corrected it in the text on lines 34-37 as follows: Indonesia is one of where dengue hemorrhagic fever (DHF) is an infectious disease and an unresolved health problem. In 2020, there were 15,132 DHF cases in Indonesia, with a death rate of 145 people and a DHF incident rate of 31.23[9].. This implies problems in efforts to control the disease.
Comment (AB4): It is not relate between vaccine, insectiside, vector. Author can delete this paragraph. Focus, please...	Thank you for your suggestion regarding there is no relationship between vaccines , insecticides, vectors we have corrected in the text on lines 38-41 as follows: However, long-term application and extensive use of synthetic insecticides cause the accumulation of residues in food, water, soil and give adverse health effects on humans and ecosystems[10], [11]. This leaves residues that pollute the environment [3], [12]. increasing population resistance of *Ae. aegypti*[4], [5], [13]–[17].
Comment (AB5): Explain the dsitribution this plant, in the world and in Indonesia	Thank you for your suggestion to explain the distribution of this plant in the world and Indonesia, we have added it to the text on lines 57-63 as follows:

	Polygala L. is one of the largest genera belonging to the *Polygalaceae* tribe. This genus consists of 500 species and can be found in tropical, sub-tropical, temperate and mountainous areas throughout the world except New Zealand. Most of these species grow in Central and South Tropical America. Several types of *Polygala L.* that can be used as medicine such as :*Polygala chinensis L., Polygala paniculata L., Polygala polifoliaPresl., and Polygala sibirica L.*[24].*Polygala paniculata*, also known as vetiver in Riau, Indonesia, is a good-smelling annual herbaceous plant belonging to the family Polygalaceae and the genus Polygala.
Comment (AB6): Use good english please	Thank you for We have corrected the correction regarding the subtitles to use good English in the text on line 91 as follows Extraction Process
Comment (AB7): Explain the kind of testing	Thank you for the correction regarding the type of test we have corrected and added to the text on line 100-109 as follows: **Bio-insecticide's Extract of *P. paniculata* Test against *Ae. Aegypti* Mosquitos** Bio-insecticide's extract of *P. paniculata* tests against *Ae. aegypti* was carried out by transferring 20 *Ae. aegypti* in each test boxes, then sprayed with various concentrationsof *P.* paniculata plant extract with four repetitions, as well as for K (+) and K(-).The effect of the concentrations on the *A. aegypti* mosquito was observed by looking at the changes in behavior, movement, and physical condition until death. *Ae. Aegypti* mosquitoes death were counted every five minutes for one hour. Moreover, the insecticide score of *P. paniculata* was determined from the number of *Ae. Aegypti* mosquitoes considered deadat 5-minute intervals. *Ae. Aegypti* mosquitoes that remained alive were left to die or killed with Baygon.
Comment (AB9): Use good English please	Thank you for the correction to use correct English, we have corrected the text on line 134-143 as follows: Based on Figure 1 In the treatment group, the *P. paniculata* plant extract was sprayed with 10%, 15%, 20% and 25% concentration in the first 5 minutes of observation. *Ae.aegypti* mosquitoes death rate with 4 repetitions in a row was 11, 18, 27 and 54 individuals. In the 60 minutes, the total mortality of *Ae. aegypti* mosquitoes in 4 repetitions was 52, 80, 80 and 80. In positive control (K+) using synthetic insecticide baygon (cypermethrin) in less than 5 minutes *Ae. aegypti* mosquitoes fell and died in 4 repetitions, namely 76 individuals.While in the negative control (+) using distilled water did not cause death.There was 80 *Ae. aegypti*

<table>
<tr><td></td><td>mosquitoes were death at a concentration of 15% occurred at 51-55 minutes, a 20% concentration of 80 total deaths occurred at 41-45 minutes and a 25% concentration of 80 total deaths occurred at 21-25 minutes.</td></tr>
<tr><td>Comment (AB10): Not clear, please make in 2 dimension graph</td><td>Thank you for the correction regarding the three (3) dimensional graph we have corrected to a two (2) dimensional graph in the text on lines 162-168 as follows:

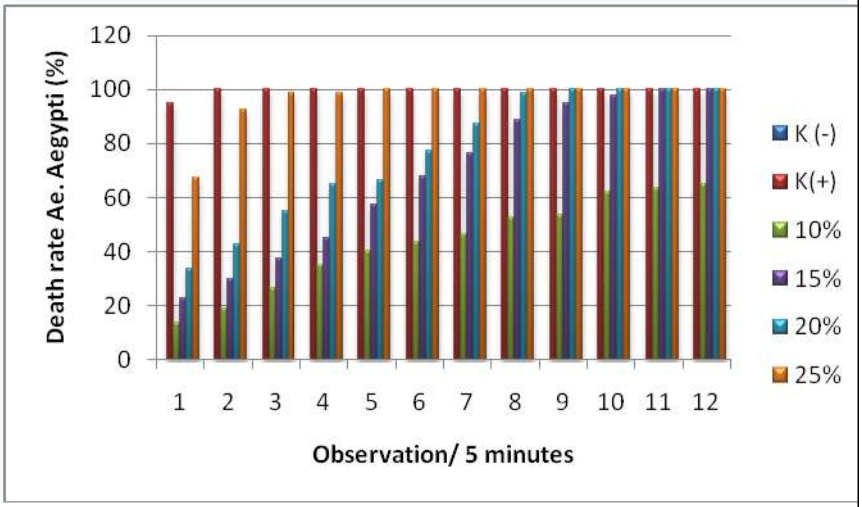

Figure 2: The Average Mortality of *Ae. aegypti* at each Concentration of Five Minutes Observation</td></tr>
<tr><td>Comment (AB11); (AB12); (AB13): No need to put the statistical anaylisis in here. Write as result please</td><td>Thank you for the correction to not include statistical analysis, we have changed it to include only the results in the text on lines 169-188 as follows:

Based on the results of study in figure 1 and 2, explained that the different concentrations of *P. paniculata* plant extracts gave different effects on number of *Ae. aegypti* mosquitoes death in each treatment and repetition. The number of *Ae.aegypti* mosquitoes death tends to increase along with the increase in the concentration of *P. paniculata* plant extracts. It's means that the higher used of the concentration, the higher of potency of the *P. paniculata* plant extract as a bioinsecticide against the *Ae. Aegypti* mosquito.During one hour observations showed the increasing number of *Ae. Aegypti* mosquitos's death. It's explained the longer an observation time, the greater potential as a bioinsecticide. This is supported by the results of the Kruskall-Wallis test (p-value of 0.001 <0.05), which means that there is a significant difference between the death rate of the *Ae. aegypti* mosquito and the difference in the concentration of the *P. paniculata* plant extract (10%, 15%, 20%, 25 %) of Knockdown Time acceleration. The results of the Kruskall-Wallis test in this study were correct and continued with the Spearman correlation test with the results of p-value 0.008 <0.05 there was a significant correlation between the increase in the concentration</td></tr>
</table>

of the extract (treatment group) and the knockdown time of the *P. paniculata* plant extract. From the results of the Kruskall-Wallis test and the Spearman correlation test, it can be concluded that the greater of concentration, the faster the time of death of *Ae. aegypti* mosquito, the strength of the correlation is denoted by a correlation coefficient 0.312 (31.2%).Correlation coefficient occurred with positive value means that correlation is moderate. The higher concentrations of *P. paniculata* plants extract then the faster of knockdown time

Comment (AB14): Please make the table more clear. Explain the highlighting data!

Thanks for the suggestion we have corrected the explanation on lines 194-198 in the manuscript

Table 1 Average of *Ae. Aegypti* Falling Down At Various Treatment Concentrations With Knockdown Time$_{50}$ (KT$_{50}$)

Time	10%	15%	20%	25%	K (+)	K(-)
5	2,75	4,5	6,75	13,5	19	0
10	3,75	6	8,5	18,5	20	0
15	5,25	7,5	11	19,75	20	0
20	7	9	13	19,75	20	0
25	8	11,5	13,25	20	20	0
30	8,75	13,5	15,5	20	20	0
35	9,25	15,25	17,5	20	20	0
40	10,5	17,75	19,75	20	20	0
45	10,75	19	20	20	20	0
50	12,5	19,5	20	20	20	0
55	12,75	20	20	20	20	0
60	13	20	20	20	20	0

: *Ae. Aegypti* Knockdown Time(KT$_{50}$);

Comment (AB15) untuk tabel 5. What mean +?

Thank you we tried to explain Regarding the meaning of +
In table 5 : Insecticide Score of *P. paniculata* Plant Extract Based on KT$_{50.}$

The knockdown effect has been defined by WHO 2006 in the book Guidelines for Testing Mosquitos Adulticides for Indoor Residual Spraying and Treatment of Mosquitos Nets
That: Insectiside Score Based on KT 50

KT50 (minute)	**Score**	**Knockdown effect**	**Interpretation**
>50	0	-	-
31-49	1	-	-
16-30	2	-	-
11-15	3	+	Weak Knockdown
5-10	4	++	Strong Knockdown
<5	5	+++	Quick Knockdown

source: WHO, 2006

A value of 3 means the median knockdown is in the range of 11-15 minutes which is interpreted to have a knockdown

	effect (+) but is weak. A value of 4 means the median knockdown is in the range of 5-10 minutes which is interpreted to have a strong knockdown effect (++). A value of 5 means the median knockdown is in the range of less than 5 minutes which is interpreted to have a knockdown effect (+++) that the insecticide has a "Quick Knockdown Effect" (WHO, 2006). So the + sign means explaining the Knockdown effect status of Bioinsecticide
Comment (AB16): Please change the scopus non-indexed reference to the scopus indexed reference	Thank you for the correction to change all non-Scopus references to Scopus-indexed references, all of which we have changed to Scopus-indexed references in the script as follows **References** [1] L. H. Guimarães de Oliveira *et al.*, "Agave sisalana extract induces cell death in Aedes aegypti hemocytes increasing nitric oxide production," *Asian Pac. J. Trop. Biomed.*, vol. 6, no. 5, pp. 396–399, May 2016. [2] D. A. Fernandes *et al.*, "Larvicidal compounds extracted from helicteres velutina K. Schum (Sterculiaceae) evaluated against aedes aegypti L.," *Molecules*, vol. 24, no. 12, p. 2315, 2019. [3] L. Scalvenzi *et al.*, "Larvicidal activity of Ocimum campechianum, Ocotea quixos and Piper aduncum essential oils against Aedes aegypti," *Parasite*, vol. 26, no. 23, pp. 1–8, 2019. [4] D. Valle, D. F. Bellinato, P. F. Viana-Medeiros, J. B. P. Lima, and A. D. J. Martins Junior, "Resistance to temephos and deltamethrin in aedes aegypti from Brazil between 1985 and 2017," *Mem. Inst. Oswaldo Cruz*, vol. 114, no. 3, pp. 1–17, Mar. 2019. [5] Z. H. Amelia-yap, C. D. Chen, M. Sofian-azirun, and V. L. Low, "Pyrethroid resistance in the dengue vector Aedes aegypti in Southeast Asia : present situation and prospects for management," *Parasit. Vectors*, vol. 11, no. 332, pp. 1–17, 2018. [6] S. Leta, T. Jibat, E. M. De Clercq, K. Amenu, M. U. G. Kraemer, and C. W. Revie, "International Journal of Infectious Diseases Global risk mapping for major diseases transmitted by Aedes aegypti and Aedes albopictus," *Int. J. Infect. Dis.*, vol. 67, pp. 25–35, 2018. [7] M. Y. Lee, "Essential Oils as Repellents against

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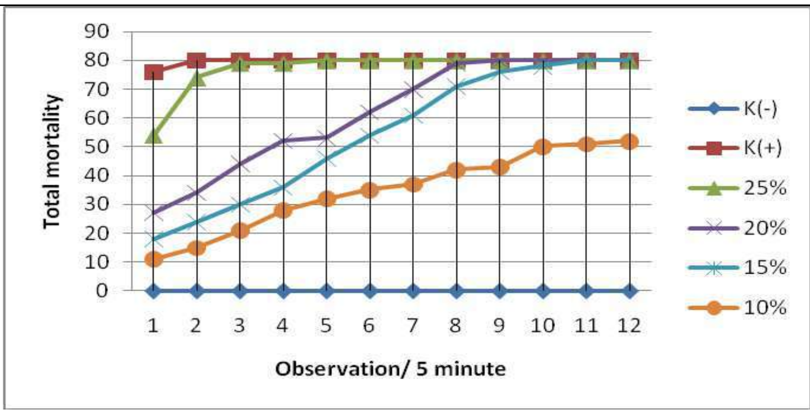
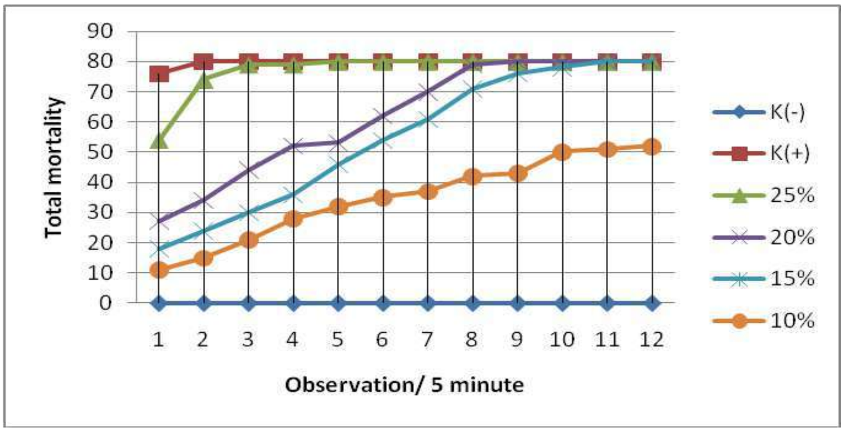
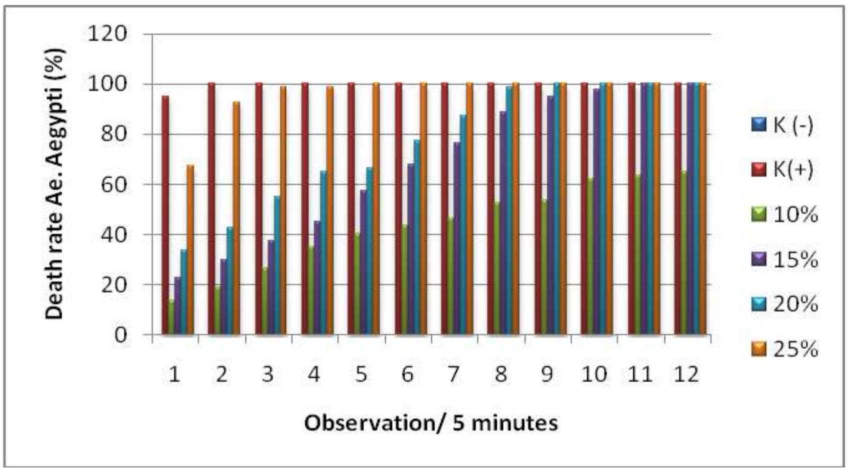
Reviewer 2

Reviewer's Comments	Author's Comments
1. Consider using *Ae. aegypti* as an abbreviation for *Aedes aegypti.*	Thank you for providing corrections to change the abbreviation Aedes aegypti to Ae. Aegypti, we have corrected this suggestion in the manuscript
2. The abbreviations YFV, ZIKV, CHIKV and DENV are used to refer to viruses (etiological agents) and not to diseases. Consider tweaking your text (page 01)	Thank you for the suggestion to consider changing the text regarding the abbreviations YHF, ZIKV, CHIKV and DENV in paragraph 1. We have changed this in the text on lines 23-25 as follows: The *Aedes aegypti* mosquito (L) belongs to the Order Diptera and family Culicidae, the main vector often neglected as a transmitter of diseases in humans. The diseases include Yellow Fever, Zica, Chikungunya, Dengue Haemorhaegic Fever and other arbo viruses[1]–[4].
3. There is redundancy between the Materials and Methods topic presented in the subtopics "Preparation of Materials and Tools" and "Extract Making". The text needs to be adjusted to avoid such redundancies (page 2-3).	Thank you for the correction Regarding the redundancy between the topic of Materials and Methods presented in the subtopic Preparation of Materials and Tools and Preparation of Extracts in the text, we have corrected it on lines 78-83 in the text as follows: **Preparation of Materials and Tools** This study used 2500 gram *P*. paniculata, 5 litre of 96% ethanol, 5 litre of distilled water, 1 bottle of synthetic insecticide Baygon (cypermethrin), and 480 *Ae. aegypti* mosquito. The equipment used in this study includes a blender, analytical scales, Rotary Vacuum Evaporator, stopwatch, shaker water bath, thermometer, hygrometer, Buchner funnel, stick, basin, test box, syringe, and a spray bottle.
4. In Figure 1, only one caption should be kept for an image. The descriptions that are in the figure must be placed in the legend (page 4).	Thank you for the correction regarding the information in Figure 1, we have corrected it on line 144-147 as follows:

	 Figure 1 *Ae. Aegypti* mosquitoes total mortality at each concentration with four repetitions
5. When presenting the data in Figure 01, it is interesting to consider the average number of deaths by concentration and not the sum of deaths (Line 135).	Thank you for the advice. Figure 01 is retained but the average mortality is made in Figure 02 as follows: on lines 144-147 and lines 164-168  Figure 1 *Ae. Aegypti* mosquitoes total mortality at each concentration with four repetitions  Figure 2: The Average Mortality of *Ae. aegypti* at each Concentration of Five Minutes Observation
6. Note the writing of	Thank you for the advice regarding scientific writing Aedes

the scientific name "*Aedes aegypti*" on pages 5 and 6.	aegypti " on pages 5 and 6 we have corrected it as suggested
7. In table 01 the date presented in the table (0.000) are different from those discussed in the text (0.001) (line 167)	Thank you for the advice. Based on input from other reviewers that this table is not needed but the results from the table. This is conveyed in the script narrative only on lines 169-188 as follows: Based on the results of study in figure 1 and 2, explained that the different concentrations of *P. paniculata* plant extracts gave different effects on number of *Ae. aegypti* mosquitoes death in each treatment and repetition. The number of *Ae.aegypti* mosquitoes death tends to increase along with the increase in the concentration of *P. paniculata* plant extracts. It's means that the higher used of the concentration, the higher of potency of the *P. paniculata* plant extract as a bioinsecticide against the *Ae. Aegypti* mosquito.During one hour observations showed the increasing number of *Ae. Aegypti* mosquitos's death. It's explained the longer an observation time, the greater potential as a bioinsecticide. This is supported by the results of the Kruskall-Wallis test (p-value of 0.001 <0.05), which means that there is a significant difference between the death rate of the *Ae. aegypti* mosquito and the difference in the concentration of the *P. paniculata* plant extract (10%, 15%, 20%, 25 %) of Knockdown Time acceleration. The results of the Kruskall-Wallis test in this study were correct and continued with the Spearman correlation test with the results of p-value 0.008 <0.05 there was a significant correlation between the increase in the concentration of the extract (treatment group) and the knockdown time of the *P. paniculata* plant extract. From the results of the Kruskall-Wallis test and the Spearman correlation test, it can be concluded that the greater of concentration, the faster the time of death of *Ae. aegypti* mosquito, the strength of the correlation is denoted by a correlation coefficient 0.312 (31.2%).Correlation coefficient occurred with positive value means that correlation is moderate. The higher concentrations of *P. paniculata* plants extract then the faster of knockdown time
8. The value 0.08 is greater than 0.05, not less. (line 185)	Thanks for the correction. We have deleted this information because the related table has been changed to a new narrative
9. The Time required for 50% knockdown of mosquitoes (KT50) could be accurately estimated using probit analysis (table 4).	Thank you for the suggestion, to use probit analysis to determine the time required for 50% mosquito knockdown (KT50) in Table 4 We try to explain Knockdown time (KT50) means the time it takes for each concentration of P. paniculata Bioinsecticide to drop 50% of the average Ae. aegypti.We did not use the probit analysis in Table 4, because here we only looked at how many minutes the average number of mosquitoes fell by 50% for each concentration of P. paniculata Bioinsecticide.

10. In obtaining table 05, were the mortality averages considered?	Thank you for the correction. We try to explain, We have changed Table 05 to Table 02 because Tables 01, 02, 03 have been omitted according to input from other reviewers. For Table 02, we do not use the average mortality, but we use KT_{50} (Time) which is the time it takes to drop 50% of Ae. aegypti after being sprayed with P. paniculata Bioinsecticide at each test concentration.
11. In line 196 refers to table 04 and in line 207 refers to table 05.	Thank you for the correction. We have corrected the script on line 201-202 as follows: Knockdown $Time_{50}$ (KT_{50}) is the times to knockdown of *Ae*, *aegypti* mosquitoes. In Table 1, the average repetition of the study show during the 10% concentration of *P. paniculata* plant
12. According to the presentation of the text, I suggest that the Results section be presented separately from the Discussion section.	Thank you for the advice. We have corrected the manuscript according to the following suggestions: A. Results pada line 117 B. Discussion pada line 229

Reviewer 3

Reviewer's Comments	Author's Comments

5. Proofreading (4 Juli 2022)

Dokumen: a. Tracked proofreading

b. Respon letter-Proofreading

5.a. Tracked Proofreading

Bio-insecticide's Extract of Scented Root (*Polygala paniculata*) in Controlling The Mosquito *Aedes aegypti* ~~Mosquito~~(L.)

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ABSTRACT~~–~~

Controlling *Aedes aegypti* mosquitoes with chemical insecticides causes resistance ~~effects~~ on humans, environmental residues, ~~as well as~~ and contaminates food and water. *Polygala paniculata* ~~has~~ is a potential ~~as an~~ alternative ~~of insecticide~~to insecticides in controlling *Ae.* ~~*Aegypti* mosquitoes.~~*aegypti.* This study ~~further~~ aimed to determine the effect of the Insecticide Score of *P. paniculata* ~~extract~~ extracts on ~~*Ae. Aegypti* mosquitoes'~~ the mortality of *Ae. aegypti* mosquitoes based on KT_{50}. ~~The~~ A total of 20 mosquitoes for each concentration were used to examine the effectiveness of 10%, 15%, 20%, and 25% concentration of *P. paniculata* ~~concentration with~~ extracts compared to a positive and a negative ~~controls in~~ control with four ~~repetitions every five minutes for one hour.~~replications. The results showed ~~the effect of extract~~ that *P. paniculata* extracts had an effect on *Aeaegypti* ~~mosquitoes'~~mosquito mortality. ~~The~~ There were significant differences in mortality rate between concentrations (Kruskal-Wallis test ~~resulted in a~~, p~~-value of~~ = 0.001~~< 0.05, while the~~).Spearman Correlation test gave a p-value of 0.008 < 0.05. ~~Furthermore, the~~Similarly, there was a weak but significant correlation ~~strength was +~~between plant extract concentration and mortality (Spearman correlation: r = +0.312, p = 0.~~312 or 31.2%, with~~ 0,008). The 10% ~~concentration, while~~ and 15% concentrations had KT50, with Insecticide Scores of 1 and 2, respectively, implying that both had no knockdown effect. A 20% concentration had ~~KT50with~~a KT50 with an Insecticide Score of 3, indicating a weak knockdown effect, ~~while~~whereas a 25% concentration had KT50, with an Insecticide Score of 5, signifying a quick knockdown effect. ~~This shows~~These results show that a 25% concentration has a quick knockdown ~~timein*Ae*~~time on *Ae*. *Aegypti* mosquitoes. Therefore, extract *P. paniculata* ~~may have the~~ extract at a concentration of 25% has a potential for use as a bio-~~insecticidesin~~insecticide in controlling *Ae. aegypti* mosquitoes.

Keywords: extract *P. paniculata*, *Aedes aegypti* mosquito, bio-insecticides

1. Introduction

The *Aedes aegypti* mosquito (L~~)~~), which belongs to the Order Diptera and family Culicidae, is the main vector, often neglected ~~as a transmitter of~~, transmitting human diseases ~~in humans.~~ ~~The diseases include~~including Yellow Fever, Zica, Chikungunya, ~~-~~Dengue Haemorhagic Fever and other arbo viruses [1~~] [~~–4]~~. The~~. *Aedes aegypti* transmits the human arboviral disease

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Comment [Editor1]: Remark: It is standard practice to indicate the authority the first time you mention a species.

Comment [Editor2]: Remark: Please check your target journal guidelines for the correct style of citing references within text. Put a space between the last word and the open bracket. Usually, references are cited like this: [1-4], not like this: [1] – [4].

~~transmitted by *Ae.aegypti* is~~, a global public health threat[5]~~. This disease~~ that causes significant morbidity and mortality in developing countries [1]. The transmission of dengue fever is increasing in urban and semi-urban areas in tropical countries worldwide [6]~~.~~. It is estimated that 40% or 50~~–~~ to 528 million people worldwide are at risk of becoming infected with dengue fever and around 10,000–20,000 people die yearly [7]. According to WHO, about 390 million cases of dengue virus infection occur every year, of which 96 million manifest clinically with high severity. Furthermore, WHO reports that about 3.9 billion people are at risk of being infected with the dengue virus [8].

Indonesia is one of the countries where dengue hemorrhagic fever (DHF) occurs and is an ~~infectious disease and an~~ unresolved health problem. In 2020, there were 15,132 DHF cases and 145 deaths in Indonesia, ~~with a death rate of 145 people~~ and a DHF incident rate of 31.23 % [9]~~.~~. ~~This implies~~; indicating ~~-~~problems in ~~efforts to control~~controlling the disease.

~~However, long~~Long-term application and extensive use of synthetic insecticides cause ~~the~~ accumulation of residues in food, water, and soil, ~~-~~and ~~give adverse~~affect human and ecosystem health ~~effects on humans and ecosystems~~[10~~], [~~ 11]. ~~This~~Use of insecticides leaves residues that pollute the environment [3~~], [~~ 12]~~. increasing population~~ and cause resistance ~~of~~in *Ae.* ~~*aegypti*~~*Aegypti* populations [4~~], [~~, 5~~], [~~, 13~~], [~~ 17].

Controlling *Ae. aegypti* mosquitoes using synthetic insecticides involves fumigation with pyrethroids and larvacides containing temephos [18].~~Pyrethroid~~ *Aedes aegypti* develops resistance ~~causes *Ae. aegypti*~~ to ~~keep growing,~~pyrethroid and multiply rapidly increasing the incidence of dengue fever ~~(DF)~~ worldwide. This ~~increases the risk of~~ puts almost half of the world's population at the risk of being infected with the disease [5]. ~~Similarly, using~~Using temephos to kill larvae ~~increases the resistance of mosquitoes larvae, *A. aegypti*.~~has a similar effect. This has been reported in several countries, such as Brazil [4], Mexico [19] and ~~Southeast Asia~~ countries in south-east Asia, including Indonesia, Malaysia, Philippines, Thailand, Singapore, Laos, and Myanmar [5].

There is a need to obtain alternative insecticides effective in controlling the ~~mosquito~~*A. aegypti* population ~~*A. aegypti*~~[20]. This could be achieved by using natural plant chemical compounds ~~with the potential to control the mosquito population effectively. The~~that are environmentally friendly ~~method would guarantee plant protection~~ and avoid the side effects of synthetic insecticides~~,~~ [10~~], [~~ 21~~], [~~22]. Bioactive plant compounds are biodegradable, environmentally friendly, and non-toxic to ~~other~~non-target insects [2].~~This is because bio-insecticides~~ They only affect the target insect without destroying beneficial natural enemies. ~~Additionally~~Generally, they are a safe, economical, target-specific, biodegradable, and residue-free ~~environmental food source~~[23].

Polygala L. is one of the largest genera belonging to the Polygalaceae tribe. This genus consists of 500 species and can be found in tropical, sub-tropical, temperate, and mountainous areas throughout the world except New Zealand. Most of these species grow in Central and South Tropical America. ~~Several types~~There are several species of *Polygala L.* that can be used as medicine ~~such as :~~including *Polygala chinensis L.*, *Polygala paniculata L.*, *Polygala* ~~*polifoliaPresl*~~*polifolia Presl*., *and Polygala sibirica L*.[24]. *Polygala paniculata*, also known as vetiver in Riau, Indonesia, is a good-smelling annual herbaceous plant belonging to the family Polygalaceae ~~and the genus Polygala.~~. The plant is often used ~~as~~ in traditional medicine~~,~~ as a tonic, and ~~in inflammation~~for controlling inflammatory cases of asthma, bronchitis, arthritis, and ~~other pathologies, as well as~~ kidney disorders [25]. Moreover, *P. paniculata* is used for *in- vivo* protection against the neurotoxic effects of ~~Methylmercury~~methylmercury (Hg~~)~~) [26], bronchitis,

Comment [Editor3]: Remark: Please check this this. I think incidence rate should be reported as a percentage or decimal.

Comment [Editor4]: Remark: It is obvious from the species name that it belongs to this genus. Hence, we have deleted it.

neurahenia, inflammation, amnesia, topical anesthetic, and expectorant drugs [27]. ~~The~~ *Polygala* ~~extract produces~~extracts contain secondary metabolites, including alkaloids, saponins, flavonoids, phenols, tannins, steroids, and terpenoids [27].~~Therefore, these~~ These bioactive compounds make *P. paniculata* potentially useful as a bio-~~insecticides. This becomes an interesting study topic because the~~insecticide. The bioactive compounds extracted from *P. paniculata* have ~~never~~not yet been explored as bio-insecticides in controlling the *Ae. aegypti* mosquito. Therefore, ~~it is important~~the main objective of this study was to ~~examine~~evaluate the ~~I~~insecticide ~~S~~score of the toxin contained in the bioactive compounds of *P. paniculata* in killing the *Ae. aegypti* mosquito.

2. Materials and Methods

~~Preparation of~~ Materials and ~~Tools~~equipment

This study used 2500 ~~gram~~g *P. paniculata*, 5 lit~~re~~er of 96% ethanol, 5 lit~~re~~er of distilled water, ~~1~~one bottle of synthetic insecticide Baygon (cypermethrin), and 480 *Ae. aegypti* ~~mosquito.~~mosquitoes. The equipment used in this study ~~includes~~included a blender, analytical scales, Rotary Vacuum Evaporator, stopwatch, shaker water bath, thermometer, hygrometer, Buchner funnel, stick, basin, test box, syringe, and a spray bottle.

Comment [Editor5]: Remark: Do you mean "a shaking water bath"?

Test ~~Animal Preparation~~animal preparation

Test animals were bred ~~using~~in media containing clean water in a cool place and protected from direct sunlight for the *Ae.* ~~*Aegypti*~~*aegypti* mosquitoes to lay their eggs. The larvae were reared in an aquarium at 24.~~2^{0}C~~2 - 24.~~4^{0}C~~4 ^{0}C, with a relative humidity of 67~~-~~–70%. The larvae ~~werefed~~were fed with coconut water [28] ~~to become larvae~~until they reached instar III and IV stages before becoming mosquitoes. The adult *Ae. aegypti* mosquitoes were then used as test animals.

Extraction ~~Process~~process

A mass of 2500 ~~grams~~g of *P. paniculata* plants were washed and air-dried at room temperature, and blended ~~them~~ to ~~form~~give a final mass of 400 ~~grams~~g of powder. The powder was macerated with 96% ethanol until completely submerged for three days. The resulting solution was filtered using a Buchner funnel and placed in a dark bottle. ~~Furthermore, the~~ The dregs from the first filtering were ~~then~~ soaked again for one day, filtered, and the process repeated ~~in~~for the third ~~immersion.~~time. The ~~results~~filtrates from the three maceration processes were combined and concentrated using a Vacuum Rotary Evaporator to evaporate 96% ethanol and obtain an extract. The extract obtained was stored in the refrigerator to be used later [29].

~~Bio-insecticide's Extract~~Tests of extracts of *P. paniculata* ~~Test~~ against *Ae. Aegypti* ~~Mosquitos~~mosquitoes

~~Bio-insecticide's extract~~Tests of *P. paniculata* ~~tests~~extracts against *Ae. aegypti* ~~was carried out~~were performed by transferring 20 *Ae. aegypti* in each test ~~boxes, then sprayed~~box before spraying —with ~~various concentrations of~~different concentrations of *P. paniculata* ~~plant extract~~extracts with four repetitions per concentration, as well as for C (+) and C (-) K (+) and K(-). The effect of the ~~concentrations~~ extracts on the *A. aegypti* mosquito was observed by looking at the changes in behavior, movement, and physical condition until death. Deaths of *Ae.*

Comment [Editor6]: Remark: It is not clear what these are. Please clarify.

Aegypti mosquitoes death were counted every five minutes5 min for one hour. Moreover, the Iinsecticide Sscore of *P. paniculata* was determined from the number of *Ae. Aegypti* mosquitoes considered deadatdead at 5-minute intervals. *Ae. AegyptiAedes. aegypti* mosquitoes that remained alive were left to die or killed with Baygon.

Data analysis

Data were analyzed using the Statistical Test of One-Way Analysis of Variance with RAL, followed by the One Way ANOVA test. However, when the One Way ANOVA test doesdata did not meetconform to the assumptions of parametric tests the requirements, thenon-parametric Kruskal-–Wallis Non-Parametric Test test and the Test Spearman Analysis arethe Spearman test were conducted to examine the relationship between the independent and dependent variables.

3. Results and Discussion

A. Results

The ResultsObservations on deaths of Observing *Ae.aegypti* Mosquitoes' Death After SprayingMosquitoes after spraying the *P. paniculata* Plant Extractplant extracts

The *Ae. aegypti* mosquitoes died after spraying the *P.* exposure to concentration of 10% and 15 % *P. paniculata* plant extractat 10% and 15% concentrations.extracts. They died slowly by flying irregularly and actively and before falling in a tilted body position. The After falling, the mosquitoes appeared weak, with some legs still movinglimited leg movements, before becoming paralyzed, and eventually dying, and their bodies remaining stiff.. The death was faster at 20% and 25% concentrations. In the positive control (K+), death occurred in less than five minutes, while in the negative control (K-), there was no death.

the *Ae. Aegypti* mosquitoes tried to avoid the spray during the four experiment repetitions.

Comment [Editor7]: Remark: Check if this is correct. You later refer to deaths at 20% and 25%.

Comment [Editor8]: Remark: For a balanced comparison you should state whether death occurred or not in the negative control.

Total mortality of *Ae. aegypti* Mosquitoes Total Mortalitymosquitoes at Each Concentration With Four Repetitionseach concentration

Based on Figure 1 In the treatment group, the After exposure to *P. paniculata* plant extract was sprayed with at 10%, 15%, 20%, and 25% concentration, total mortalities of 11, 18, 27 and 54 individuals, respectively, were recorded in the first 5 minutesmin of observation. (Figure 1). After 60 mins, the total mortalities of *Ae.aegypti* mosquitoes death rate with 4 repetitions in a row was 11, 18, 27 and 54 individuals. In the 60 minutes, the total mortality of *Ae. aegypti* mosquitoes in 4 repetitions waswere 52, at 10%, 80, 80 each at 15%, 20%, and 80.25%. In the positive control (K+) using+), which was a synthetic insecticide baygon (called cypermethrin) in less than 5 minutes, 76 *Ae. aegypti* mosquitoes fell and died in 4 repetitions, namely 76 individuals.While in the less than 5 min (Figure 1). In the negative control (+) using (K-), which was distilled water did not cause death.There was 80 *Ae. aegypti* , mosquitoes were deathdid not die. There were 80 *Ae. aegypti* mosquitoes deaths after 51–55 min at a concentration of 15% occurred at 51-55 minutes,%, after 41–45 min at 20% concentration of 80 total deaths occurred at 41-45 minutes and a 25% concentration of 80 total deaths occurred atafter -21-–25 minutes. at 25% concentration

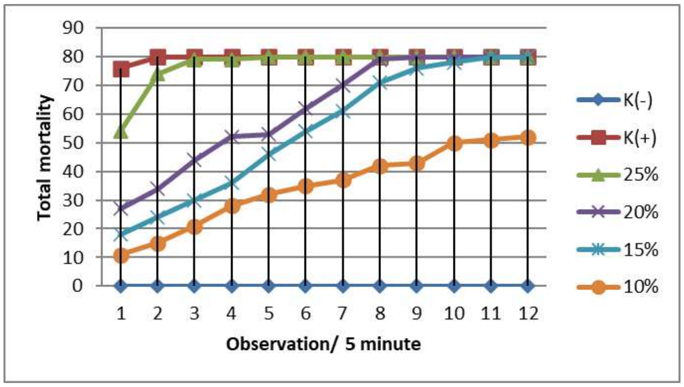

Figure 1: ~~*Ae.*~~ Total mortality *of* *Aegypti* mosquitoes ~~total mortality~~ at each concentration ~~with four repetitions~~ at five-minute intervals

~~(Primary Data. 2020)~~

~~The Average~~Mean mortalities of *Ae. aegypti* ~~Death Rate~~ at ~~Each Concentration Every Five Minutes Observation~~each concentration at five-minute intervals

Comment [Editor9]: Remark: 'Mean' is more academic than 'average'.

~~Based on Figure 2, the treatment group was sprayed with~~After exposure to *P. paniculata* plant extract ~~with a concentration~~at concentrations of 10%, 15%, 20%, and 25~~%~~ %, the mean mortality of *Ae. aegypti* mosquitoes were 2.75 individuals (13.75%), 4.5 individuals (22.5%), 6.75 individuals (33.75%), and 13 individuals (67.5%), respectively, in the first 5 ~~minutes~~min of observation~~, the average~~ (Figure 2). Mean mortality rate of *Ae.*–*aegypti* mosquitoes ~~with 4 repetitions in a row was 2.75 tails (13.75%), 4.5 tails (22.5%), 6.75 tails (33.75%) and 13 tails (67.5%).*Ae.aegypti* mosquitoes death rate in~~after 60 ~~minutes with 4 repetitions at a concentration of 10% was~~min of exposure were 13 ~~tails~~individuals (65~~%), while the~~ %) at 10%, 20 individuals (100%). at each of concentrations of 15%, 20%, and 25% ~~were 20 tails~~(Figure 2). All (100~~%). 95%~~%) *Ae. aegypti* ~~mosquito~~ individuals fell and died in positive control (K+) ~~using synthetic insecticide Baygon (cypermethrin)~~ in less than 5 ~~minutes with 4 repetitions.While~~ min. In the negative control ~~(-) using distilled water did not cause death.*Ae.Aegypti mosquitos* totally~~(K-) no deaths (0% mortality) were recorded. All (100%) of *Ae. aegypti* individuals died after 51–55 min at 15% concentration ~~in 51 until 55 minutes,~~, after 41–45 min at 20% concentration ~~of 80 total deaths occurred at 41-45 minutes,~~ and after 21–25 min at 25% concentration ~~of 80 total deaths occurred at 21-25 minutes.~~(Figure 2).

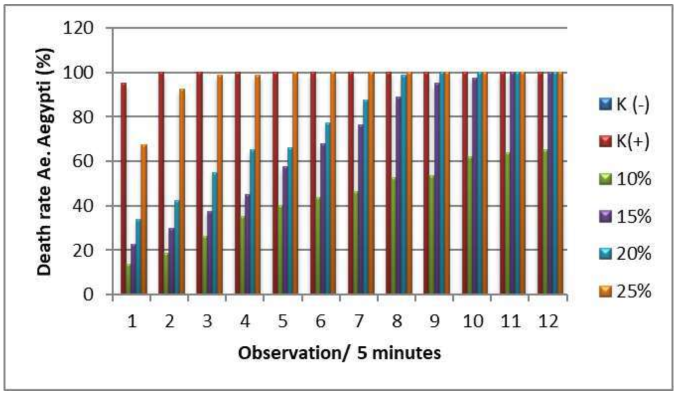

Figure 2: ~~The Average Mortality~~Mean mortality of *Ae. aegypti* at each ~~Concentration of Five Minutes Observation~~concentration at five-minute intervals

~~Based on the~~These results ~~of study in figure 1 and 2, explained~~indicate that the different concentrations of *P. paniculata* plant extracts ~~gave~~had different effects on ~~number of *Ae. aegypti* mosquitoes death in each treatment and repetition. The number of *Ae.aegypti* mosquitoes death tends~~mortlaity of *Ae. aegypti* individuals. Mortality tended to increase ~~along~~ with ~~the increase in~~ the concentration of *P. paniculata* plant extracts~~. It's means~~, indicating that the higher the concentration of extract used ~~of the concentration~~, the higher of potency of the *P. paniculata* plant extract as a bio-insecticide~~insecticide~~ against the *Ae.* ~~*Aegypti* mosquito.During one hour observations showed the increasing number of *Ae. Aegypti* mosquitos's death. It's explained the longer an observation time, the greater potential as a bioinsecticide.~~*Aegypti*. –This ~~is~~was supported by the results of the Kruskal~~l~~-Wallis test (p ~~value of~~= 0.001 ~~<0.05~~), which means that there ~~is~~ was a significant difference mortality rate of the *Ae. aegypti* mosquito between the ~~death rate of the *Ae. aegypti* mosquito and the difference in the concentration of the~~concentrations of *P. paniculata* plant ~~extract~~extracts (10%, 15%, 20%, 25 ~~%) of Knockdown Time acceleration.~~%). The results of the Kruskal~~l~~-Wallis test ~~in this study were correct and continued with~~concurred with those of the Spearman correlation test ~~with the results of p-value 0.008 <0.05 there was~~ which showed a significant correlation (p = 0.008) between ~~the increase in the~~ concentration of the ~~extract (treatment group)~~extracts –and the knockdown time ~~of the *P. paniculata* plant extract. From the results of the Kruskall-Wallis test and the Spearman correlation test, it can be concluded~~. These results indicate that the ~~greater of~~higher the concentration, the ~~faster~~shorter the time ~~of~~to death of *Ae. aegypti* mosquito, the strength of the correlation is denoted by a correlation coefficient 0.312 (31.2%).~~Correlation coefficient occurred with positive value means that correlation is moderate.~~ The higher concentrations of *P. paniculata* plants extract then the ~~faster~~shorter of knockdown time. It was also noted that mortality increased with increased duration of exposure to the plant extracts

Comment [Editor10]: Remark: Please check this. I think there must be a negative correlation between concentration and time to death.

Insecticide Score of *P. paniculata* ~~*Plants*~~*plant extracts*

Table 1 ~~Average~~Mean falling down times of *Ae*. ~~*Aegypti* Falling Down At Various Treatment Concentrations With~~*aegypti* at various treatment concentrations with Knockdown Time$_{50}$ (KT$_{50}$)

Time	10%	15%	20%	25%	K (+)	K(-)
5	2,75	4,5	6,75	13,5	19	0
10	3,75	6	8,5	18,5	20	0
15	5,25	7,5	11	19,75	20	0
20	7	9	13	19,75	20	0
25	8	11,5	13,25	20	20	0
30	8,75	13,5	15,5	20	20	0
35	9,25	15,25	17,5	20	20	0
40	10,5	17,75	19,75	20	20	0
45	10,75	19	20	20	20	0
50	12,5	19,5	20	20	20	0
55	12,75	20	20	20	20	0
60	13	20	20	20	20	0

~~–~~: *Ae. Aegypti* Knockdown Time (KT$_{50}$);
Source ~~:~~: (Primary Data, 2020)

Knockdown Time$_{50}$ (KT$_{50}$) is the ~~times~~time to knockdown of *Ae*~~,~~. *aegypti* mosquitoes. ~~In Table 1,the average repetition of the study show during the 10% concentration of *P. paniculata* plant extract had a~~The highest KT$_{50}$ of between 36 ~~until~~ and 40 minutes~~, 15~~ was recorded for the10% concentration of *P. paniculata* plant extract followed by that of the15% concentration ~~had a KT$_{50}$~~ of between 21 ~~until~~and 25 ~~minutes, in~~min, and that of the ~~*P. paniculata* plant extract with~~ 20% concentration ~~the KT$_{50}$ was obtained~~ between 11 and 15 ~~minutes~~min, and ~~in~~that of the ~~*P. paniculata* plant extract with a~~25% concentration of ~~25%, the KT$_{50}$ was~~ less than 5 ~~minutes. In the average iteration of the study, there~~min (Table 1). There were no *Ae. aegypti* mosquitoes that ~~fell on~~died in the negative control and the ~~Knockdown Time$_{50}$ (KT$_{50}$) was less than 5 minutes in~~KT$_{50}$ of the positive control~~. This means~~ was less than 5 min (Table 1). These results indicate that the different concentrations of *P. paniculata* plant extracts ~~gave~~ had different ~~effects~~KT$_{50}$ on ~~the number of~~ *Ae. aegypti* ~~that fell, as well as on each treatment and repetition~~mosquito.

Table 2: ~~Bio~~ Insecticide ~~Extract~~scores of e of *P. paniculata* plant extracts based on Knockdown Time$_{50}$

Group Control	KT50 (Time)	*Knockdown Effect*	Insecticide Score	Interpretation
Concentration 10%	36–40	-	1	-

Concentration 15%	21–25	-	2	-
Concentration 20%	11–15	+	3	Weak Knockdown
Concentration 25%	< 5	+++	5	Quick Knockdown
Positive Control	< 5	+++	5	Quick Knockdown

Interpretation ~~Data~~of data (+ *weak Knockdown* +++ *Quick Knockdown*)) [30]
(Primary Data, 2020)

Table 2 shows the effectiveness level of the four *P. paniculata* plant extract concentrations and positive control based on ~~Insecticide Knockdown Time$_{50}$ (~~(KT_{50})~~.~~. A 10% concentration of the *P. paniculata* plant extract had a ~~KT of$_{50}$~~KT_{50} between 36- and 40 ~~minutes. This implies~~min giving an Insecticide Score of 1 or no knockdown effect. Similarly, a 15% concentration of the plant extracts had a ~~KT of$_{50}$~~KT_{50} of between ~~21-~~21and 25 ~~minutes~~min, indicating an Insecticide Score of 2 or no knockdown. A 20% concentration of the plant extract had a ~~KT of$_{50}$between~~KT_{50} of between 11–15 ~~minutes, implying~~min, giving an Insecticide Score of 3 or a weak knockdown effect. Furthermore, a 25% concentration of *P. paniculata* plant extract had a KT_{50} ~~in~~ of less than 5 ~~minutes. This indicates~~min, indicating an Insecticide Score of 5 or a quick knockdown effect. The 25% concentration had a KT_{50} ~~in~~of less than 5 ~~minutes~~min, similar to that of the positive control, with an Insecticide Score of 5 or a quick knockdown effect. Therefore, the 25% concentration of *P. paniculata* plant extract was the most effective in knocking out *Ae. Aegypti* mosquitoes, with an Insecticide Score of 5 or a quick knockdown effect.

B. Discussion

The results ~~in Figures 1 and 2 show~~showed that the number and percentage of *Ae. aegypti* mosquito deaths increased with the concentration of *P. paniculata* plant extract. ~~Higher~~This was expected as higher concentrations ~~increase~~ of the ~~accumulation of~~ *P. paniculata* plant ~~extract's toxic~~extract a higher exposure of *A. aegypti* mosquitoesto higher levels of the bioactive compounds ~~in *A. aegypti* mosquitoes' bodies, increasing their mortality~~.

Kosini ~~examined the effect of the*Gnidia kaussiana (Thymeleaceae)* extracton~~ reported similar results for *Callosobruchus maculatus*~~. The study explained that~~ larvae exposed to *Gnidia kaussiana* plant extracts and attributed them to an increased absorption of toxic compounds in by the larvae at higher concentration of the ~~*Gnidia kaussiana*~~plant extract, which accelerates the mortality process of larvae ~~*Callosobruchus maculatus*by melanizing the cuticle. This~~. Absorption of toxic plant bioactive compounds disrupts the endocrine system due to the presence of secondary metabolites such as terpenoids, alkaloids, and flavonoids [31].~~The study of~~ da Botas et al ~~explained that essential oil~~ attributed the larcide effect of Baccharisreticularia DC and *limonene* ~~as a larvacide agent~~ on ~~Controlling *Ae. Aegypti* (Diptera: Culicidae),it's also able~~*aegypti* to its ability to inhibit the formation of acetylcholinesterase ~~enzyme by blocking the nerve signal of transduction,~~ which can cause ~~the~~ death and paralysis in *Ae.* ~~*aegypti*larvae.~~*Aegypti* larvae. The more the larvae of *Ae. aegypti* ~~absorbs~~absorb the toxic compounds in *B. reticularia* essential oil, the ~~more~~ higher the *Ae. aegypti* ~~death.~~mortality rate.

Comment [Editor11]: Remark: It is discouraged to refer to figures in the Discussion.

Also, the longer the exposure to *B. reticularia* essential oil compounds ~~will increase~~the higher the level of toxicity~~.~~ [32].

~~Many~~Our results corroborate those of many previous studies ~~stated~~stating that ~~the~~ bioactive plant compounds have insecticidal, larvicidal, ~~repellent, and environmentally friendly~~ effects ~~useful for insect control.~~insects. According to Suluvoy, essential oils, flavonoids, alkaloids, glycosides, esters, and fatty acids ~~have anti-insect effects. Therefore, they could~~can be used as an alternative to chemical compounds ~~in insect control as repellents, feeding deterrents or antifeedants, toxicants, inhibitors, growth factors, chemosterilants, and attractants~~because they have anti-insect effects [21].

Previous studies ~~explained~~reported that *Polygala* plants contain bioactive compounds with various biological activities~~,~~ such as alkaloids, saponins, flavonoids, phenols, tannins, steroids, and terpenoids [27]. ~~Tannins and flavonoids are phenolic plant compounds that act as primary antioxidants or free radical scavengers[33].The bioactive compounds of *P. paniculata* able to affect the mortality of *Ae.*~~ The bioactive compounds in *P. paniculata* extracts able to cause mortality of *Ae.* ~~-~~*aegipty* mosquitoes are flavonoids, saponins, tannins, alkaloids, steroids, and terpenoids. In this study, the mortality of *Ae. aegypti* mosquitoes ~~death~~ was most probably caused by compounds in the *P. paniculata* plant extract~~. The compounds~~ they entered the mosquito's body through contact or respiratory poisoning and the mouth and digestive tract, causing stomach poisoning.

The ~~analysis showsthat~~results showed that the ~~number~~mortality rate of *Ae. aegypti* mosquitoes ~~that died~~ when exposed to the *P. paniculata* plant extract varied with the ~~extract's~~ concentration~~. Variations in~~ of the ~~concentrations affected the mortality of *Ae.aegypti* mosquitoes differently in each treatment and repetition. The results in Figures 1 and 2 show that spraying a 25% concentration of the *P. paniculata* plant~~ extract ~~for 60 minutes kills 80 *Ae. Aegypti* mosquitoes in less than the first five minutes. This is the largest number of deaths that occurred faster than other concentrations.~~ . Therefore, the higher the concentration of the plant extract ~~increased its effectiveness~~the more effective it was as ~~a vegetable~~an insecticide against mosquitoes.

This study compared the effectiveness ~~level~~ of the four *P. paniculata* plant extract concentrations with that of the positive ~~controls~~control based on Insecticide Knockdown Time$_{50}$ (KT_{50}). A 25% concentration of the plant extract had a KT_{50} of less than five minutes, ~~implying~~which was similar to that of the positive control. This KT_{50} implies an Insecticide Score of 5 or a quick knockdown effect. This is in line with the 2006 WHO standard, which stated that an insecticide ~~is~~ has a knockdown time required to drop a vector when the median knockdown ranges between 3~~ -~~–5 min [30]. Furthermore, ~~it~~ an insecticide has a quick knockdown effect when it has a KT_{50} of less than ~~five minutes.~~5 min. According to Norris, a good insecticide requirement for controlling disease vector insect species is that it must cause a rapid knockdown of the target species~~, especially active pathogens. Additionally, it must quickly intervene and kill adult mosquitoes~~ [17].

~~The 25% concentration had a KT_{50} of less than five minutes, similar to a positive control Baygon containing cypermethrin. They both had an Insecticide Score 5, implying a quick knockdown effect.~~. Therefore, the 25% concentration of the *P. paniculata* plant extract was the most effective concentration in knocking down *Ae. aegypti* mosquitoes~~. This is because~~ as it had an Insecticide Score of 5 or a quick knockdown effect. The positive control treatment was ~~intended to compare~~a benchmark for comparing the quality of *P. paniculata* plant ~~extract concentration.~~extracts. In contrast, the negative control treatment was used to compare its

Comment [Editor12]: Remark: This is not relevant to your study. Hence, we have deleted this.

Comment [Editor13]: Remark: We have deleted this as it is a repetition of the results.

effectiveness with that of the plant extract. ~~The results showed that no mosquito died after 60 minutes of observation.~~

Chang highlighted the need to use bio-insecticides as an alternative insect controller. ~~Using inappropriate insecticides causes insects to adapt easily by metabolic detoxification quickly and survival. This causes synchronization of insect immunity passed on to their offspring. Therefore, it~~It is necessary to develop safe alternative insecticides, larvicides, and repellents effective for humans, animals, the environment, and the ecosystem. Natural insecticides are needed to suppress vector resistance and slow down their genetic adaptation [34]. According to Sulovoy and Grace~~., botanical~~, plant insecticides only affect target insects, do not destroy beneficial natural enemies, and are a safe and residue-free food source. [21]. It is more environmentally friendly, effective, cheap, and naturally available [12].

Conclusion

The ~~active substance~~ bioactive compounds contained in the *P. paniculata* plant ~~extract has~~extracts have the ability to drop and kill *Ae. aegypti* mosquitoes. ~~This is because more compounds in the plant extracts exposed to the mosquitoes increase the knockdown effect time.~~ Therefore, the plant ~~extract is~~extracts *P. paniculata* are a potential alternative insecticide for controlling *Ae. aegypti* mosquitoes. ~~It does~~Like other plant insecticides, it is expected that *P. paniculata* extracts will not leave residues in the environment and ~~is safe for other living beings.~~will be environmentally friendly. Also, the ~~extract's~~bioactive compounds ~~do~~in the extracts will not cause resistance against *Ae. aegypti* mosquitoes~~, and the plant has economic value and is beneficial to cultivate.~~.

Acknowledgments

This study was funded ~~byDIPASTIKes~~by DIPA STIKes Hang TuahPekanbaru in 2020 with the title "Bio-insecticides Extract of Scented Root (*Polygala paniculata*) in Controlling *Aedes aegypti* m~~M~~osquito" with Contract Number No. 08/STIKes-HTP/VI/2020/0147, A.

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Comment [Editor14]: Remark: Reference 33 has not been cited in the manuscript. Please check and revise.

Comment [Editor15]: Remark: There is no clear link of this paragraph to your results. Please check and revise.

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