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RESEARCH ARTICLE

# Larvicidal Activity of *Piper nigrum*, *Pandanus amaryllifolius*, *Cymbopogon nardus*, *Cinnamomum burmanii* and *Azadirachta indica* Herbs Extracts on *Aedes aegypti* Larvae

Nur Nabilah Mohamad, Muniandy M Nathan*, Muhammad Ashraf Amzah

*Institut Latihan Kementerian Kesihatan Malaysia Sungai Buloh*

**Corresponding author: nmun61@yahoo.com*

**Abstract**

Dengue fever is an endemic disease in Malaysia. Existing control measures are aimed at important species of mosquitoes that are focused on adult mosquitoes or mosquito larvae. Mosquito control is difficult when there are many factors such as resistance to insecticides used in mosquito control and causing health effects to non-target organisms. One way to combat dengue fever is to eliminate *Aedes aegypti* mosquitoes. One way is to use plant extracts as an additional insecticide as it has larvacidal potentials that contain different phytochemicals, especially in killing mosquito larvae without affecting other organisms and the environment. This study aimed to study the use of herbs in the control and prevention of the breeding of *Aedes aegypti* mosquito larvae. The study was conducted in the form of an experimental quasi study. The sample for this study was instar IV larvae of 10 larvae with 4 readings with a sample size of 240 larvae. Results of larval mortality analysis using herbal species using the Kruskal Wallis test showed that this value was smaller than the alpha value of 0.05. It can be concluded that there are related herbicides (*Piper nigrum*, *Pandanus amaryllifolius*, *Cymbopogon nardus*, *Cinnamomum burmanii* and *Azadirachta indica*) in the control and reproduction of *Aedes aegypti* mosquito larvae.

***Keywords*:** *Mosquito Aedes Aegypti, Piper nigrum, Pandanus amaryllifolius, Cymbopogon nardus, Cinnamomum burmanii, Azadirachta indica*



## INTRODUCTION

In Malaysia Dengue fever regarded as predominantly as an urban disease due to high densities susceptible hosts. High population growth in urban areas, improper disposal of waste, lack of efficient solid waste management and increased movement of dengue virus in infected human through current modern transportation contribute to a marked increase in dengue cases in Malaysia. Mosquitoes are a significant general wellbeing danger as a typical vector that can make genuine sickness people for particular kinds of dangerous ailments, for example, intestinal sickness, filariasis, chikungunya, yellow fever, dengue fever and encephalitis that add to significant mortality (World Health Organization) WHO) 2015). The use of synthetic insecticides, organophosphates and pyrethroids, is the most commonly used approach worldwide. An important issue in this regard is the occurrence of resistance against mosquitoes especially *Aedes aegypti* and *Aedes albopictus* after widespread use of chemical insecticides (Rohani et al. 2001). According to (Benelli et al. 2015) environmental friendly devices have been implemented to improve mosquito control. Significant efforts have been made to investigate the effectiveness of botanical products and most plant compounds have been reported to be highly toxic to mosquitoes and act as an adult or larval mosquito repellents. This phenomenon has encouraged the use of natural products. Plants can be an alternative source for controlling mosquito vectors as they are rich in various phytochemicals that are specialized in killing mosquito larvae without affecting other organisms and the environment (Pedro et al. 2014).

## PROBLEM STATEMENT

Malaysia recorded the highest number of dengue cases in four years at over 130,000, rising 61 percent compared to 2018. Meanwhile 182 deaths were recorded in 2019. There were many strategies used in prevention and control of dengue in the Vector borne Disease Control Programme. This includes anti larval measures, inspection of premises, identify and source reduction of larvae breeding habitats, anti-adult mosquito's activity, health education, community participation and enforcement activities. All these strategies to combat dengue have to constantly monitored and reviewed as their effectiveness and to modify if necessary. Therefore, this study was conducted to use alternative home based herbs extract for larviciding in the control and prevention of the breeding of Aedes Aegypti mosquito larvae.

## METHODOLOGY

This study was conducted at the Bukit Mertajam Clinical Training Center in Seberang Perai Tengah. This study is based on a quasi experimental study in which it evaluated the effect of herbal extract used to determine the mortality of *Aedes aegypti* mosquito larvae. The ingredients used in this study are herbs such as *Piper nigrum*, *Pandanus amaryllifolius*, *Cymbopogon nardus*, *Cinnamomum burmanii* and *Azadirachta Indica*. Equipments used in this study are based on the WHO Guideline for Laboratory and Field Testing of Mosquito Larvicides (2005). The dosage of each herb extract in study is from 0 grams (control). 2 grams, 3 grams, 5 grams and 10 grams were placed into an ovitrap container containing 10 larvae. According to the WHO Guideline For Laboratory and Field Testing Of Mosquito Larvicides (2005), the solvent should be dissolved in a screw-cap bottle with aluminium foil over the mouth of the bottle. Shake the bottle to spread the material in the solvent. The concentration was tested by adding 0.1–1.0 ml of the appropriate dilution to 100 ml or 200 ml of deionized water or distilled water. The lowest dose should be provided in advance. Observation is done every 6 hours by taking 4 readings in 24 hours.

## RESULTS

Mortality rates of dengue mosquito larvae using herbs (*Piper nigrum*, *Pandanus amaryllifolius*, *Cymbopogon nardus*, *Cinnamomum burmanii*, *Azadirachta Indica*) with different doses were analyzed using non-parametric test. Therefore, the Kruskal Wallis test was used as in table 1. For *P. nigrum* analysis showed there is no significant difference in mean rank for each dose tested with a value of 8.50.

Table 1: Mortality of Aedes Larvae using *Piper nigrum*.

| Dos (gram) | N | Mortality Mean Rank | Test Statistic | P |
|---|---|---|---|---|
| 2 | 4 | 8.50 | | |
| 3 | 4 | 8.50 | 0.000 | 1.000 |
| 5 | 4 | 8.50 | | |
| 10 | 4 | 8.50 | | |

P< 0.05**

*P. amaryllifolius* analysis in table 2 showed that there was a mean difference in the mean of each dose tested where the dose of 10 grams recorded higher mortality of 14.50 compared to the other doses.

Table 2: Mortality of Aedes Larvae using *Pandanus amaryllifolius*.

| Dos (gram) | N | Mortality Mean Rank | Test Statistic | P |
|---|---|---|---|---|
| 2 | 4 | 4.38 | | |
| 3 | 4 | 6.00 | 10.653 | 0.014 |
| 5 | 4 | 9.13 | | |
| 10 | 4 | 14.50 | | |

P< 0.05**

Whereas for *C. nardus* analysis there is a mean difference in the mean of each dose tested where the dose of 5 grams and the dose of 10 grams respectively recorded the highest death of 12.00.

Table 3: Mortality of Aedes Larvae using *Cymbopogon nardus*.

| Dos (gram) | N | Mortality Mean Rank | Test Statistic | P |
|---|---|---|---|---|
| 2 | 4 | 2.88 | | |
| 3 | 4 | 7.13 | 12.458 | 0.006 |
| 5 | 4 | 12.00 | | |
| 10 | 4 | 12.00 | | |

P< 0.05**

For *C. burmanii*, the analysis showed that there was a mean difference in the mean of each dose tested where doses of 3 grams, doses of 5 grams and doses of 10 grams each recorded high mortality of 9.00.

Table 4: Mortality of Aedes Larvae using *Cinnamomum burmanii*.

| Dos (gram) | N | Mortality Mean Rank | Test Statistic | P |
|---|---|---|---|---|
| 2 | 4 | 7.00 | | |
| 3 | 4 | 9.00 | 3.000 | 0.392 |
| 5 | 4 | 9.00 | | |
| 10 | 4 | 9.00 | | |

P< 0.05**

As in table 5 for *A. indica* analysis showed that there was a mean difference in the mortality of each dose tested. The dose of 10 grams recorded the highest mortality of 13.63 and it is significant with p below 0.05.

Table 5: Mortality of Aedes Larvae using *Azadirachta Indica*.

| Dos (gram) | N | Mortality Mean Rank | Test Statistic | P |
|---|---|---|---|---|
| 2 | 4 | 3.75 | | |
| 3 | 4 | 6.88 | 9.598 | 0.022 |
| 5 | 4 | 9.75 | | |
| 10 | 4 | 13.63 | | |

P< 0.05**

Mortality rates of mosquito larvae using herbs (*P. nigrum*, *P. amaryllifolius*, *C, nardus*, *C. burmanii*, *A.*

*Indica*) with different duration of larviciding hours were analyzed using non-parametric test. The Kruskal Wallis test was used to test to determine the most effective duration of larviciding. Results in table 6 revealed that *P. nigrum* does not shows any significant difference in mean rank between 6 to 24 of contact hours with p value above then 0.05.

Table 6: Mortality of Aedes Larvae with duration of contact hours using *Piper nigrum*.

| Time (hour) | N | Mortality Mean Rank | Test Statistic | P |
|---|---|---|---|---|
| 6 | 4 | 5.50 | | |
| 12 | 4 | 7.50 | 3.571 | 0.312 |
| 18 | 4 | 9.50 | | |
| 24 | 4 | 11.50 | | |

P< 0.05**

For *P. amaryllifolius* analysis showed the mean rank in the 24 hours recorded the highest mortality with a value of 11.50 but it is significant with p value above then 0.005 as it in table 7.

Table 7: Mortality of Aedes Larvae with duration of contact hours using *Pandanus amaryllifolius*.

| Time (hour) | N | Mortality Mean Rank | Test Statistic | P |
|---|---|---|---|---|
| 6 | 4 | 5.50 | | |
| 12 | 4 | 7.50 | 3.571 | 0.312 |
| 18 | 4 | 9.50 | | |
| 24 | 4 | 11.50 | | |

P< 0.05**

Table 8 reveal result for *C. nardus* analysis. The mean difference in the meantime over the 24 hours recorded the highest mortality with a mean of 10.38 but it is significant as the p value above then 0.05.

Table 8: Mortality of Aedes Larvae with duration of contact hours using *Cymbopogon nardus*.

| Time (hour) | N | Mortality Mean Rank | Test Statistic | P |
|---|---|---|---|---|
| 6 | 4 | 7.00 | | |
| 12 | 4 | 9.00 | 3.000 | 0.392 |
| 18 | 4 | 9.00 | | |
| 24 | 4 | 9.00 | | |

P< 0.05**

While for *C. burmanii* analysis showed in table 9. a mean mortality difference between 6 hours of exposure compared above then 12 hours. The p value recorded 0.392 means the difference is not significant.

Table 9: Mortality of Aedes Larvae with duration of contact hours using *Cinnamomum burmanii*.

| Time (hour) | N | Mortality Mean Rank | Test Statistic | P |
|---|---|---|---|---|
| 6 | 4 | 7.00 | | |
| 12 | 4 | 9.00 | 3.000 | 0.392 |
| 18 | 4 | 9.00 | | |
| 24 | 4 | 9.00 | | |

P< 0.05**

For *A. Indica* the analysis showed (Table 10) the highest mean time recorded time period of 24 hours with 11.63 hours. Even though each time period shows different mortality mean rank but it is not significant with p value above then 0.05.

Table 10: Mortality of Aedes Larvae with duration of contact hours using *Azadirachta Indica*.

| Time (hour) | N | Mortality Mean Rank | Test Statistic | P |
|---|---|---|---|---|
| 6 | 4 | 4.88 | | |
| 12 | 4 | 7.38 | 4.853 | 0.183 |
| 18 | 4 | 10.13 | | |
| 24 | 4 | 11.63 | | |

P< 0.05**

Mortality rates of mosquito larvae using different herbs were analyzed using non-parametric. Therefore, the Kruskal Wallis test was used again to compare mortality with different type of herbs extract in larviciding. The analysis showed a mean difference between the tested herbs and the black pepper recorded the highest mortality with a value of 59.00 while the lowest was a mean of 14.63. The p value shows that the mortality is significantly difference.

Table 11: Kruskal Wallis test to determine mortality of Aedes Larvae with duration of contact hours using Azadirachta

| Types Of Herbs | N | Mortality Mean Rank | Test Statistic | P |
|---|---|---|---|---|
| Black pepper | 16 | 59.00 | | |
| Pandan | 16 | 27.44 | | |
| Fragrant | 16 | 43.88 | 52.191 | 0.000** |
| Lemongrass | 16 | 57.59 | | |
| Cinnamon | 16 | 59.00 | | |

P< 0.05**

## DISCUSSION

In this proposal, there are several improvements that can be made that through future studies should be tested the effectiveness of herbs studied using other plant organs such as roots or seeds as larvicidal. Also, future studies may use mosquito larvae tested in large quantities as this study uses small numbers. Besides, the temperature is also a factor to be considered as temperature affects larval breeding where the larvae cannot reproduce normally if the temperature is above 10 ° C. Subsequently, society is encouraged to use natural larvicidal as it is readily available and has no health effects on humans.

## CONCLUSION

This study concludes that extracts of home based herbs can be used as an alternative to the control and prevention of aedes mosquito larvae. It can be seen that all herbs demonstrate as a lethal concentration (LC) at a specific dose. Among them, black pepper extracts produce the most effective lethal concentration as aedes mosquito larvae larvicide. With this invention the villagers can use

these herbs in the absence of abates. This herbs are easily available in their environment and does not cost much. More herbs should be tested for this purpose to identify the active ingredient in each herb to be used as an alternative measure in dengue control.

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