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The Potency of *Moringa oleifera* Leaves Extract as Larvicide for *Aedes aegypti*

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ABSTRACT. The main vector of dengue fever is the *Aedes aegypti*. The use of synthetic insecticides, especially larvicides, has several effects, including resistance and environmental pollution. Therefore, researchers are interested in examining the potential of Moringa plants as natural larvicides. This study was conducted for knowing influence administration of *M. oleifera* leaf extract against the death of *Aedes aegypti* larvae and effective concentration as a larvicide against *Aedes aegypti* larvae. Method. This research was a true experiment, using design randomized post-test only control group design. The research test was conducted by dividing the sample into 6 groups, where 1 group was the control and 5 groups were treated with *M. oleifera* leaf extract, consentrations of 100 ppm, 200 ppm, 300 ppm, 400 ppm and 500 ppm. Results. The results of the Kruskal-Wallis statistical test on the effect of *M. oleifera* leaf extract on larval mortality during 24 hours of exposure showed a significant effect (p value 0.002), while at 48 hours exposure it also showed the same result (p = 0.004). This result showed that there was a significant difference between control group and treatment groups at concentration 100 ppm, 200 ppm, 300 ppm, 400 ppm, 500 ppm (p value 0.000). Conclusion: There is an effect of giving *M. oleifera* leaf extract on the mortality of *Aedes aegypti* larvae. Various concentrations were effective in causing the death of *Aedes aegypti* mosquito larvae. It is needed to observe the mortality of *Aedes aegypti* larvae at shorter observation intervals and varying extract concentrations.

Keywords: *Aedes aegypti,* larvicide*, M. oleifera*.

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1. Introduction

Dengue Hemorrhagic Fever (DHF) has become a public health problem in Indonesia. Of the several provinces in Indonesia that were affected by DHF, one of them the only province of East Nusa Tenggara. East Nusa Tenggara province consists of various large and small islands. Disease this give adverse impact because could cause death. Fever bloody also give burden economy for public. (Nadjib *et al.*, 2019) (Murugesan and Manoharan, 2019).

The main vector of dengue fever is the *Aedes aegypti* mosquito, while the potential vector is *Aedes albopictus*. One way to control dengue vectors is to use insecticides. The use of synthetic insecticides, especially larvicides, has several effects, including resistance and environmental pollution. Literature review about review ecology and resistance Aedes aegypti in various region has conducted and show different results.(Egid *et al.*, 2022) Development product larvicide from diversity biological start developed which are known to have several biological activities. (Faustino *et al.*, 2020)(Rampadarath, Puchooa and Ranghoo-Sanmukhiya, 2014)(Ishak *et al.*, 2014).

Moringa is a plant that grows in various regions in Indonesia. Moringa growing in East Nusa Tenggara has the advantage of being easy to cultivate and resistant to heat. This plant is also able to grow on various islands in East Nusa Tenggara. Moringa plants contain a variety of beneficial compounds. (Kou *et al.*, 2018) Almost all parts of this plant, namely leaves, flowers, roots, stems and fruit have benefits. Another study on Moringa leaves in Mexico found *phenolic acids* (*gallic* and *chlorogenic acids*) and *flavonoids* (routine, *luteolin, quercetin, apigenin* and *kaempferol*). (Valdez-Solana *et al.*, 2015) Research on the use of Moringa as a larvicide has also been carried out. (Al-Barty and Hamza, 2015) (Kumar *et al.*, 2016) (Chinenyenwa and Godson, 2017).

Moringa plants contain compounds found in leaves, flowers and roots, including alkaloids and flavonoids. This compound indicates its potential as a larvicide. Alkaloids have the ability to act as stomach poisons and inhibit the work of the cholinesterase enzyme in larvae, while flavonoids act as respiratory poisons, causing the death of larvae. Because of the content of these various compounds, the Moringa plant has the potential as a larvicide against mosquito larvae, especially *Aedes aegypti* . Flavonoids interfere with the respiratory system, alkaloids interfere with the digestive system which can cause inflammation result in larval death. Research using Moringa seeds shows potential as a larvicide. (Silva *et al.*, 2020).

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Therefore, researchers are interested in examining the potential of Moringa plants as natural larvicides against *Aedes aegypti* larvae. Study this conducted for knowing influence administration of *M. oleifera* leaf extract against the death of *Aedes aegypti* mosquito larvae and effective concentration as a larvicide against *Aedes aegypti* mosquito larvae. The results of this study are expected can provide scientific information about the potential of *M. oleifera* in controlling disease vectors, especially *Aedes aegypti* larvae and as a basis for developing knowledge in the health sector as an effort to prevent vector-borne diseases, especially the *Aedes aegypti* mosquito and use natural larvicides as an alternative vector control.

2. Materials and Methods

2.1 Research Design

This research was a true experiment using design randomized post test only control group design.

2.2 Plant Material

M. oleifera obtained from Kupang East Nusa Tenggara.

2.3 Extraction of M. oleifera

Maceration method was used to make moringa leaves extract. Solvent used was ethanol 96%. To determine the compounds contained in the extract, phytochemical testing was carried out using the thin layer chromatography (TLC) method.

2.4 Larvae Rearing

The larvae of *Aedes aegypti Instar* III was used in this study. The *Aedes aegypti* was caught in Oebobo Village on the grounds that it is an endemic location for dengue cases in Kupang City. The captured mosquitoes were put in mosquito cages and installed with ovitraps as egg catchers. Eggs were incubated in plastic trays filled with clean water for rearing mosquito larvae. The eggs hatch within 2 days and after hatching are fed with chicken liver powder. In approximately 4 days, mosquito larvae grow to reach the third instar.

2.5 Larvae Rearing

The larvae of *Aedes aegypti* were maintained in the Entomology Laboratory of the Environmental Health Department, Poltekkes, Ministry of Health, Kupang. Extract was devided into several concentration. The research test was conducted by dividing the sample into 6 groups, where 1 group was the control group and the other 5 groups were treated with Moringa leaf extract. Each group was 20 larvae.

Control: 0 ppm
Group I: 100 ppm
Group II: 200 ppm
Group III: 300 ppm
Group IV: 400 ppm
Group V: 500 ppm

This test was repeated 4 times. Larva mortality was evaluated at the end of the 24 and 48 hours exposure.

2.6 Statistical Analysis

Data normality tested with One-sample Kolmogorov test, test homogeneity using Levene Test. One Way ANOVA test is used if the data is normally distributed and homogeneous, then the LSD post hoc test is continued. If the data is not normal or homogeneous, it will be analyzed using the Kruskal-Wallis test.

2.7 Ethical Clearance

This study was approved by animal ethic committee, Faculty of Veterinary Medicine, Nusa Cendana University (KEH/FKH/NPEH.2019/023).

3. Result and Discussion

The results of plant identification used in this study is Moringa oleifera Lamk. The identification results showed that the ethanol extract of Moringa leaves found the presence of compounds alkaloids, flavonoids, tannins and saponins.

Table 1 shows that no larvae death in the control group, while exposure to moringa extract caused larval death. Figure 1 shows a graph of the mortality of larvae exposed to the extract for 24 hours in each treatment group.

Table 2 shows that the mortality in the control group was lower than in the group exposed to moringa leaf extract. Figure 2 shows that the mortality after 48 hours in the control group was less than the treatment group. The treatment group that was exposed moringa leaf extract at various concentrations showed higher mortality.

Table 1
The mortality of *Aedes aegypti* larvae after 24 hours of exposure

Group	Replication				Rate of mortality
	1	2	3	4	
Control	0	0	0	0	0
100 ppm	7	8	8	4	6.8
200 ppm	6	9	5	8	7.0
300 ppm	9	6	8	8	7.8
400 ppm	9	9	10	10	9.5
500 ppm	9	9	9	10	9.3

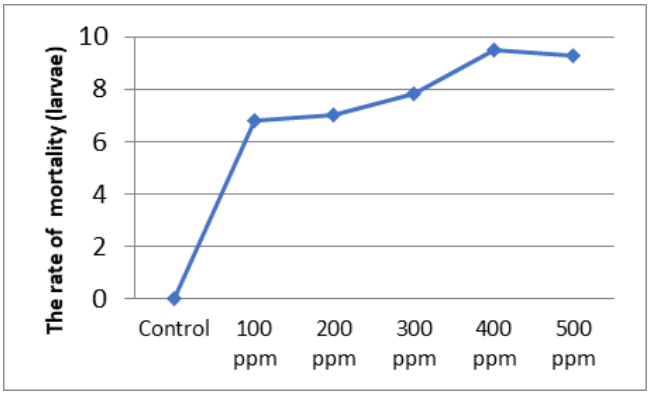

Fig. 1 The mortality of Aedes aegypti larvae after 24 hours of exposure.

Table 2
The mortality of *Aedes aegypti* larvae after 48 hours of exposure

Group	Replication				Rate of mortality
	1	2	3	4	
Control	1	1	1	0	0.8
100 ppm	7	9	10	4	7.5
200 ppm	7	9	5	8	7.3
300 ppm	9	9	8	8	8.5
400 ppm	10	10	10	10	10.0
500 ppm	9	9	9	10	9.3

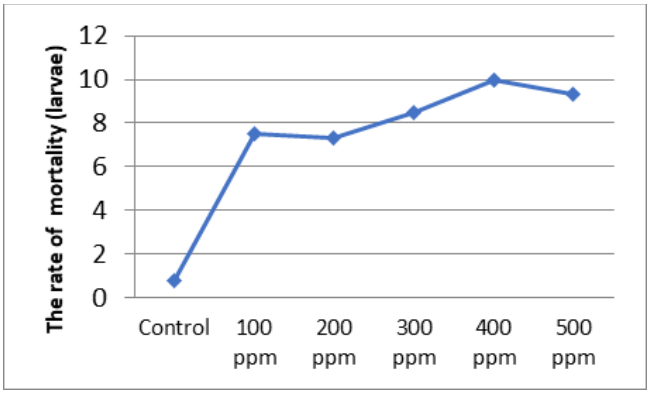

Fig. 2 The mortality of *Aedes aegypti* larvae after 48 hours of exposure.

The normality test was carried out using the One-sample Kolmogorov test, it was said to be normal if p>0.05. The mortality in 24 hours obtained p value = 0.000 (p <0.05) while the mortality in 48 hours obtained p value = 0.000 (p <0.05). This means that the data was not normally distributed. Homogeneity test using Levene Test, said to be homogeneous if p>0.05. The results of the homogeneity test showed that the data was not homogeneous. The mortality in 24 hours obtained p value = 0.011 (p < 0.05) while the mortality in 48 hours obtained a value of p = 0.005 (p < 0.05).

Because the data are not normally distributed and not homogeneous, it is continued with the nonparametric statistical test of the Kruskal-Wallis test. The results of statistical tests on the effect of Moringa leaf extract on larval mortality for 24 hours obtained p value = 0.002 (p < 0.05), while 48 hours mortality obtained p value = 0.004 (p < 0.05). These results indicate that there is a significant effect of giving moringa leaves on the mortality of mosquito larvae. The results of the Post Hoc LSD test showed that there was a significant difference between control and group I, control with group II, control with group III, control with group IV and control with group V (p-values = 0.000).

Aedes aegypti is one of the vectors carrying the dengue virus which causes dengue hemorrhagic fever. In many areas, outbreaks are still common due to this disease and can often lead to death. Decreased immunity is a factor that increases the incidence of dengue infection (Ndii, 2022).

The growth of this mosquito in its life cycle undergoes a larval form that lives in water. One of the efforts that can be done to prevent dengue fever is to control the mosquito life cycle. Surveilance larval resistance to insecticide need conducted (Hamid *et al.*, 2018). Research in Jakarta shows that Aedes aegypti resistant to insecticide with the mortality rate not reaching 90% (Hamid *et al.*, 2017). The material commonly used to control the mosquito life cycle is abate powder. However, many studies have also been carried out to test natural ingredients in inhibiting larval growth. Various plants contain bioactive compounds which in certain concentrations are able to inhibit the mosquito life cycle. One of them is *M. oleifera*.

Moringa leaf extract used in this study contains various compounds including flavonoids, saponins, alkaloids and tannins. The alkaloid and flavonoid compounds in Moringa leaves indicate their role as larvicides. Alkaloids have the ability to act as stomach poisons and inhibit the work of the cholinesterase enzyme in larvae, while flavonoids act as respiratory poisons, causing larval death and disrupting the nervous system. This indicates that secondary metabolites of alkaloids and flavonoids are capable of providing a larvicidal effect on mosquito larvae. One of the mechanisms of action of larvicides is to interfere with the respiratory system. A study showed that M. oleifera seeds as a source of insecticide lectin have high potential to be used in the control of A. aegypti because at the same time stimulating oviposition by females, it can kill eggs or larvae that may emerge from them (de Oliveira *et al.*, 2016).

Observations in 24 hours there was a significant mortality in larvae given Moringa leaf extract. Likewise for the next 24 hours. Based on larval mortality for 48 hours, it was shown that the higher the concentration of Moringa leaf extract solution, the greater the ability of Moringa leaf extract to kill larvae (figure 1). The relationship between the concentrations of Moringa leaf extract with mortality is thought to be related to the toxic load contained in the larvae. Larvae that get a high concentration of poison have a faster chance of dying compared to larvae that were treated with lower concentrations. Another study that gave Moringa leaf extract to Anopheles larvae also showed that there was a correlation between the concentration given and larval mortality (Sharma *et al.*, 2013). Based on its activity, it appears that the ethanol extract of Moringa leaves can be used as a larvicide. The content of secondary metabolites such as alkaloids, tannins and flavonoids found in Moringa leaves affects the nervous system and respiratory system of the larvae, causing death. Tannins can reduce the intensity of eating which results in disruption of growth. The higher the concentration of the Moringa leaf extract solution, the more it causes the death of the larvae.

Use of natural materials as larvicides must be developed because the use of abate powder still has drawbacks, namely the distribution of abate powder is uneven and not always available in the market. Study use ingredient experience star fruit wuluh as larvicide has conducted also.(Rohmah, Subekti and Rudyanto, 2020) Other research too researching papaya leaf extract as larvicide for Aedes (Ilham *et al.*, 2019).

The results of this study prove that the larvicidal activity of Moringa leaf extract can kill *Aedes aegypti* larvae. These results are supported by research showing that *M. oleifera* extract has larvicidal activity against *Anopheles gambial* (Chinenyenwa and Godson, 2017). Therefore, the production of larvicides based on local resources is expected to reduce costs. To reduce this effect, it is necessary to use natural larvicides from plants as an alternative to control *Aedes aegypti* larvae. Because it is made from plants, it will be more easily decomposed. Prolonged use of synthetic pesticides can pollute the environment and increase larval resistance to insecticides.

Several studies indicate that extracts from several parts of the Moringa plant, namely *M. oleifera* seed extract are effective as mosquito vector control agents. The intervention using this plant extract showed some developmental abnormalities in larvae and pupae. The study also tested flower, seed and leaf extracts (Prasad and Sharma, 2014). *M. oleifera* extract can also be used with TiO2NPs as larvicides (Al-Barty and Hamza, 2015). *M. oleifera* is one of plants used for mosquito prevention in northwestern Ethiopia. Besides that there is also plant other. They have larvicidal potential that are effective against all four instar larvae of An. Arabiensis (Ejeta, Asme and Asefa, 2021).

Several studies have shown that each part of the plant exhibits a different larvicidal effect. The difference in effect can also be caused by the difference in the solvent used to extract. A study showed that there were no larvicidal properties of aqueous extracts from the leaves, flowers and fruit of *M. oleifera* (Saini, Sivanesan and Keum, 2016). These results are supported by other studies which conclude that the extract

solvent affects the effectiveness of larvicidal.(Yuliasih and Widawati, 2017). This can happen because with different solvents, the components of the dissolved compounds are also different. Larval death too influenced by many factors. This factors are larval age, larval density, habitat, volume of water, time, temperature, humidity. In this study, these variables were controlled.

Moringa seed product development in tablet form as a larvicide has been done. Tablets are able to induce larval death and reduce hatching eggs of *A. aegypti*, in addition to having a repellent effect on oviposition. The results presented in this study demonstrate the potential of this new product for use in population control of A. aegypti, a vector of several arboviruses of global importance (Silva *et al.*, 2021).

Other study showed that Moringa leaf extract solution could kill larvae. The rate of larvae mortality at each concentration is different. This result also show that content of alkaloid compounds, tannins and flavonoids in Moringa leaf extract can kill *Aedes aegypti* larvae (Yasi and Harsanti, 2018).

The weakness of this study was that it did not observe the death of larvae every hour, but only observed 2x24 hours. This study also did not specifically observe the changes that occurred in the surviving larvae. This is important to do in order to know the phases of changes in larvae due to the administration of Moringa leaf extract.

4. Conclusion

There is an effect of giving Moringa leaf extract on the mortality of *Aedes aegypti* larvae. Concentrations of 100 ppm, 200 ppm, 300 ppm, 400 ppm and 500 ppm were effective in causing the death of *Aedes aegypti* mosquito larvae. We recommend that further research is needed to observe the mortality of *Aedes aegypti* larvae at shorter observation intervals and varying extract concentrations. We also need to examine the morphological changes that occur in larvae that do not die.

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