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# The effect of $LC_{50}$ granula mixed extracts of *Coriandrum sativum* L. and *Allium sativum* on mortality of *Larvae of Aedes aegypti* L. causing dengue fever

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## Abstract

*Aedes aegypti* L. is a type of mosquito that has the potential to be a vector for carrying Dengue Hemorrhagic Fever (DHF). It is necessary to control mosquito vectors with environmentally friendly materials, namely natural larvicides such as coriander (*Coriandrum sativum* L.) and garlic (*Allium sativum*). This study aims to determine the $LC_{50}$ of a mixture of coriander and garlic extract granules on the mortality of *Aedes aegypti* L. mosquito larvae as a vector for dengue hemorrhagic fever within 48 hours. Extract preparation was carried out using the maceration method with 70% ethanol solvent then evaporated to obtain a thick extract. Granules were made using aerosol and avicel in a ratio of 20%: 20%. Serial preparation of granule concentrations is 10 ppm, 25 ppm, 50 ppm, 75 ppm and 100 ppm. Extract granule testing was carried out by inserting 20 larvae into the extract granule concentration which had been dissolved using distilled water at each predetermined concentration. The results of the research show that the higher the concentration used, the higher the death rate of *Aedes aegypti* L. larvae with the $LC_{50}$ probit analysis results being 32.95 ppm with a lower limit of 18.22 ppm and an upper limit of 85.09 ppm. This shows that the mixture of coriander and garlic extracts used is toxic to *Aedes aegypti* L larvae.

**Keywords:** $LC_{50}$; Granule extract; *Aedes aegypti* L; *Coriandrum sativum* L; *Allium sativum*

## 1. Introduction

Dengue fever is transmitted by the *Aedes aegypti* mosquito as its vector. Until now, dengue hemorrhagic fever (DHF) is still a serious health problem that must be addressed immediately. . Many people choose to control using synthetic insecticides because they are more practical, effective, the time period required is not too long and easy to apply. However, the use of synthetic insecticides has an unfavorable impact on the environment, coupled with excessive use. These losses include causing pollution to the environment, and can cause poisoning in humans and livestock (Ekawati et al., 2017).

Overcoming these problems can be done by natural means, namely by utilizing plants or plant-based insecticides that are more selective and safe. Some of the advantages of vegetable larvicides include easy application, no pollution to the environment and harmless to humans and livestock (Ekawati et al., 2017). In addition to a safer level of safety, vegetable larvicides are also biodegradable so they do not pollute the surrounding environment, are safe for humans and other organisms (Noshirma and Ruben, 2016). Plant-based larvicides are easy to find in the surrounding environment and are very affordable. The materials to be used in this experiment are coriander (*Coriandrum sativum* L.) and garlic (*Allium sativum*).

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Coriander (*Coriandrum sativum* L.) has been known only as a food spice, as well as garlic (*Allium sativum*). However, it turns out that both ingredients can act as ingredients for mosquito larvae control. The potential of garlic to act as a larvicide is due to the content of compounds in garlic, including Allicin, Flaphonoids, Sulfur Ammonia Acid Allin and Garlic oil (Ikhtiar et al., 2019). Coriander has the potential as a vegetable larvicide because it contains tannin, flavonoids, alkaloids, and saponins. In a plant extract, in addition to the main active compounds, there are usually also many other compounds that are less active, but their presence can increase the overall activity of the extract (synergistic) (Syahrir et al., 2017).Based on the explanation of the two ingredients, it is known that each material to be used has different active compounds that are larvicidal, namely allicin in garlic and flavonoids in coriander. Both compounds are toxic to *Aedes aegypti* larvae L. Mixing several active compounds in plants will form new compounds that are synergistic, antagonistic, or neutral. Where in a plant extract, in addition to the main active compound there are usually also many other compounds that are less active, but their presence can increase the overall activity of the extract (synergistic), antagonistic or neutral (Syahrir et al., 2017). Synergistic means that the mixture of these compounds can increase toxicity characterized by the smaller concentration value needed to kill the larvae, while antagonistic means that the mixture of these compounds is opposite in nature so as to reduce the level of toxicity. Neutral is the nature of the mixture of the two compounds is the same so that it does not affect the nature of both (Asnan et al., 2018).

## 2. Material and method

The study of the toxicity of a mixture of coriander (*Coriandrum sativum* L.) and garlic (*Allium sativum*) extracts on the mortality of *Aedes aegypti* L. mosquito larvae is a laboratory experimental study using a completely randomized design (CRD) conducted in the Toxicology Laboratory of the Biology Education Study Program, Faculty of Teacher Training and Education, University of Jember. This research was conducted from February to June 2022.

### 2.1. Tools and Materials

The tools used in this study include the following: plastic trays for colonizing test larvae, analytical scales, blenders, plastic jars, spoons (stirrers), sieves, filter paper, funnels, measuring cups, rotary evaporators, spatulas, jam bottles, glass plates, ovens, refrigerators, tissues, cameras, label paper, measuring cups, dropper pipettes, aluminum foil and plastic cups, lumping, mesh 14 sieves. As for the materials used in this study include: mixture of coriander and garlic extracts, 97% ethanol, aeracil, avicel, larvae of *Aedes aegypti* L. instar III late to instar IV early, tween 20, abate (positive control) and distilled water (negative control).

### 2.2. Maintenance Stage

*Aedes aegypti* L. mosquito larvae were placed on trays with water provided at the Entomology Laboratory of the East Java Provincial Health Office, Surabaya. Larval feeding was done daily, in the form of mashed fish pellets. Observation of *Aedes aegypti* L. mosquito larvae was carried out every day to determine the molting process so that the larval stage could be determined. The larvae that were ready to be used were healthy, mobile, and homogeneous instar III and early instar IV larvae.

### 2.3. Larval Identification Stage

Larval identification was carried out microscopically and macroscopically. Microscopic identification is done by observing the morphology of the larvae including color, size, and side spines of the anal tooth. While macroscopic observations were made by observing the resting phase of the larvae.

### 2.4. Extract Preparation Stage

The stage after identifying the test larvae is to make coriander and garlic extracts. Making extracts begins with preparing the ingredients, namely coriander and garlic. For garlic, the part used is the bulb that has been separated from the skin, then pasrah or thinly sliced and wind-dried so that later it becomes powder after being mashed. After drying, then each ingredient is mashed using a blender until it becomes powder. The next step is weighing the powder and adding 97% ethanol in a ratio of 1: 3 which is then stirred until homogeneous using a spatula and then tightly closed. Next extract using the maceration method for 3 days, which during the maceration process is stirred manually every 6 hours so that the extract is mixed with the solvent. The next stage is filtering using a funnel and filter paper, then the results of the filter are evaporated using a rotary evaporator at $50^{O}$C for ± 3 hours at a speed of 50 rpm. Furthermore, the rotary results were put into the oven for ± 4 hours with a temperature of $40^{O}$C so that the results obtained in the form of concentrated thick extracts that will be used in research. Furthermore, the extract is placed in the refrigerator until the time of application.

### 2.5. Granule Making Stage

The thickened extract of Coriander (*Coriandrum sativum* L.) and Garlic (*Allium sativum*) Extract Mixture was put into lumping, then mixed with aerosol and 20% avicel: 20% little by little until it becomes a compact mssa and can be braided. Then passed on a mesh 14 sieve. The dried granules were passed again on a mesh 14 sieve.

### 2.6. Toxicity Test Stage of Granule Mixture of Coriander (*Coriandrum sativum* L.) and Garlic (*Allium sativum*) Extracts against *Aedes aegypti* mosquito larvae

The toxicity test stage of the granule extract was carried out in two stages, namely preliminary testing and final testing. The preliminary testing stage was carried out to find concentrations that killed the test larvae by 5% and 95%. The final testing stage was carried out by making a concentration series based on the results of preliminary testing. In this study the concentrations used were concentrations of 10 ppm, 25 ppm, 50 ppm, 75 ppm and 100 ppm. In addition to using a series of extract concentrations, positive and negative controls were also used. The positive control used abate and the negative control used distilled water. The number of *Aedes aegypti* L. mosquito larvae used was 20 larvae in each treatment. The number of treatments in this test was 5 treatments with 4 repetitions.

The stages of the extract granule toxicity test are started by inserting 20 test larvae using a drop pipette into a plastic cup. The plastic cup already contains a mixed solution of extract granules with a predetermined concentration. The deduction time was carried out for 48 hours. Observations were made on the movement and number of dead larvae by touching the larval body using a stick or pipette. Then record the number of dead larvae and determine the toxicity ($LC_{50}$) using probit analysis with SPSS software.

## 3. Results and Discussion

Based on the results of the research conducted, the average mortality of *Aedes aegypti* L. mosquito larvae using a mixture of coriander (*Coriandrum sativum* L.) and garlic (*Allium sativum*) extract granules can be seen in Table 1.

**Table 1** Final Test Results of granule Mixture of Coriander (*Coriandrum sativum* L.) and Garlic (*Allium sativum*) Extracts on Mortality of *Aedes aegypti* L. Mosquito Larvae within 24 Hours

| | **Mortality of *Aedes aegypti* L. Mosquito Larvae (%)** | | | | |
|---|---|---|---|---|---|
| **Treatment** | **Repeat** | | | | **Average** |
| | **1** | **2** | **3** | **4** | |
| K- | 0 | 0 | 0 | 0 | 0 |
| K+ | 20 | 20 | 20 | 20 | 100 |
| 10 ppm | 1 | 0 | 1 | 0 | 2.5 |
| 25 ppm | 4 | 6 | 4 | 6 | 27.5 |
| 50 ppm | 10 | 13 | 12 | 13 | 60 |
| 75 ppm | 15 | 15 | 14 | 16 | 80 |
| 100 ppm | 19 | 20 | 20 | 19 | 97.5 |

Table 1. shows that the higher the concentration of the mixture of coriander (*Coriandrum sativum* L.) and garlic (*Allium sativum*) extracts, the higher the percentage of mortality of *Aedes aegypti* L. mosquito larvae. The test results showed that the negative control using distilled water did not cause death to the test larvae after 48 hours of observation. While the positive control using abate with a concentration of 100 ppm caused 100% mortality of the total test larvae after 48 hours of observation.

**Table 2** Probit Analysis of **$LC_{50}$** of Mixed Extracts of Coriander (*Coriandrum sativum* L.) and Garlic (*Allium sativum*) in 48 Hours Deduction Time

| **Concentration (ppm)** | | | |
|---|---|---|---|
| ***Lethal Concentration* 50% (LC )$_{50}$** | **$LC_{50}$ (ppm)** | **Lower Limit (ppm)** | **Upper Limit (ppm)** |
| *Coriander* (*Coriandrum sativum* L.) andGarlic (*Allium sativum*) Extract Blend | 42.95 | 18.22 | 85.09 |

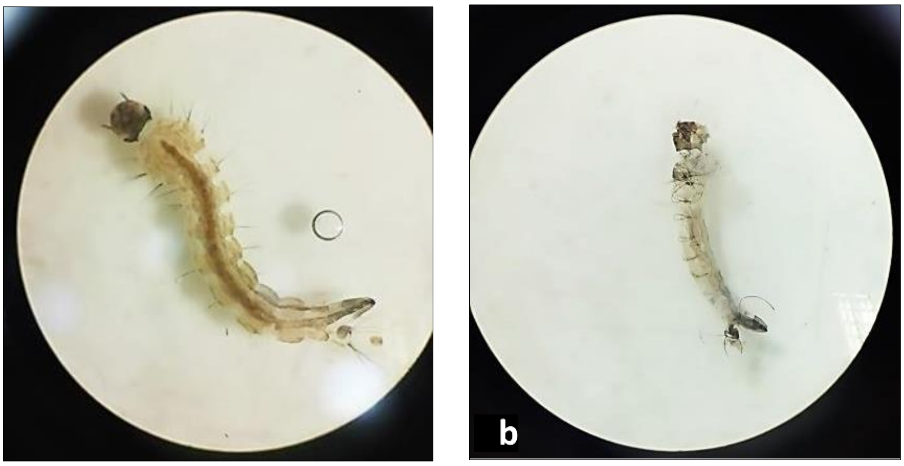
50Based on the results of probit analysis in Table 2. It can be seen that the LC value of the mixture of coriander (*Coriandrum sativum* L.) and garlic (*Allium sativum*) extracts is 42.95 ppm in 48 hours with a lower limit of 18.22 ppm and an upper limit of 85.09 ppm where the concentration that kills 5% is 10 ppm and the concentration that can kill 97.5% is 100 ppm.

$LC_{50}$ can be interpreted as a situation where the concentration of the extract granule can kill the test larvae by 50%. This indicates a toxic or poisonous effect contained in a material as a single dose or mixed preparation (Mappasomba et al., 2019). In this study, the toxicity test was carried out by putting 20 test larvae into a container that already contained a solution of a mixture of coriander (*Coriandrum sativum* L.) and garlic (*Allium sativum*) extract granules with several concentration series that were based on preliminary testing, namely 10 ppm, 25 ppm, 50 ppm, 75 ppm and 100 ppm. The mixture of coriander (*Coriandrum sativum* L.) and garlic (*Allium sativum*) extract granules had a toxic effect on *Aedes aegypti* L mosquito larvae with an $LC_{50}$ of 42.95 ppm. And in the same concentration as abate, namely 100 ppm, the number of deaths of test larvae can almost match the mixture of coriander (*Coriandrum sativum* L.) and garlic (*Allium sativum*) granule extracts of 97.5% and abate 100%. This can be interpreted that The mixture of coriander (*Coriandrum sativum* L.) and garlic (*Allium sativum*) extract granules is toxic to the mortality of *Aedes aegypti* mosquito larvae (between 30 _- 1000 ppm) so that the extract The mixture of coriander (*Coriandrum sativum* L.) and garlic (*Allium sativum*) extract granules can be used as a natural larvicide instead of abate with a lower limit of 18.22 ppm and an upper limit of 85.09 ppm.

The level of toxicity value can be seen from the $LC_{50}$ value, namely the smaller the $LC_{50}$ value obtained, the toxic effect of the activity of a compound in killing test animals will be greater. Conversely, when the $LC_{50}$ value obtained is greater, the toxic effect given will be smaller. A compound is categorized as highly toxic if the $LC_{50}$ value is less than 30 ppm, said to be toxic if the $LC_{50}$ value is at 30-1000 ppm, and said to be non-toxic if the $LC_{50}$ value is above 1000 ppm (Herfayati et al., 2020). The content of compounds in plants, in addition to the main active compounds, usually also contains many other compounds that are less active, but their presence can increase the overall activity of the extract (synergistic) (Syahrir et al., 2017). Based on thin layer chromatography results from previous research it is known that garlic contains Allicin, Flaphonoid, Sulfur Ammonia Acid Allin and Garlic oil compounds. Coriander has the potential as a vegetable larvicide because it contains tannin, flavonoids, alkaloids, and saponins. (Ikhtiar et al., 2019). In this study The mixture of coriander (*Coriandrum sativum* L.) and garlic (*Allium sativum*) extract granules is synergistic because the toxicity of the resulting coriander granules is much higher than the toxicity of coriander (*Coriandrum sativum*) granules alone or garlic (*Allium sativum*) extract granules alone The mixture of coriander (*Coriandrum sativum* L.) and garlic (*Allium sativum*) extract granules at the time was not mixed.

The mode of action of toxic compounds from the granule mixture of coriander (*Coriandrum sativum* L.) and garlic (*Allium sativum*) extracts is contact poison and respiratory poison. Contact poison, this granule works by entering the body of the target insect through the skin (cuticle) and transported to the part of the insect's body where the active gtanula works. In systemic insecticides, the insect will die after eating or sucking the liquid that has been inserted. Metabolic poisons, the granules in this study are also metabolic poisons that kill through the digestive tract after swallowing water mixed with granules.

Based on the results of observations, there are morphological differences in *Aedes aegypti* mosquito larvae before and after treatment of granule mixture of coriander (*Coriandrum sativum* L.) and garlic (*Allium sativum*) extracts in 48 hours deduction time can be seen in Figure 1 as follows:




a b

**Figure 1** (a) Morphology of *Aedes aegypti* L. larvae before treatment; (b) Morphology of *Aedes aegypti* L. larvae after treatment. 4x10 magnification (Personal Documentation)

There were morphological differences in *Aedes aegypti* mosquito larvae before and after treatment. Before treatment, the mosquitoes looked normal and other body parts were still intact. Whereas after the treatment there were several changes such as the color of the larval body which was originally brown turned into a paler color, and there was some damage to the larval body parts such as damage to the eyes and antennae, detachment of spines on the thorax, detachment of lateral hairs on the abdomen. Damage also occurs in the abdomen and respiratory tract, there is a change in the color of the siphon which is initially blackish brown turning pale. In addition to morphological changes, there were also changes in the behavior of the test larvae. Before treatment, the behavior of the test larvae was still lively, very active, moving up and down on the water surface. Whereas after treatment, the test larvae turned inactive, tended to be at the bottom of the test container and some died.

The results of the phytochemical test showed that coriander (*Coriandrum sativum* L.) has the potential as a larvicide because it is known to contain secondary metabolite compounds such as alkaloids, flavonoids, saponins, tannins, terpenoids, and steroids. In addition, coriander also contains essential oils such as linalool by 60-70%. This content can cause convulsions and even paralysis in insects, because linalool works as a contact poison by increasing sensory activity in insects (Fitriani et al., 2019). Linalool has a fragrant aroma but is not favored by mosquitoes. Because in the content of linalool itself there is geraniol content. Geraniol is an active ingredient that is disliked and highly avoided by insects including mosquitoes. So the use of materials that have geraniol content is very useful as a mosquito repellent (Jubaedah et al., 2017). The more concentrated the concentration of the solution, the more secondary metabolites contained in the coriander extract.

Besides coriander, garlic (*Allium sativum*) also acts as a larvicide. The potential of garlic to act as a larvicide is because it contains secondary metabolite compounds, including allicin, flavonoids, sulfur ammonia acid allin and garlic oil (Ikhtiar et al., 2019). Phytochemical test results show that garlic contains secondary metabolite compounds in the form of alkaloids, tannins and terpenoids (Sasmilati, 2017). Garlic (*Allium sativum*) also contains amino acid bonds in the form of allin which can turn into allicin (Amirullah et al., 2019). Allicin can inhibit the development of further larvae that will become pupae, allicin can also damage cell membranes which will cause death in larvae (Adenan et al., 2018).

The compounds contained in the extract enter the larval body through contact toxins (natural holes of the larval body), stomach toxins (digestive organs) and respiratory toxins (siphon). The active compounds each have specifications to attack the larval body organs according to the way the compounds enter the larval body. Secondary metabolite compounds contained in a mixture of coriander and garlic extracts include alkaloids, flavonoids, saponins, tannins, terpenoids, and steroids. Alkaloids have properties as stomach poisons and contact poisons that can degrade cell membranes through the skin and digestive tract. The alkaloid group will generally inhibit the acetylcholinesterase enzyme so that acetylcholine will be deposited at synapses. The accumulation of acetylcholine can cause chaos in the impulse delivery system to muscle cells (Bisyaroh, 2020).

Flavonoids work as respiratory toxins. Flavonoids attack the larval respiratory organs (siphon) causing damage, making it difficult for the larvae to breathe. The mechanism of action is by causing nervous exhaustion and causing damage to the siphon, so that the respiratory system in the larvae is disrupted, causing the larvae to be unable to breathe and experience death (Sigit et al., 2022). Saponin is contact toxic to larvae, if saponin comes into direct contact with the outer skin of larvae, then little by little saponin molecules will enter the larval body which is then absorbed through the body cell wall, and causes larval death. In addition, saponin is also a neurotoxin that can cause convulsions in the larval body. The spasm results in disrupted larval movement as well as disrupted oxygen uptake (Wahyuni, 2016).

Tannin compounds can also interfere with the digestive system in larvae, because tannins bind to proteins in the digestive system that larvae need for growth, thus causing larval death (Ayal et al., 2021). In addition, tannin compounds can cause a decrease in the activity of protease enzymes in converting amino acids. Tannin compounds can bind to protease enzymes, where the binding of enzymes bound by tannins causes enzyme work to be inhibited, so that cell metabolic processes are disrupted and larvae will lack nutrients. If this process continues, it will result in larval death (Bisyaroh, 2020). Terpenoid compounds have the potential as antifeedants against insects, are larvicidal, and are repellent (Masadi et al., 2018). Antifeedant compounds do not kill, but work as appetite inhibitors in larvae by causing a bitter and sharp taste that can cause larvae to have no appetite, thus causing larval death (Sasmilati et al., 2017). In addition to being antifeedant, terpenoids are neurotoxic which can damage the nervous system in insects (Amelia et al., 2017). Steroid compounds can inhibit the larval molting process (Wulandari and Ahyanti, 2018). In addition, steroids can also inhibit growth, namely changes from the larval stage to the pupa and the stage from the pupa to the adult mosquito. With the content of steroid compounds in the extract, it will affect the thickening of chitin in the larval body cell wall, so that larval growth becomes abnormal (Suari et al., 2021).

There are several phases when mosquito larvae experience poisoning caused by larvicides, including: starting from stimulation, convulsions, paralysis and ending with death. Stimulation of larvae is indicated by symptoms of behavioral changes, followed by paralysis and continues in the respiration organs and then death (Dinata, 2018). The impact of giving a mixture of extracts on the mortality of *Aedes aegypti* larvae can be seen by observing changes in morphology and behavior. Larvae that are poisoned by the extract turn transparent compared to the body color of healthy *Aedes aegypti* L. mosquito larvae. This indicates that the extract has properties as a contact toxin against *Aedes aegypti* L. mosquito larvae. Contact toxins can degrade cell membranes through their body surface to enter and damage cells, causing damage to the cuticle. Damage to the cuticle is characterized by a change in color on the larval body to become more transparent (Husnawati, 2018).

## 4. Conclusion

Based on the results of the study, a mixture of coriander (*Coriandrum sativum* L.) and garlic (*Allium sativum*) extracts is toxic to the mortality of *Aedes aegypti* L. mosquito larvae with $LC_{50}$ of 42.95 ppm. Where a compound is categorized as very toxic if the $LC_{50}$ value is less than 30 ppm, said to be toxic if the $LC_{50}$ value is at 30-1000 ppm, and said to be non-toxic if the $LC_{50}$ value is above 1000 ppm. Larvae that experience death are caused by the inability of the larvae to detoxify toxic compounds that enter and accumulate in the larval body. mixture of coriander (*Coriandrum sativum* L.) and garlic (*Allium sativum*) extracts is toxic to the mortality of *Aedes aegypti* mosquito larvae.

Based on the results of this study that the mixture of coriander (*Coriandrum sativum* L.) and garlic (*Allium sativum*) extracts is toxic to the mortality of *Aedes aegypti* mosquito larvae still on a laboratory scale, it needs to be continued with field testing. The number of mosquito larvae needs to be added more than 20 with different breeding times.

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