



## Repellent and Larvicidal Activity of Patchouli Oil and *Curcuma aeruginosa* Rhizome Extract against *Aedes aegypti* L (Diptera: Culicidae)

Sutrisnawati Mardin<sup>a\*</sup>, Achmad Ramadhan<sup>a</sup>, Nurul Afiat<sup>a</sup>, Ika Istadewi<sup>a</sup>



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Departement of Biology Education, Faculty of Teacher Training and Education, Universitas Tadulako. Jl. Soekarno Hatta Km. 9, Tondo, Palu 94148, Central Sulawesi, Indonesia. Tel./fax.: +62-813-41001765

### Abstract

The purpose of this study was to test the effectiveness of a combination of *Curcuma aeruginosa* Roxb rhizome extract and patchouli oil as a larvicide and repellent against *Aedes aegypti* mosquitoes. The method used was experimental, based on procedures recommended by the World Health Organization Pesticides Evaluation Scheme (WHOPES, 2009). The concentration ratio of *C. aeruginosa* Roxb extract and patchouli oil used was 0%:100%; 25%:75%; 50%:50%; 75%:25%; and 100%:0%. For larvicidal and repellent tests, 30 *Ae. aegypti* larvae and 30 adult female mosquitoes were used. Data were analyzed with the Shapiro-Wilk test, then analyzed with ANOVA, and continued with the posthoc test. The results showed that the combination of *C. aeruginosa* extract and patchouli oil was effective as a larvicide and repellent against *Ae. aegypti*. The most effective concentration of the combination of *C. aeruginosa* rhizome extract and patchouli oil as a larvicide on *Ae. aegypti* mosquitoes are 75%:25% and as a repellent at a combination of concentrations of 50%:50% and 75%:25%.

**Keywords:** Larvicide, Repellent, *Curcuma aeruginosa* Roxb rhizome extract, patchouli oil, *Aedes aegypti* L.

### 1. Introduction

The female *Aedes aegypti* mosquito is a major vector of dengue hemorrhagic fever (DHF) [1]. These mosquitoes are frequently active throughout the day and prefer to feed on human blood rather than animal blood [2]. The *Ae. aegypti* mosquito is found worldwide [3] and transmits the dengue virus, a member of the Arthropod-Borne Virus (Arbovirus) group, from the genus *Flavivirus* and family *Flaviviridae*, to humans through its bite. The virus causes Dengue Fever, Dengue Hemorrhagic Fever, and Dengue Shock Syndrome, and has four serotypes: DENV-1, DENV-2, DENV-3, and DENV-4 [4][5] [6].

Dengue Hemorrhagic Fever cases in Indonesia are present in 472 cities across 34 provinces. Deaths from dengue fever have been reported in 219 cities. Since 2020, 73.35% or 377 districts/cities have achieved an Incident Rate (IR) of less than 49/100,000 population. To anticipate and reduce dengue fever cases, various methods have been implemented, including eliminating the virus, isolating sufferers, preventing mosquito (vector) bites, and vector control. Dengue fever vector control is considered one of the

most effective methods to help break the chain of dengue fever transmission in Indonesia. The most common method of vector control is eradicating vector larvae using laticed [7]. In addition, vector control also utilizes natural predators and chemical methods, such as employing chemicals with larvicidal effects. Vector control also relies on the application of insecticides to both mosquito larvae and adult mosquitoes [8] [9].

Temephos is a commonly used larvicide that has been employed in public health programs with high efficacy in reducing mosquito vectors in the population [10]. However, frequent use of this pesticide can lead to resistance [11]. Indiscriminate use of insecticides can also harm non-target insect populations [12], making larvicide necessary to prevent this issue. The ideal pesticide should be effective, efficient, and ecologically friendly. In addition to being harmless to non-target animals.

Several plant-derived chemicals have been found as bioactive molecules in arthropod pests, such as poisons, repellents, antifeedants, and/or possible growth and development inhibitors [13] [14]. Essential oils are a type of plant product made up of volatile

\*Corresponding author e-mail: [watikmardin10@gmail.com](mailto:watikmardin10@gmail.com); (SutrisnawatiMardin).

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compounds found in many species [15]. Essential oils are easy to extract, environmentally safe and biodegradable, have little toxicity to animals, and work against a wide variety of insect pests [16]. Many plants, including *C. aeruginosa* and patchouli oil (*P. cablin*), possess essential oils. *C. aeruginosa*'s advantages have been thoroughly explored and utilized as an herbal medicine, and it includes a high concentration of turmerone and zingiberene [17]. As a result, in this study, a test was performed to demonstrate the effectiveness of the combination of the two plants as a larvicide and repellent.

## 2. Materials and Methods

### 2.1 Preparation of *C. aeruginosa* Roxb (black ginger rhizome) Extract

Dried black ginger rhizome extract was used to make simplicia powder. 500 g of simplicia powder was used and the maceration method was employed with 70% ethanol solvent until the solvent became clear. The maceration results were filtered, and the maceration was concentrated using a rotary vacuum evaporator at 40°C until a thick extract was obtained [18].

### 2.2 Preparation of Patchouli Plant (*P. cablin*) Samples

To assist the process of refining patchouli oil, the patchouli plants are first chopped to obtain fine patchouli plants. The samples were then dried for roughly 8 hours under sunshine to minimize the water content of the patchouli plants.

#### 2.2.1 Water Content Measurement

Patchouli plants are air-dried before the water content is assessed. The empty cup was first dried in an oven at 105°C and weighed again after cooling in a desiccator. The procedure was repeated until a constant weight was attained, then 5 grams of patchouli plant were weighed in the cup and dried in the oven at 105°C for 3 hours. Then it was chilled in a desiccator and weighed again. This procedure is repeated until a steady weight of the cup containing the sample is obtained.

#### 2.2.2 Isolation of patchouli oil using liquid steam distillation method

Dried patchouli plants weighing 3 kg are inserted in liquid steam distillation equipment. The mixture is then heated at 100 ° C for 4 hours till distillation occurs. The distillation, which contains both oil and water, is poured into the separating funnel. One layer of patchouli oil is obtained by separating the water layer at the bottom of the separator funnels.

#### 2.2.3 Release of patchouli oil from water molecules

Patchouli oil is dried by adding MgSO<sub>4</sub> to the water molecules in it. Then, the patchouli oil is filtered to remove any remaining water.

#### 2.2.4 The determination of the refractive index

At 30°C, the refractive index of patchouli oil was determined using a refractometer. The refractive index at 25°C is computed using the formula:  $R = R' - k [T - T']$ . The patchouli oil refractive index per degree correction factor is 0.00045.

#### 2.2.5 Determination of specific gravity of patchouli oil

Determination of the specific gravity of patchouli oil was carried out using a refractometer at a temperature of 30°C. The specific gravity at a temperature of 25°C is calculated using the formula ( $\rho = \rho' - k [T - T']$ ). The correction factor for the specific gravity of each degree is 0.00070 [19].

#### 2.2.6 Analysis Using a Gas Chromatography-Mass Spectrophotometer (GC-MS)

A 1 mL sample of patchouli oil was injected into a Shimadzu GC-MS-QP2010S gas chromatograph-mass spectrophotometer under the following operating conditions: The column type employed was DB-5MS, and the temperature was set at 50°C for 30 minutes before increasing the temperature by 10°C/minute to 200°C and leaving for 25 minutes. The injector temperature is 300°C, which is the same as the detector temperature. Helium is the carrier gas used. Meanwhile, electron impact (EI 70 Ev) is the sort of ionization.

### 2.3 Preparation of *Ae. aegypti* Mosquitoes

The larvae and mosquitoes used as test animals were the *Ae. aegypti* mosquito, which was obtained from the Research and Development Center (P2B2).

### 2.4 Larvicidal activities

Black ginger and patchouli oil were tested for larvicidal effectiveness against *Aedes aegypti* mosquitoes using WHO-approved techniques [20]. 1 mL of patchouli oil and black ginger extract were dissolved in 100 mL of distilled water using acetone for the experimental treatment. In a volume of 100 ml of distilled water, the concentrations of black ginger extract to patchouli oil employed are 0%:100%, 25%:75%, 50%:50%, 75%:25%, and 100%:0%. Then, by sprinkling, 25 late third instar larvae were gradually moved to the test medium. In addition, control tests were done in tandem with each replicate. After a 24-

hour exposure period, larval mortality was determined. The Abbott formula was used to compute the percentage of corrected deaths. Abbott's formula is a converter to evaluate the susceptibility status of larvae to pesticides, specifically that if larval/adult fatalities exceed 80%, the population is declared resistant, tolerant between 80-97%, and sensitive between 98%-100%. If the control larval mortality is between 5% and 20%, data correction is performed using the Abbot formula; if the control larval mortality is greater than 20%, the test must be redone. % Mortality=  $x \frac{100(\% \text{ mortality in treatment} - \% \text{ mortality in control})}{(100 - \% \text{ mortality of control})}$ .

### 2.5 Repellent activities

The effectiveness of a combination of black ginger extract and patchouli oil as a repellent was evaluated using the human bait technique [21], which mimicked the conditions of human skin where the repellent would be used. The study took place in net cages (45 cm x 30 cm x 25 cm) containing 100 female mosquitoes aged 3-4 days in hungry conditions, with relative humidity between 65% and 80%. Volunteers did not use lotions, fragrances, oils, or scented soaps on the day of the test. Only the top 25 cm<sup>2</sup> of each volunteer's arm was exposed, while the rest was covered with rubber gloves. Black ginger extract, patchouli oil, and a control were all applied to the exposed forearm area. The untreated arm served as the control. Both arms were simultaneously placed into the mosquito cage, and by gently tapping the sides of the experimental cage, the mosquitoes were activated. Each test concentration was repeated three times [22].

Table 2. Results of distillation of patchouli plants using liquid steam distillation

| Distillery | Dry weight of patchouli (Kg) | Water Volume (ml) | Time (minute) | The oil produced (ml) | Rendement (%) |
|------------|------------------------------|-------------------|---------------|-----------------------|---------------|
| 1          | 3                            | 5000              | 240           | 60                    | 1.20          |
| 2          | 3                            | 5000              | 240           | 54                    | 1.08          |
| 3          | 3                            | 5000              | 240           | 62                    | 1.24          |

The observation period lasted 5 hours. Mosquitoes landing on the hand were recorded and then shaken off before feeding. The repellency percentage was calculated using the formula: % Repellency=  $[(Ta - Tb)/Ta] \times 100$ .

Where Ta represents the number of mosquitoes in the control group and Tb is the number of mosquitoes in the treatment group.

### 2.6 Data analysis

The data obtained will be processed using statistical data processing software. The data will be analyzed using the Shapiro-Wilk test, the aim is to determine the distribution of the data. Data is said to have a normal distribution if the p-value is > 0.05. The data will be subjected to ANOVA testing, followed by a post hoc test. Results will be considered significant if the p-value is less than 0.05.

## 3. Results

### 3.1 Black Curcuma Rhizome Extraction

500g of dried *Simplicia* rhizome of black ginger (*Curcuma aeruginosa*) was weighed and then extracted for 5 days in an ethanol solvent using a maceration procedure. Table 1 summarizes the extraction results.

Table 1. Results of extracting black ginger rhizomes using ethanol solvent

| Sample          | Simplicity weights used(g) | Weight of the resulting extract(g) | Rendement (%) |
|-----------------|----------------------------|------------------------------------|---------------|
| Ethanol extract | 500                        | 65                                 | 13            |

Note: The extract yield obtained was declared good because the yield was >10%.

### 3.2 Making Patchouli Oil

Patchouli plants that have been chopped are then subjected to the process of refining patchouli oil. Patchouli weighed 3 kg and then put it into a liquid steam distillation apparatus. Then heated at a temperature of 100 °C for 3-4 hours until the distillate is obtained. The results of the distillation of patchouli plants are presented in Table 2. The results of distilling

the oil using the liquid steam distillation method for 3 repetitions produced 176 milliliters of patchouli oil during 4 hours of distillation, which shows that the amount of oil produced is proportional to the distillation time.

### 3.3 Physico-Chemical Characteristics of Patchouli Oil

Table 3 shows the results of the analysis of the physicochemical properties of patchouli oil.

Table 3. Physico-chemical characteristics of patchouli oil

| Characteristics  | Requirements (SNI-06-2385-2006) | Checkup result |
|--|---------------------------------|----------------|
| Color  | Light yellow-reddish brown      | Light yellow   |
| Index Bias (nD20)  | 1,507-1,515                     | 1,510          |
| Specific gravity (g/cm3)   | 0.950-0.975                     | 0.9480         |
| Patchouli alcohol (C <sub>15</sub> H <sub>26</sub> pH <sub>12</sub> O) (%) | Minimum 30%                     | 36.13          |

This research produced patchouli oil with a characteristic light yellow color. This is by SNI 06-2385-2006, which states that good patchouli oil is light yellow to reddish brown. Specific gravity (g/cm<sup>3</sup>) 0.9480, this value is still within the requirements of SNI-06-2385-2006.

One very important criterion for determining the purity and quality of patchouli oil is its specific gravity. The refractive index (nD20) of patchouli oil as a result of the examination was 1.510 with a specific gravity of 0.9480. The results of the Patchouli alcohol examination were above the minimum limit, namely 36.13%. Based on the results of these characteristic checks, the patchouli oil produced in this research is still within the SNI requirements.

#### 3.4 Gas chromatography-mass spectrophotometer (GC-MS) analysis

The chromatogram resulting from GC-MS analysis of the essential oil components of the patchouli plant produced through water vapor distillation is shown in Figure 1. Based on Figure 1, 33 peaks were detected with 8 dominant component peaks which are constituents of the patchouli plant essential oil. The 33 Peak (compounds) detected can be seen in Table 4 below. The patchouli alcohol compound is one

of the main ingredients that make up patchouli oil. The quality of patchouli oil is determined by patchouli alcohol (36.13%), a compound that determines its

Table 4. Gas chromatography-mass spectrophotometer (GC-MS) analysis results

| Peak# | R. Time | L. Time | F. Time | Area      | Area%  | Height  |
|-------|---------|---------|---------|-----------|--------|---------|
| 1     | 10.336  | 10.275  | 10.408  | 207438    | 0.07   | 6373    |
| 2     | 12.051  | 11.958  | 12.142  | 519585    | 0.17   | 16027   |
| 3     | 24.113  | 24.050  | 24.208  | 433566    | 0.14   | 13154   |
| 4     | 25.484  | 25.417  | 25.550  | 135396    | 0.04   | 4014    |
| 5     | 25.663  | 25.550  | 25.825  | 7494241   | 2.44   | 123967  |
| 6     | 26.639  | 26.483  | 26.758  | 10244765  | 3.34   | 246125  |
| 7     | 27.059  | 26.908  | 27.158  | 44672765  | 14.57  | 1115972 |
| 8     | 27.553  | 27.392  | 27.617  | 16921858  | 5.52   | 415876  |
| 9     | 27.644  | 27.617  | 27.733  | 1359195   | 0.44   | 42905   |
| 10    | 27.871  | 27.733  | 27.917  | 12502731  | 4.08   | 302001  |
| 11    | 27.949  | 27.917  | 28.017  | 4486198   | 1.46   | 126516  |
| 12    | 28.066  | 28.017  | 28.192  | 2976215   | 0.97   | 58792   |
| 13    | 28.256  | 28.192  | 28.350  | 391911    | 0.13   | 6259    |
| 14    | 28.415  | 28.350  | 28.450  | 327165    | 0.11   | 8888    |
| 15    | 28.503  | 28.450  | 28.575  | 781737    | 0.25   | 19701   |
| 16    | 28.675  | 28.575  | 28.750  | 7564802   | 2.47   | 162997  |
| 17    | 28.904  | 28.750  | 28.975  | 66106583  | 21.56  | 1416730 |
| 18    | 29.041  | 28.975  | 29.200  | 1647985   | 0.54   | 30378   |
| 19    | 29.347  | 29.200  | 29.408  | 662624    | 0.22   | 13351   |
| 20    | 29.558  | 29.408  | 29.658  | 310662    | 0.10   | 4469    |
| 21    | 30.669  | 30.600  | 30.725  | 272178    | 0.09   | 7413    |
| 22    | 30.792  | 30.725  | 30.900  | 1082378   | 0.35   | 23181   |
| 23    | 30.989  | 30.900  | 31.133  | 1187496   | 0.39   | 21651   |
| 24    | 31.829  | 31.733  | 31.942  | 667321    | 0.22   | 9193    |
| 25    | 32.098  | 31.942  | 32.300  | 2079351   | 0.68   | 29475   |
| 26    | 32.386  | 32.300  | 32.508  | 408286    | 0.13   | 7962    |
| 27    | 32.613  | 32.508  | 32.683  | 275865    | 0.09   | 5016    |
| 28    | 32.923  | 32.800  | 33.117  | 6116176   | 1.99   | 83738   |
| 29    | 33.175  | 33.117  | 33.217  | 624923    | 0.20   | 12102   |
| 30    | 33.420  | 33.217  | 34.367  | 110792106 | 36.13  | 1643185 |
| 31    | 33.867  | 33.825  | 33.950  | 94920     | 0.03   | 2879    |
| 32    | 34.113  | 34.000  | 34.333  | 2992152   | 0.98   | 58738   |
| 33    | 35.832  | 35.733  | 35.942  | 291518    | 0.10   | 6287    |
|       |         |         |         | 306631912 | 100.00 | 6045330 |

smell. Due to its properties as a repellent and inhibitor of insect growth, the compounds alpha-pinene (0.07%) and beta-pinene (3.34%) in patchouli oil also act as a strong antiseptic. These properties allow the use of patchouli oil to control insect populations. Apart from that, alpha guaiene (14.57%) and delta guaiene (21.56%) compounds are usually used as air fresheners in industry.

#### 3.5 Larvicidal Activity Test

In this study, the combination of *C. aeruginosa* Roxb rhizome extract and patchouli oil showed significant larvicidal activity against *Ae. aegypti* larvae (Table 5).

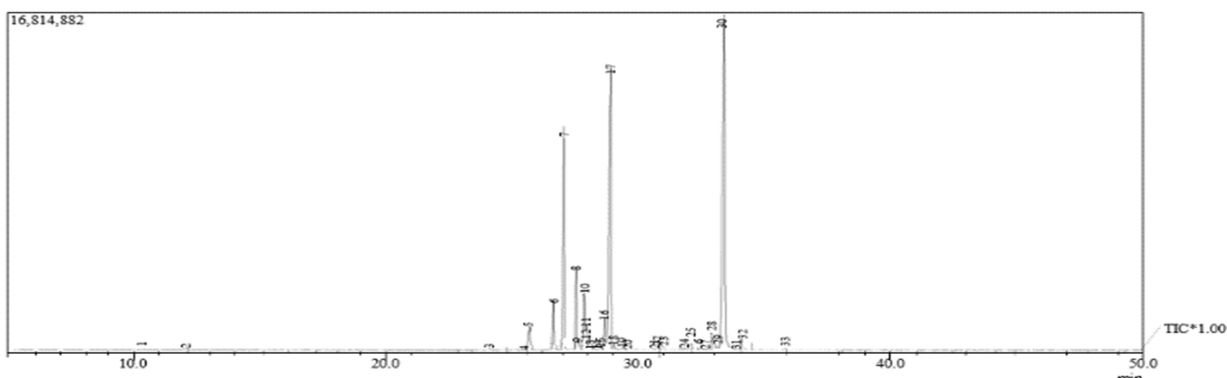


Figure 1: Chromatogram of essential oil obtained from isolation of patchouli plants

Table 5. Kruskal Wallis Test Results Treatment for mortality of *Ae. aegypti* larvae

| Treatment | Mean Rank | Significance Level |
|-----------|-----------|--------------------|
| T0        | 2.00      | 0.005              |
| T1        | 7.50      |                    |
| T2        | 10.50     |                    |
| T3        | 15.67     |                    |
| T4        | 17.50     |                    |
| T5        | 6.33      |                    |
| T6        | 17.50     |                    |

Overall, there was larval mortality but 100 percent larval mortality was found at a combination concentration of 75%:25% (Figure 2). Thus, the concentration of 75% *C. aeruginosa* rhizome extract: and 25% patchouli oil was considered the most influential on the mortality of *Ae—aegypti* larvae.

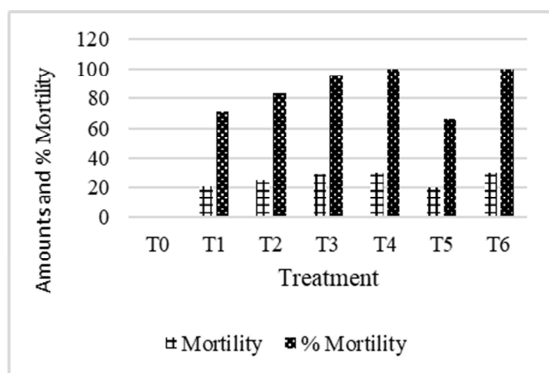


Figure 2: Amounts and Percentage of Larval Mortality *Ae. aegypti*

Note: R1= First Replication; R2= Second replication; R3= Third replication; T0= Aquadest control; T1= Black Ginger Extract (100%); T2= Comparison of black ginger extract: patchouli oil (25%:75%); T3= Comparison of black ginger extract: patchouli oil (50%:50%); T4= Comparison of black ginger extract: patchouli oil (75%:25%); T5= Patchouli Oil (100%); T6= Temephos(Control).

### 3.6 Repellant Activity Test

The results of the repellent activity test are based on the frequency of *Ae. aegypti* mosquitoes landing on the hands of positive control respondents, normal controls, and respondents' hands, which were treated/smear with patchouli oil extract (*P.cablin*Benth) and black ginger rhizome extract (*C. aeruginosa*Roxb) with various concentration ratios and time intervals. The test results can be seen in Figure 3 below:

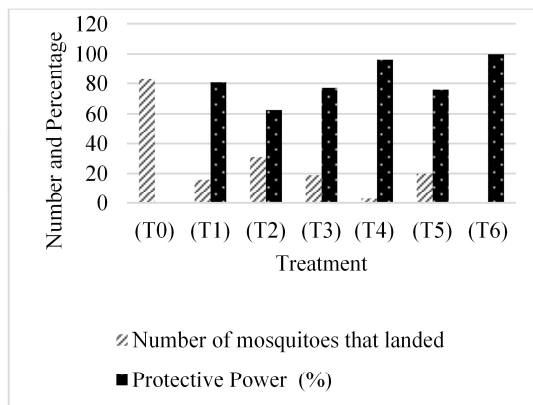


Figure 3: Protective power (Repellant) of a combination of black ginger rhizome extract and patchouli oil against the *Ae. aegypti* mosquito.

Note: T0 = Negative Control; T1 = Black ginger rhizome extract (100%); T2 = BG:PO (25%:75%); T3 = BG:PO (50%:50%); T4 = BG:PO (75%:25%); T5 = PO (100%); T6 = Commercial mosquito repellent. BG=Black Ginger; PO=Patchouli Oil.

Based on Figure3, the repellency of the combination of black ginger extract and patchouli oil (25%:75%) is 62.65%, the combination of black ginger extract and patchouli oil (50%:50%) is 77.11%, against mosquitoes *A aegypti* average was 82.20%, the combination of black ginger extract and patchouli oil (75%:25%) was 96.39, black ginger extract without the combination (100%) had a repellency of 80.72%, patchouli oil without the combination (100%) of 75.9%. The negative control (without treatment) had a repellency percentage of 0% and the positive control (treated with commercial mosquito repellent) had a repellency of 100%. These findings indicate that the combination of black ginger extract and patchouli oil has a high proportion of repellent power.

### Efficacy Test of Repellent Combination of *C.aeruginosa* Extract and Patchouli Oil Against *Ae. aegypti. L* Mosquitoes.

The results of the calculation of the repellent power of the combination of black ginger extract and patchouli oil against *Ae. aegypti* mosquitoes, using one-way ANOVA can be seen in Table 6.

Table 6. Analysis of Variance Repellency of the Combination of *C. aeruginosa* Extract and Patchouli Oil against the *Ae. aegypti* mosquito.

| Repellency     | Sum of Squares | df | Mean Square | F     | Sig.  |
|----------------|----------------|----|-------------|-------|-------|
| Between Groups | 1549.905       | 6  | 258,317     | 27,53 | ,0006 |
| Within Groups  | 131,333        | 14 | 9,381       |       |       |
| Total          | 1681.238       | 20 |             |       |       |

Based on Table 7, the results of the analysis of variance show the repellency of the combination of black ginger extract and patchouli oil with various comparisons against the *Ae. aegypti* mosquito has a significant difference (Sig. Value  $0.000 < 0.05$ ). Next, a post-hoc test was carried out to determine which groups were significantly different in this treatment. For the post-hoc test, the Bonferroni-post-hoc test was used. The results of the Bonferroni-post-hoc test are presented in the following table.

Table 7. Post-hoc Bonferroni Test Results

| Treatment | Treatment        | Mean difference | Sig. |
|-----------|------------------|-----------------|------|
| T0        | BG (100%)        | 22.333*         | .000 |
|           | BG:PO (25%:75%)  | 17.333*         | .000 |
|           | BG:PO (50%:50%)  | 21.333*         | .000 |
|           | BG:PO (75%:25%)  | 26.667*         | .000 |
|           | PO (100%)        | 21.000*         | .000 |
|           | Positive Control | 27.667*         | .000 |
| T1        | Negative Control | -22.333*        | .000 |
| T2        | BG:PO (75%:25%)  | 9.333*          | .047 |
|           | Positive Control | 10.333*         | .021 |
|           | Negative Control | -17.333*        | .000 |
| T3        | Negative Control | -21.333*        | .000 |
| T4        | BG:PO (25%:75%)  | -9.333*         | .047 |
|           | Negative Control | -26.667*        | .000 |
| T5        | Negative Control | -21.000*        | .000 |
| T6        | BG:PO (25%:75%)  | -10.333*        | .021 |
|           | Negative Control | -27.667*        | .000 |

Note: BG=Black Ginger; PO=Patchouli Oil.

Based on the results Post-hoc Bonferroni test, there was a significant difference (Sig 0.000) between the negative control (T0) and the treatment group (T1, T2, T3, T4, T5, T6) and the positive control group, treatment group 2 (T2) and treatment group 4 (T4), and positive and negative control groups, positive control group and treatment group 2 (T2).

#### 4. Discussion

The results of the larvicidal activity test obtained in this study showed that the combination of black ginger rhizome extract and patchouli oil affected the mortality of *Ae. aegypti* larvae. From the various comparisons of treatment concentrations tested, the ratio of 75% black ginger extract to 25% patchouli oil was the best for killing *Ae. aegypti* mosquito larvae with an average mortality percentage of 100%. Thus, the larvicidal activity of the combination of black ginger extract and patchouli oil with this concentration

ratio shows greater toxicity against *Ae. aegypti* larvae. The compounds contained in black ginger extract, such as alkaloids, flavonoids, and saponins, are stronger than the levels of these compounds in patchouli oil, causing acute toxicity and causing the death of *Ae. aegypti* mosquito larvae. However, at a combined concentration of 20.90%, this concentration was able to kill *Ae. aegypti* mosquito larvae. Saponins have been shown in previous investigations to exhibit membrane permeabilization and hemolytic capabilities. Saponins are soluble in aqueous and organic solvents and can be extracted. Saponins target the cuticular membrane of larvae, disrupting it and causing larval mortality [23][24].

The efficacy of *Zingiberofficinale* essential oil in repelling *Culexquinquefasciatus* mosquitoes was tested in earlier research at a dose of 4 mg/cm<sup>2</sup>. got 100% protection power up until minute 120 at a concentration of 4 mg/cm<sup>2</sup> [25]. In a previous patchouli oil study, the combination of sage oil and patchouli oil increased the total protection time for *Anopheles dirus* to an average of 270 minutes [26]. The compounds found in *P. cablin* were as follows: patchouli alcohol (33.25%), norpatchoulenol (5.72%),  $\alpha$ -bulnesene (4.11%),  $\gamma$ -bulnesene (6.12%), and gostol (6.33%). These compounds worked in concert with one another to significantly increase the larvicidal action of the species, with an LC<sub>50</sub> of 28.43  $\mu\text{g}\cdot\text{mL}^{-1}$  [27].

Patchouli alcohol compounds were shown to be the most efficient mosquito repellents, with 2,000 mg/cm<sup>2</sup> providing 100% protection for up to 280 minutes against *Aedes aegypti*, *Anopheles stephensi* Liston, and *Culexquinquefasciatus* [28]. Based on these studies, patchouli alcohol may be the main reason for the mosquito repellency of patchouli oil.

The compounds contained in these two test materials can disrupt the digestive system of *Ae. aegypti*, causing gastric poisoning in the larvae, which ultimately causes larval death [29]. The flavonoids contained in black ginger extract and patchouli oil also affect the insect's respiratory system. When flavonoids enter the insect's body, they can paralyze the respiratory nerves which can ultimately cause death [30].

Based on the results of gas chromatography-mass spectrophotometer (GC-MS) analysis, 33 peaks were detected with 8 dominant component peaks which are constituents of the essential oil of the patchouli plant. The patchouli alcohol compound is

one of the main ingredients that make up patchouli oil. The quality of patchouli oil is determined by patchouli alcohol, which is a compound that determines its smell. The results of this research produced patchouli alcohol compounds of 36.13%, alpha guaiene (14.57%) and delta guaiene (21.56%), alpha-pinene (0.07%) and beta-pinene (3.34%) compounds. According to Donnellian et al. that guaiene, patchoulene, and caryophyllene are the main components of patchouli oil. This component is thought to repel mosquitoes.

The results of the Bonferroni-post-hoc test showed that the combination of black ginger extract and patchouli oil had a larvicidal effect comparable to temephos in treatment (P4). The organophosphate pesticide Temephos has been registered with the EPA (United States Environmental Protection Agency) since 1965 [31]. Temephos has resistance to *Ae. aegypti* mosquito larvae of 0.001 ppm [32]. For this reason, as an alternative, black ginger extract and patchouli oil can be used as natural larvicides.

The results of the analysis of variance show the repellency of the combination of black ginger extract and patchouli oil with various comparisons against the *Ae. aegypti* mosquito has a significant difference (Sig. Value 0.000 < 0.05). Furthermore, based on the Bonferroni-post-hoc test, the concentration of the combination of black ginger extract and patchouli oil with a ratio of 50%:50% and 75%:25% was not significantly different from the positive control (authentic). The results of this test show that the combination of black ginger extract and patchouli oil at this concentration has the same potential as the mosquito repellent sold on the commercial mosquito repellent.

The research was also conducted [33] regarding the anti-mosquito gel formulation of patchouli essential oil (*P. cablin*) based on Na CMC and the activity test stated that at a concentration of 8% w/v it had good repellent activity against mosquitoes. Furthermore, research was also carried out by [34] on the potential of the essential oils of Patchouli leaves (*P. cablin*), Bandotan leaves (*Ageratum conyzoides* L), Kenanga flowers (*Cananga odorata* hook F & Thoms), and Rosemary leaves (*Rosmarinus officinalis* L) as a repellent against *Ae. aegypti* mosquitoes say that with a concentration of 55% v/v patchouli essential oil has the potential as a repellent against mosquitoes.

## 5. Conclusion

This study concludes that the combination of *C.*

*aeruginosa* Roxb rhizome extract and patchouli oil (*P. cablin* Benth) with concentrations of 50%:50% and 75%:25% is effective as larvicide and repellent against *Ae. aegypti* mosquitoes.

## 6. Conflicts of interest

The authors confirm that they do not have any competing interests.

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