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# The differences in the toxicity of jackfruit (*Artocarpus heterophyllus* lam.) seed extract and cocoa fruit (*Theobroma cacao* l.) extract on mortality of C*ulex* spp. mosquito larvae: Elephant foot disease vector

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## Abstract

*Culex* spp. mosquitoes are the main vectors of filariasis. According to the East Java Provincial Health Office on 2018, the total number of filariasis (elephantiasis) cases in East Java Province was 134 cases distributed across 38 districts/cities. So far, the control of *Culex* mosquitoes has been carried out using synthetic insecticides. The use of synthetic insecticides is considered effective, practical, potent, and economically advantageous. However, continuous and repeated use of synthetic insecticides can lead to environmental pollution, the death of various types of living creatures, and resistance. Synthetic insecticides are hazardous to human and domestic animal health, as well as having an impact on other non-target organisms. An alternative that can be pursued is the use of natural insecticides derived from plants. Jackfruit seeds contain secondary metabolites such as tannins, flavonoids, alkaloids, steroids, and saponins. Meanwhile, cocoa fruit husks contain tannins, saponins, alkaloids, and flavonoids. The utilization of compounds present in the extracts of jackfruit seeds (*Artocarpus heterophyllus* Lam.) and cocoa fruit husks can be used to control the population of *Culex* spp. mosquitoes, the main vectors of filariasis. The methods employed in this research include extraction, preparation of serial concentrations, colonization of test larvae, preliminary testing, and final testing. The research results reveal the toxicity or $LC_{50}$ value of jackfruit seed extract (*Artocarpus heterophyllus* Lam.) towards *Culex* spp. mosquito larvae are 430.303 ppm, while the toxicity ($LC_{50}$) value of cocoa fruit husk extract (*Theobroma cacao* L.) is 306.742 ppm. The conclusion drawn from this research is that the cocoa fruit husk extract (*Theobroma cacao* L.) is more toxic compared to the jackfruit seed extract (*Artocarpus heterophyllus* Lam.) towards *Culex* spp. mosquito larvae.

**Keywords:** Culex spp.; Jackfruit seeds; Cocoa seeds; Toxicity; Elephant Foot Disease Vector.

## 1. Introduction

Filariasis disease or elephantiasis, transmitted by *Culex* spp. mosquitoes as its vectors, is a national health problem in Indonesia that needs to be promptly addressed. The synthetic insecticides that have been used thus far have generated negative impacts that endanger human health. WHO (1995) recommends searching for native Indonesian plants to be used as biological or natural insecticides. The jackfruit tree (*Artocarpus heterophyllus* Lam.) is a perennial plant. The jackfruit's seeds are enveloped by a thick, yellowish fruit flesh. The cotyledon of the jackfruit seed (*Artocarpus heterophyllus* Lam.) is covered by a thin, brownish seed coat (Ranasinghe, *et al*., 2019). Jackfruit seeds (*Artocarpus heterophyllus* Lam.) contain secondary metabolites such as tannins, flavonoids, alkaloids, steroids, and saponins (Dwitiyanti, *et al*., 2019). Cocoa, which is a prominent plantation commodity in Indonesia, has a significant production volume. One of the major cocoa-producing regions in Indonesia is East Java, with a production amounting to 28,575 tons. Cocoa fruit husks (*Theobroma cacao* L.) are known to contain active chemicals, including tannins, saponins, alkaloids, and flavonoids (A'yun et al., 2018). Tannins and saponins act as stomach poisons by disrupting the digestive system (Mutaali & Purwani, 2015). Meanwhile, flavonoids act as contact poisons and can damage nerves (Cania &

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Setyaningrum, 2013). According to Burci et al. (2019), based on the brine shrimp lethality assay, jackfruit seed extracts exhibit toxic properties. The brine shrimp lethality assay serves as an initial test to assess the toxic nature of an extract.

*Culex* spp. mosquitoes are the main vectors of filariasis. The *Culex* genus is also known as a vector for avian malaria and arboviruses (Ahdiyah & Purwani, 2015). Two species of Culex mosquitoes that serve as filariasis vectors are *Culex quinquefasciatus* and *Culex bitaeniorrhynchus*. In Kansas, California, and Central America, the *Culex quinquefasciatus* species acts as a vector for diseases caused by the West Nile Virus, which is transmitted by crows. Various species of *Culex* mosquitoes have been discovered in several regions in Indonesia (Sholichah, 2009).

According to the East Java Provincial Health Office (2018), the total number of filariasis (elephantiasis) cases in East Java Province was 134 cases spread across 38 districts/cities. So far, insect control has commonly been carried out using synthetic insecticides. The use of synthetic insecticides is considered effective, practical, potent, and economically advantageous. However, continuous and repetitive use of synthetic insecticides can lead to environmental pollution, the death of various types of living organisms, and the development of resistance in the targeted pests (Ahdiyah & Purwani, 2015). Synthetic insecticides are harmful to human health and domestic animals, as well as influencing other non-target organisms (Sembel, 2015). An alternative approach is to use natural insecticides derived from plants, one of which involves using jackfruit seed extracts. According to Pratiwi (2014), natural insecticides are easily biodegradable, as their residues degrade quickly. Natural insecticides also have low toxicity to mammals, making them highly suitable for application in various contexts.

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## 2. Methodology

This research was conducted in the Pharmacy Laboratory of the Faculty of Pharmacy and the Toxicology Laboratory of the Biology Education Study Program, Faculty of Education and Teacher Training, University of Jember. The subject of this study was the late third instar to early fourth instar larvae of *Culex* spp. mosquitoes. Jackfruit seed extraction was performed using the maceration method. The collected and clean jackfruit seeds were thinly sliced and then dried until a constant weight was achieved. After reaching a constant weight, the jackfruit seeds were blended into a fine powder. The measured amount of jackfruit seed powder was placed in a container and 96% ethanol was added, followed by stirring to achieve homogeneity, and then left to stand. The maceration process was carried out for 3 days, with daily stirring. The macerated mixture was filtered using filter paper and a Buchner funnel to separate it from the sediment. To obtain a concentrated extract, the process of evaporation was performed using a rotary evaporator. The extract was then placed in an oven to further concentrate it. Compound detection was performed using thin-layer chromatography.

This study was conducted in the Pharmacy Laboratory, Faculty of Pharmacy, and the Toxicology Laboratory, Biology Education Program, Faculty of Education and Teacher Training, University of Jember. The subject of this research was the final-stage larvae of *Culex* spp. mosquitoes, from the late third instar to the early fourth instar. Jackfruit seed extraction was carried out using the maceration method. The collected and clean jackfruit seeds were thinly sliced and then dried until a constant weight was achieved. Once a constant weight was obtained, the jackfruit seeds were blended into a fine powder. The measured amount of jackfruit seed powder was placed into a container, and 96% ethanol was added, followed by thorough stirring to achieve homogeneity, and then left undisturbed. Maceration was performed for 3 days, with daily agitation during the maceration process. The macerated mixture was filtered using filter paper and a Buchner funnel to separate it from the sediment. To obtain a concentrated extract, the process of evaporation was conducted using a rotary evaporator. Subsequently, the extract was placed in an oven to achieve further concentration. Compound detection was carried out using thin-layer chromatography.

The extraction of cocoa fruit husk (*Theobroma cacao* L.) was performed using the maceration method. In the first stage, the grated cocoa fruit husk was dried until it reached a constant weight while covered with black cloth to reduce the oxidation reaction of polyphenolic compounds, and then it was roasted. The cocoa fruit husk was roasted for 1 hour at

a temperature of 50°C, then blended into a powder. Subsequently, 200 grams of the powder were weighed and placed into a container, and 800 ml of 97% ethanol (at a ratio of 1:4) was added. The mixture of the powder and 97% ethanol was stirred to homogeneity using a spatula and then covered. It was then subjected to extraction for 24, 36, and 48 hours. Afterward, filtration was carried out using a Buchner funnel lined with filter paper to separate the sediment and the desired liquid using a pressure of 0.06-0.08 MPa. The 97% ethanol solvent was separated from the cocoa fruit husk extract by evaporating the solvent using a rotary evaporator at a temperature of 50°C for 3 hours, at a speed of 50 rpm, until no solvent droplets remained, resulting in a liquid extract. The extract was then oven-dried at a temperature of 40°C for 4 hours to obtain a concentrated extract.

In the preliminary test, 20 larvae were used for each concentration without replication and the concentration of jackfruit seed extract that could kill 1 *Culex* spp. mosquito larva was found to be 0.03%, while the concentration that could kill 19 mosquito larvae was 1.05%. The serial concentrations used for the final test were 0.03%, 0.285%, 0.54%, 0.795%, and 1.05% for the jackfruit seed extract. As for the cocoa fruit husk extract, the concentrations used for the final test were 50 ppm, 180 ppm, 310 ppm, 440 ppm, 570 ppm, and 700 ppm. The final test was conducted with 20 test larvae for each concentration, along with both positive and negative controls, and was repeated 4 times. The exposure time for the research was 24 hours. The determination of the $LC_{50}$ value was carried out using probit analysis with the Minitab application.

## 3. Results and discussion

**Table 1** Compound Detection Results in Jackfruit Seed Extract with TLC

| No. | System Used | Result | Information |
|---|---|---|---|
| 1. | KLT<br>Stationary phase: TLC silica gel 60 $F_{254}$<br>Mobile phase: butanol: acetic acid: water (4:5:1) | There are stains with an intense yellow color | Flavonoid (+) |
| 2. | KLT<br>Stationary phase: TLC silica gel 60 F254<br>Mobile phase: N-hexane: ethyl acetate (4:1)<br>Detection: anisaldehyde-sulfate reagent | There are purple stains | Terpenoid/<br>Saponin (+) |
| 3. | KLT<br>Stationary phase: TLC silica gel 60 F254<br>Mobile phase: toluene:acetone: formic acid (6:6:1)<br>Detection: reagent $FeCl_3$ | There are stains with a black color | Polifenol/ tannin (+) |

**Table 2** Larvae Mortality of Culex spp. In Final Testing Using Jackfruit Seed Extract

| Concentration (%) | Number of Test Larvae | Mean±SD |
|---|---|---|
| 0.03 | 20 | 8.75±4.787 |
| 0.285 | 20 | 25±4.082 |
| 0.54 | 20 | 48.75±6.291 |
| 0.795 | 20 | 82.5±2.886 |
| 1.05 | 20 | 93.75±2.5 |
| Control (+) | 20 | 100±0 |
| Control (-) | 20 | 0±0 |

Table 2 shows that the higher the concentration of jackfruit seed extract, the higher the number of test larvae that also die. The lowest mortality rate of test larvae was observed at a concentration of 0.03%, with an average mortality rate of

8.75 and a standard deviation of 4.787 within a 24-hour exposure period. The highest mortality rate of test larvae was recorded at a concentration of 1.05%, with an average mortality rate of 93.75 and a standard deviation of 2.5 within a 24-hour exposure period. The treatment applied to the positive control, using 0.01% abate, was able to kill 100% of the test larvae, whereas, in the negative control, where only distilled water (aquades) was used, no mortality of test larvae was observed within the 24-hour exposure period.

**Tabel 3** $LC_{50}$ Toxicity of Jackfruit Seed Extract on Mortality of Culex spp. Mosquito Larvae.

| **Lethal Concentration ($LC_{50}$)** | **$LC_{50}$ (%)** | **Lower Limit (%)** | **Lower Limit (%)** |
|---|---|---|---|
| Jackfruit Seed Extract (*Artocapus heterophyllus*) | 430.37 | 364.5 | 489.6 |

Table 3 shows that the value of $LC_{50}$ is 430.3 with a lower limit of 364.5% and an upper limit of 489.6%. The $LC_{50}$ value signifies the concentration required to kill 50% of the test larvae within a 24-hour exposure period. The lower limit value represents the lowest concentration needed to kill 50% of the test larvae, while the upper limit value represents the highest concentration to achieve the death of 50% of the test larvae within a 24-hour exposure period.

**Table 4** Final Test Results of Cocoa Fruit Bark Extract (*Theobroma cacao* L.) on *Culex* spp. Mosquito Larvae Mortality Mortality within 24 hours of discharge

| **Concentration** | **Amount of Test Lava** | **Average** |
|---|---|---|
| 50 ppm | 20 | 1,25 |
| 180 ppm | 20 | 5,5 |
| 310 ppm | 20 | 9,75 |
| 440 ppm | 20 | 13,25 |
| 570 ppm | 20 | 16 |
| 700 ppm | 20 | 19 |
| Kontol (-) | 20 | 0 |
| Kontol (+) | 20 | 20 |

Table 4 demonstrates that the higher the concentration of cocoa fruit husk extract used, the higher the average percentage of larval mortality in the tested group. The test results show that treatment in the negative control variable using distilled water (aquades) did not cause any mortality in the test larvae after 24 hours of observation. Meanwhile, treatment in the positive control variable using 50 ppm abate concentration resulted in the death of 20 test larvae, or 100% of the total tested larvae after 24 hours of observation.

**Table 5** $LC_{50}$ Toxicity of Cocoa Fruit Bark Extract (*Theobroma cacao* L.) to Mortality of *Culex* spp. Mosquito Larvae.

| **Lethal Concentration ($LC_{50}$)** | **$LC_{50}$ (ppm)** | **Concentration (ppm)** | |
|---|---|---|---|
| | | Lower Limit | Upper Limit |
| Cocoa Bark Extract | 306.742 | 271.484 | 338.798 |

Table 5. shows the results of the final test, which were subsequently analyzed using the probit analysis method with Minitab 16 software to determine the $LC_{50}$ value of cocoa fruit husk extract (*Theobroma cacao* L.) on the mortality of *Culex* spp. mosquito larvae. The probit analysis results in Table 2 reveal that the concentration of cocoa fruit husk extract (*Theobroma cacao* L.) capable of killing 50% of *Culex* spp. mosquito larvae within a 24-hour exposure period are 306.742 ppm, with a lower limit of 271.484 ppm and an upper limit of 338.798 ppm.

The $LC_{50}$ results from the testing of jackfruit seed extract on *Culex* spp. mosquito larvae indicate that the jackfruit seed extract does not possess toxic properties on *Culex* spp. mosquito larvae. After the completion of the final test, observations were made using a microscope on the test larvae before and after treatment. Significant differences were observed in the abdominal region of the test larvae.

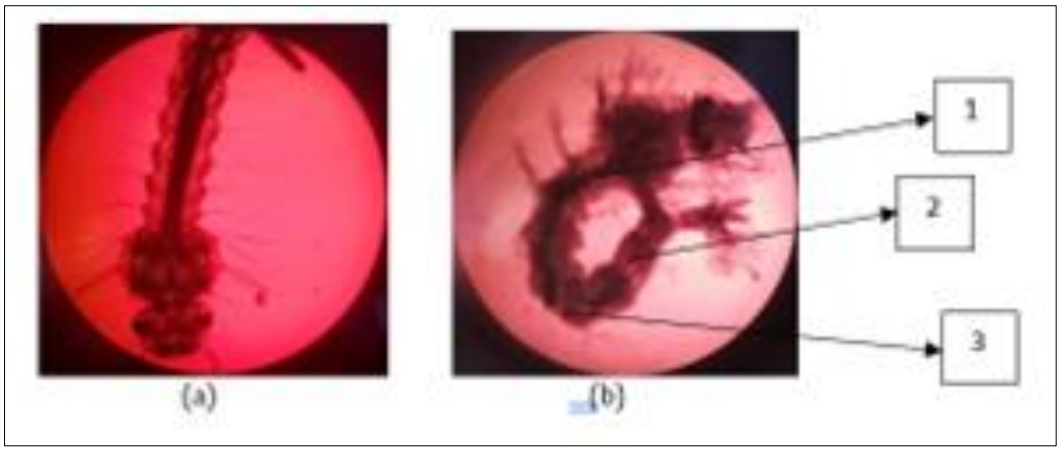


**Figure 1a** Mosquito Morphology Culex spp. Before Given Jackfruit Seed Extract**, Figure 1 b** Mosquito Morphology Culex spp. After being given Jackfruit Seed Extract

(Source: Personal Document)

Figure (b) demonstrates that jackfruit seed extract can damage the abdominal region of the test larvae. The damage to the abdominal region of the test larvae is attributed to the compounds present in the jackfruit seed extract. Compounds that are stomach toxins include saponins and tannins. According to Hidayati and Suprihatini (2020), saponin compounds can damage the abdomen of *Culex* spp. mosquito larvae, which is why in Figure 4.5, the severed abdomen of the larva is visible, as indicated by the arrow. This indicates that the compounds in the jackfruit seed extract can cause mortality in *Culex* spp. mosquito larvae. After being treated with eosin, it can be observed that the bodies of live larvae turn red, while those that are dead do not change color.

Various possibilities could occur that result in jackfruit seed extract not exhibiting toxicity to Culex spp. mosquito larvae. According to Burci et al. (2019), based on the brine shrimp lethality assay, the results indicate that jackfruit seed extract has toxic properties. The research by Burci et al. (2019) shows that the ethanol extract of jackfruit seeds has an $LC_{50}$ of 389.17 µg/mL for the brine shrimp lethality assay. According to Meyer (1982), an extract is considered toxic if the brine shrimp lethality assay results in an $LC_{50}$ value of less than 1 mg/mL (1000 µg/mL). The brine shrimp lethality assay (BSLA) is a bioassay used to test the toxic properties of phytochemical compounds in a plant extract (Waghulde, 2019). The results of the brine shrimp lethality assay conducted by Burci indicate the possibility of success if jackfruit seed extract is tested on other research subjects.

Compound detection was conducted to identify tannin, saponin, and flavonoid compounds in the jackfruit seed extract. The results of compound detection using thin-layer chromatography showed that the jackfruit seed extract contains flavonoid, saponin, and tannin compounds. These compounds have distinct characteristics. Tannins are generally toxic, as are saponins and flavonoids, although some flavonoids are non-toxic. Therefore, it is possible that the jackfruit seed extract could lead to the mortality of the research subjects.

Different results were obtained in the testing of cocoa fruit husk extract (*Theobroma cacao* L.). Based on the probit analysis results using the Minitab 16 software application, it was determined that the $LC_{50}$ value obtained is 306,742 ppm, with a lower limit of 271,484 ppm and an upper limit of 338,798 ppm. The probit analysis results indicate that a concentration of 306,742 ppm of cocoa fruit husk extract (*Theobroma cacao* L.) is required to achieve a 50% mortality rate of the test larvae within 24 hours.

The lower limit represents the lowest concentration of the extract that can cause 50% mortality in the test larvae, while the upper limit represents the highest concentration of the extract that can kill 50% of the larvae within a 24-hour exposure period. According to Tanbiyaskur et al. (2019), a plant extract is considered toxic if it has an LC50 value ≤ 1000 ppm. Therefore, the cocoa fruit husk extract (*Theobroma cacao* L.), which falls within the upper and lower range, can be considered to have toxic properties against *Culex* spp. mosquito larvae. After the completion of the final test,

observations were made using a microscope on the test larvae before and after treatment. Based on the observation results, the larval body conditions can be seen in Figure 3 as follows.

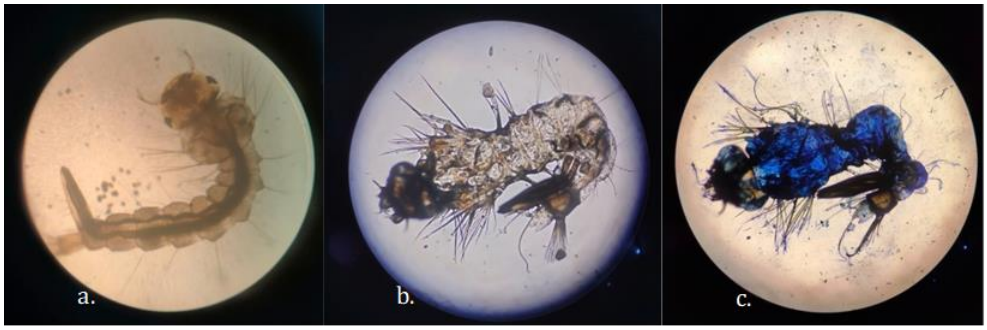


**Figure 2** (a) Morphology of *Culex* spp. Mosquito Before Treatment; (b) Morphology of *Culex* spp. Mosquito After Treatment; (c) Morphology of *Culex* spp. Mosquito After Treatment and Methylene Blue Drops.

Based on the observations under the microscope, Figure 1(a) shows a live larva with its body cells still colored and visible, and its movements are very active. Figure 1(b) depicts a dead larva that appears pale, with its body cells becoming transparent and its body organs damaged. Figure 1(c) illustrates the state of a deceased larva treated with methylene blue to confirm that the larva cells are completely dead. Dead cells are marked by the absorption of methylene blue into the larva's body, due to the presence of damaged cell organelles, causing the droplets of methylene blue to be directly absorbed into the larva's body. These observations indicate that the entire body of the mosquito larva was exposed to toxic substances from the cocoa fruit peel extract. The damage to the abdomen of the test larvae is caused by the compounds present in the cocoa fruit peel extract. Tannins are polyphenolic compounds that have an affinity for proteins, thereby disrupting the insect's digestion process due to the presence of these tannins. Saponin compounds can lead to a decrease in digestive system function (Mutaali & Purwani, 2015). Flavonoids are secondary metabolites that act as respiratory poisons. The mechanism of action of flavonoids involves entering the larva's body through the respiratory system, subsequently causing the larva to be unable to breathe and potentially damaging nerves, resulting in larval death upon exposure to flavonoid compounds (Cania & Setyaningrum, 2013). Following tests conducted on *Culex* spp. mosquito larvae, the results indicated that jackfruit seed extract is non-toxic to *Culex* spp. mosquito larvae, while cocoa fruit peel extract is toxic to *Culex* spp. mosquito larvae.

The non-toxicity of jackfruit seed extract in killing the test larvae used can be attributed to several factors. The jackfruit seed extract utilized in this study is a crude extract, which means that not only the required compounds are extracted, but other compounds are also extracted, such as glucose. The solvent employed for extraction is 96% ethanol. Ethanol is a polar solvent with a tendency to be universal, allowing for the extraction of numerous compounds, especially glucose, due to its polar nature. This aligns with the statement by Darusman et al. (2016) that polar solvents possess high polarity levels and tend to be universal as they can extract compounds with lower polarity levels as well. Another possibility is the low content of secondary metabolite compounds that are expected to kill the test larvae, thus rendering the jackfruit seed extract used ineffective in killing *Culex* spp. mosquito larvae. The effects of the extract depend on various factors, one of which is the extraction method. This aligns with Wahyuni's statement (2015) that the effects of insecticides derived from plant extracts can vary. The variation in effects can be influenced by the plant species used, mosquito species, geographical varieties, parts of the plant used, extraction methods employed, and the solvent used.

The toxicity of cocoa fruit peel extract (*Theobroma cacao* L.) to Culex spp. mosquito larvae are due to the presence of compounds within the cocoa fruit peel. According to Pappa et al. (2019), cocoa fruit peel still contains functional components such as theobromine, caffeine, and polyphenols. These compounds are phytochemical components resulting from the secondary metabolites of plants. Phytochemical components can be isolated from plants through extraction methods. The extracted compounds can then be utilized as natural larvicides to control the population of *Culex* mosquito species, in line with the findings by Pappa *et al*., (2019). The mechanism of action of larvicides in killing larvae involves the larvicide contacting the skin and then being directly applied to penetrate the insect's integument (cuticle), trachea, sensory glands, and other organs connected to the cuticle. The chemical substances present in the insecticide dissolve the fats or waxy layer on the cuticle, enabling the active ingredients within the insecticide to penetrate the insect's body. Additionally, this larvicide substance enters the larva's body through the larva's mouth (via the ingested food). Larvae die because of the toxin introduced through the ingested food, which subsequently inhibits the cellular metabolism in the mosquito's body. This inhibition prevents the conversion of nutrients from food into

energy within the cell, leading to the cells being unable to function, ultimately causing the death of the larvae (Amalia *et al*., 2021).

## 4. Conclusion

Based on the obtained research results, it can be concluded that the level of toxicity / $LC_{50}$ of jackfruit seed extract (*Artocarpus heterophyllus* Lam.) towards the mortality of *Culex* spp. mosquito larvae within a 24-hour exposure period are 430.3%, with a lower bound of 364.5% and an upper bound of 489.6%. Meanwhile, the $LC_{50}$ value of cocoa fruit peel extract (*Theobroma cacao* L.) towards the mortality of *Culex* spp. mosquito larvae are 306.742 ppm, with a lower limit of 271.484 ppm and an upper limit of 338.798 ppm. There is a difference in toxicity between jackfruit fruit extract and cocoa fruit peel extract (*Theobroma cacao* L.) towards the mortality of Culex spp. mosquito larvae. Cocoa fruit peel extract (*Theobroma cacao* L.) is more toxic than jackfruit fruit extract (*Artocarpus heterophyllus* Lam.) towards the mortality of *Culex* spp. mosquito larvae, which act as vectors for diseases such as elephantiasis.

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