POTENTIAL OF GINGER ENDOPHYTIC ACTINOBACTERIA AS *Aedes aegypti* MOSQUITO LARVICIDE

Lenni Fitri*, Nurjanah & Yekki Yasmin
Department of Biology,
Faculty of Mathematics and Natural Sciences,
Universitas Syiah Kuala, Banda Aceh,
Aceh Province, Indonesia
*Corresponding author: *lennifitri@unsyiah.ac.id*

Received: 18 April 2022; Acceptance: 19 February 2023

ABSTRACT

The Indonesian often use ginger (*Zingiber officinale*) as a spice and medicine. The plant contains potent essential oils, including Kaempferol (*flavonoids*), which are potent insect respiratory inhibitors. Since the direct extraction of bioactive compounds from ginger needs a significant amount of biomass or plant parts, bacteria are often utilized as a substitute to increase the efficiency of the process. Actinobacteria are a group of endophytic bacteria, which have been reported to have several benefits. Therefore, this study aimed to 1) assess the potential of ginger endophytic actinobacteria as *Aedes aegypti* mosquito larvicide, 2) determine the number of larvae that can change into pupa, 3) examine the morphology of mosquito larvae before and after the application of the endophytic actinobacteria. Treatments were arranged as Completely Randomized Factorial Design. The isolates used were ginger rhizome endophytic actinobacteria, namely AJ1, AJ2, AJ3, AJ4, AJ5, AJ6, and AJ7, and the inoculum volumes include 0.5 mL, 1 mL, and 1.5 mL with three recurrences each. The data obtained in this study was analyzed using the Analysis of Variance (ANOVA). The results showed that AJ7 had the highest ability in causing larvae death with an average of 27.11 individuals (90.37%). The highest number of larvae becoming pupae was found in AJ3 actinobacterial isolate. Furthermore, the larvae morphology after the treatment showed transparent white body and head, a disjointed digestive tract, and a transparent siphon. Based on these results, the isolate AJ7 has the potential to be used as a biopesticide or larvicide to reduce *Aedes aegypti* larvae. Further studies are advised on the identification, physiology, and molecular testing of ginger endophytic actinobacteria.

Keywords: Ginger, actinobacteria, secondary metabolite, *Aedes aegypti*

ABSTRAK

Masyarakat Indonesia sering menggunakan halia (*Zingiber officinale*) sebagai rempah dalam masakan dan perubatan. Tanaman ini mempunyai minyak pati iaitu Kaempferol (*flavonoids*), di mana ia berpotensi sebagai perencat respirasi dalam serangga. Memandangkan ekstrak secara langsung bahan bioaktif dari halia memerlukan jumlah biojism yang signifikan dari bahagian tanaman, bakteria kerap digunakan sebagai pengganti dalam meningkatkan

keefisienan proses. Actinobacteria merupakan kumpulan bakteria endofitik, di mana telah dilaporkan mempunyai pelbagai faedah. Oleh itu, kajian ini bermatlamatkan untuk, 1) menilai potensi halia actinobateria endofitik sebagai larvisid nyamuk *Aedes aegypti*; 2) menentukan jumlah larva yang akan berubah menjadi pupa; 3) menilai morfologi larva nyamuk sebelum dan selepas aplikasi actinobacteria endofitik. Rawatan analisis mengikut Reka Bentuk Faktor Secara Rawak Sepenuhnya telah digunakan dalam kajian ini. Pencilan yang telah digunakan dari rizom endofitik actinobacteria antaranya AJ1, AJ2, AJ3, AJ4, AJ5, AJ6, dan AJ7, serta jumlah inokulum iaitu 0.5 mL, 1 mL, and 1.5 mL dengan tiga ulangan setiap satu. Data yang diperolehi dalam kajian di analisis menggunakan Analisis Varians (ANOVA). Hasil menunjukkan AJ7 mempunyai keupayaan tertinggi dalam menyebabkan kematian larva dengan purata 27.11 individu (90.37%). Jumlah tertinggi menjadi pupa didapati pada pencilan AJ3 actinobacteria. Selain itu, morfologi larva selepas rawatan menunjukkan badan dan kepala putih jernih, salur pengkumuhan yang terasing dan sifon yang jernih. Merujuk kepada hasil kajian, pencilan AJ7 berpotensi untuk digunakan sebagai biopestisid atau larvisid bagi mengurangkan larva *Aedes aegypti*. Kajian lanjutan adalah dinasihatkan dengan merujuk kepada proses pengecaman, kajian fisiologi dan pengesahan secara molekul ke atas halia actinobacteria endofitik.

Kata kunci*:* Halia, Actinobacteria, metabolit sekunder, *Aedes aegypti*

INTRODUCTION

Ginger (*Zingiber officinale*) is a pseudo-stem plant that is widely used as medicine and spices (Tjitrosoepomo 2007). It also has several components, such as sesquiterpenoid and monoterpenoid (essential oils) that provide a practical toxic effect to kill mosquito larvae. Furthermore, one of the active ingredients of essential oil is kaempferol (flavonoid), which serves as a potent respiratory inhibitor for insects. Several studies revealed that the active compounds in ginger have the potential to be used as bio-larvicides (Suadnyani & Sudarmaja 2016). Larvicide is a class of pesticides that can kill pre-adult insects or larvae. A previous study reported that the eradication of mosquitoes using this chemical is the best method to prevent the spread of mosquitoes (Rumengan 2010).

The extraction of bioactive compounds directly from ginger requires a lot of biomass or a large portion of the plant, hence, bacteria are used as an alternative. The bacterial communities found in plants are called endophytic bacteria, and have been reported to have the ability to produce secondary metabolites that are similar to those produced by the host. Van Loon et al. (1998) stated that endophytic bacteria have been widely used as potential bio-control agents to control several types of pathogens, such as viruses, fungi, bacteria, and some insects. Several genera are also known to have the ability to produce secondary metabolites, such as antibiotics, anticancer, antifungal, antivirus, and insecticidal agents (Castillo 2002). One of the classes of endophytic bacteria is actinobacteria, such as *Streptomyces* spp. According to Ernawati (2016), *Streptomyces* spp. found in *Centella asiatica* plants can produce secondary metabolite compounds that are similar to acarbose. Furthermore, acarbose has been reported to function as an *α-glucosidase* inhibitor, which can reduce blood glucose levels. These metabolite compounds were also obtained in *C. asiatica* plants (De Sousa et al. 2004).

Rahayu et al. (2019) stated that there are seven types of ginger endophytic actinobacteria isolates. These microbes were used as biological controls because they are environmentally friendly and have larvicidal effects on *Aedes aegypti* mosquito. Suadnyani and Sudarmaja (2016) revealed that the endophytic actinobacteria of ginger essence were used

as larvicide because the plant is an alternative, which is easily obtainable, highly effective, and environmentally friendly. Kesetyaningsih and Suryani (2006) reported that *Culex quinquefasciatus* larvae exposed to *Bacillus thuringiensis* bacteria had decreased movement until they died. Fitri and Yasmin (2014) also revealed that chitinolytic bacteria in local isolates had toxic effects on *Aedes aegypti* mosquito larvae. Based on findings, there is no study on the potential of Ginger endophytic actinobacteria as larvicides. Therefore, this study aims to determine the potential of ginger endophytic actinobacteria (*Zingiber officinale*) as *Aedes aegypti* mosquito larvicides.

MATERIALS AND METHODS

Aedes aegypti Mosquito Larvae Breeding

Aedes aegypti mosquito larvae were obtained using an ovitrap, a small black tin which has a plank to store eggs. Furthermore, cans were filled with 10 mL water and placed in the corners of the house for five days. The water in the ovitrap was then placed into a larval breeding container with a dimension of 30 cm x 30 cm. The larvae were reared until they reached the pupae stage of development. During the breeding, the samples were fed with 1g of yeast tape, which was placed in a container, and the water was replaced afresh every two days. In the pupa period, the breeding containers were placed into imago cages. The male imago was fed with a mixture of 10% sugar solution, while females were supplied with the blood of mice. Blood was collected by placing the shaved-fur mice clamped into a mosquito breeding container for 15 minutes (Yasmin & Fitri 2010).

After the blood was taken, the rat and the sugar solution were removed. Subsequently, wetted water container with cone shape filter in it was placed into the breeding cage. This filter paper served as the site for laying eggs by the female mosquitoes. The eggs were then set back into the larval breeding container, left to hatch, and reared until they became third-instar larvae. Some of the larvae were taken for treatment, while others were used for further breeding (Yasmin & Fitri 2010).

Endophytic Actinobacteria Rejuvenation

The bacterial samples used were isolates of endophytic actinobacterial, namely AJ1, AJ2, AJ3, AJ4, AJ5, AJ6, and AJ7 obtained from the results of Rahayu et al. (2019) (Figure 1), on Emprit ginger plants rejuvenated using ISP2 media. Furthermore, the isolates were taken using a 14.7 cm long skewer and then etched onto the ISP2 medium. Subsequently, the Petri dish was wrapped and incubated for seven days at room temperature.

Based on the results of Rahayu et al. (2019), actinobacteria were characterized based on their shape, colony surface, as well as substrate, dissolved, and air mycelium color. Furthermore, ginger-derived endophytic actinobacterial isolates exhibited a variety of morphologies. Mycelium in AJ1 was an abnormal tint, and the colony's convex surface was covered with dissolved orange pigment. AJ2 has an aerial celium coloration, a convex shape, and its brown substrate lacked soluble pigments. The mycelium material of AJ3 isolate was brown, convex, has a grey aerial mycelium, and lacked soluble pigments. Isolates AJ4, AJ5, and AJ6 were convex, had no soluble pigment, and had yellow substrate mycelium and white air mycelium. In AJ7 isolates, the colony surface was convex, the air mycelium was grey, the substrate mycelium was green, and there were no soluble pigments, as described in Table 1.

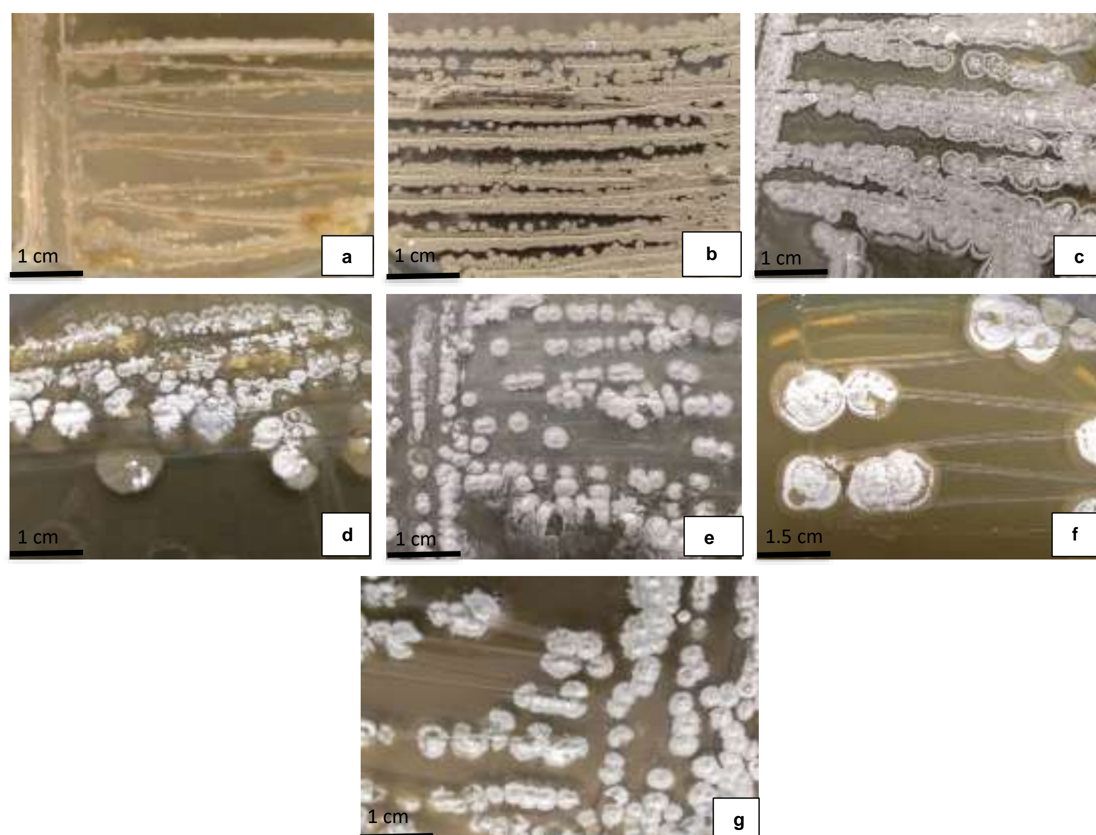

Figure 1. Ginger endophytic actinobacterial morphology, (a) AJ1, (b) AJ2, (c) AJ3, (d) AJ4, (e) AJ5, (f) AJ6, and (g) AJ7

(Source: Rahayu et al. 2019)

Table 1. Morphological characteristics of endophytic ginger actinobacteria on ISP 2 media

Isolate Code	Color of Aerial Mycelium	Color of Substrate Mycelium	The Color of the Dissolved Pigment	Surface of the Colony
AJ1	Beige	Beige	Orange	Convex
AJ2	Chocolate	Chocolate	-	Convex
AJ3	Gray	Chocolate	-	Convex
AJ4	White	Yellow	-	Convex
AJ5	White	Yellow	-	Convex
AJ6	White	Yellow	-	Convex
AJ7	Gray	Green	-	Convex

Note: AJ1 = Endophytic Actinobacteria Ginger isolate 1; AJ2 = Endophytic Actinobacteria Ginger isolate 2; AJ3 = Endophytic Actinobacteria Ginger isolate 3; AJ4 = Endophytic Actinobacteria Ginger isolate 4; AJ5 = Endophytic Actinobacteria Ginger isolate 5; AJ6 = Endophytic Actinobacteria Ginger isolate 6; AJ7 = Endophytic Actinobacteria Ginger isolates 7

Production of Supernatant

Actinobacteria isolates were inoculated through 7 holes previously made on 250 mL of ISP2 liquid medium using a plastic straw with a diameter of 8 mm. The samples were then incubated for ten days in the GallenKamp orbital shaker at 100 rpm and room temperature. Subsequently, it was filtered using filter paper to separate actinobacterial biomass from the ISP2 medium. The

filtering results were then transferred into a 50 mL centrifuge tube for centrifugation at 6000 rpm and 4°C for 30 minutes, followed by the separation of the components. The supernatant obtained was poured to the limit between the supernatant and the pellet in the Erlenmeyer flask. It was then taken for use in biological tests (Pujiati 2012).

Biological Test

A total of 150 mL sterile distilled water and 30 *Aedes aegypti* larvae were poured into a 14 cm diameter Petri dish that has been sterilized using an autoclave. Furthermore, 0.5 mL, 1 mL, and 1.5 mL of supernatant from isolates AJ1 to AJ7 was added to the Petri dish at each treatment. Dead larvae were then counted and observed using a microscope to examine the shape of the head, abdomen, and siphon before and after the treatments for seven days until the samples died.

Data Statistical Analysis

The software SPSS version 20.0 was used to analysis thedata on of mortality rate of *Aedes aegypti* larvae and the number of samples that are capable of developing into pupae. The test used in this study was the Analysis of Variance (ANOVA), and it was continued with a Turkey follow-up test with a 95% confidence interval in the presence of an effect. Meanwhile, the morphology of the larvae was examined in a descriptive manner and presented as an image.

RESULTS AND DISCUSSION

Aedes aegypti Larvae Mortality

Based on the results of the analysis of variants, the volume of actinobacterial inoculum did not significantly affect the mortality of *Aedes aegypti* larvae ($P>0.05$), while actinobacterial isolates showed significant differences in causing the death of the samples ($P>0.05$). Furthermore, the interaction between the isolates and actinobacterial inoculum volume had no significant effect in causing the death of *Aedes aegypti* larvae ($P>0.05$).

Table 2. The effect of inoculum volume on the mean mortality of *Aedes aegypti* larvae

Inoculum Volume (mL)	Mean Mortality of *Aedes aegypti* Larvae (Individual)
0.5	23.57 ± 0.723^{a}
1	24.48 ± 0.723^{a}
1.5	24.86 ± 0.723^{a}

Note: The same superscript letters show no significant difference. Mean±SD at the same column with different superscripts are significantly different ($P<0.05$)

Table 2 shows that the inoculum volume had no effect on the death of *Aedes aegypti* larvae, where the mean mortality ranged from 23.57 to 24.86 individuals. This was because several secondary metabolites released by actinobacteria into the medium had the same effect. Moreover, the volume of inoculum given in this study was minimal. This finding is consistent with Syukur et al. 2018 that the results obtained were not significantly different due to the concentrations used.

Table 3. Mean number of *Aedes aegypti* larvae mortality after the application of ginger endophytic actinobacteria

Actinobacterial Isolate Code	**Mean Mortality of *Aedes aegypti* Larvae (Individual)**
Control	0
AJ1	23.56 ± 3.25^{abc}
AJ2	26.44 ± 3.36^{bc}
AJ3	19.67 ± 3.81^{a}
AJ4	25.44 ± 2.00^{bc}
AJ5	25.89 ± 4.14^{bc}
AJ6	22.00 ± 2.92^{ab}
AJ7	27.11 ± 3.89^{c}

Note: Different letter superscripts (a, b, and c) show that there are significant differences. Mean±SD at the same column with different superscripts are significantly different ($P<0.05$)

Based on Table 3, the death of *Aedes aegypti* larvae with the administration of AJ3 actinobacterial isolate code has a significant difference from AJ2, AJ4, and AJ5, but was not different from AJ6 and AJ1. Furthermore, AJ7 was significantly different from AJ3 and AJ6, but it did not have a real difference with AJ1, AJ4, AJ5, and AJ2. This finding indicates AJ3 has a low larvicidal ability, while AJ7 has a high potential to be used as *Aedes aegypti* larvicide. The variation was suspected to be caused by the production of different secondary metabolites by each type of actinobacteria found in ginger plants. Each actinobacterium also has a distinct ability to absorb nutrients from the host plant. This difference can cause the production of varying secondary by these microbes. According to Kumala et al. (2006), different levels of bacterial toxicity on larvae was influenced by the types of bacteria and enzymes or secondary metabolites produced. Radji (2005) stated that the ability of endophytic bacteria to produce secondary metabolites is related to the physiology of their host plants.

Observations made until the 7th day revealed that the lowest mean number of *Aedes aegypti* larvae deaths was obtained in AJ3 isolates with a percentage of 65.57% or 19.67 individuals) Meanwhile, AJ7 caused the highest larval mortality compared to others, namely 90.37% or 27.11 individuals. *Aedes aegypti* larvae treated with AJ7 actinobacterial isolates had the highest number of deaths on the first day of observation. On the second and third days, the mortality began to decrease, and all samples died on the fourth day. It was suggested that AJ7 has higher secondary metabolite content, resulting in increased mortality of *Aedes aegypti* larvae compared to other isolates. Suadnyani and Sudarmaja (2016) stated that ginger rhizome extract had a toxic effect on *Aedes aegypti* larvae with a percentage of 96%. This indicates that actinobacteria can produce the same compounds similar to those in ginger. Holt (1994) also reported that these microbes have secondary metabolites that were similar to their host plants. Strobel and Daisy (2003) revealed that endophytic microbes, can produce various types of secondary metabolites with different functions possessed by the plants.

Larvae Became Pupa

The analysis of variant results showed that the treatment of actinobacterial inoculum volume had no significant effect on the formation of *Aedes aegypti* pupae ($P>0.05$). Furthermore, the actinobacterial isolates were known to significantly influence the development of pupae ($P<0.05$). The interaction between actinobacterial isolates and inoculum volume administration also had no effect on the number of *Aedes aegypti* larvae that reached this stage.

Table 4. Effect of inoculum volume on the mean ability of *Aedes aegypti* larvae changed into pupa

Inoculum Volume (mL)	**Mean Number of Larvae Changed into Pupa (Individual)**
0.5	5.14±1.912^{a}
1	5.52±1.912^{a}
1.5	6.43±1.912^{a}

Note: The same superscript letters show no significant difference. Mean±SD at the same column with different superscripts are significantly different ($P<0.05$)

Based on Table 4, the number of larvae that are capable of forming pupae was not affected by the inoculum volume. From all the inoculum volume treatments given, the mean development ranged from 5.14 to 6.43 individuals. This was because the number of secondary metabolites released by actinobacteria into the media gave the same effect. Furthermore, the volume of inoculum given was minimal. This is consistent with Syukur et al. (2018) that the differences in results were not significantly different due to the use of low concentrations.

Table 5. Mean number of *Aedes aegypti* larvae that changed into pupa after the ap plication of ginger endophytic actinobacteria

Actinobacterial Isolates Code	**Mean Number of Larvae Changed into Pupa (Individual)**
Control	30
AJ1	6.44 ±3.25^{abc}
AJ2	3.56±3.36^{bc}
AJ3	10.33±3.81^{c}
AJ4	4.56±2.01^{ab}
AJ5	4.11 ±4.14^{ab}
AJ6	8.00±2.92^{bc}
AJ7	2.89±3.89^{a}

Note: Different letter superscripts (a,b,c) show that there are significant differences. Mean±SD at the same column with different superscripts are significantly different ($P<0.05$).

Table 5 shows that the difference in actinobacterial isolates applied to *Aedes aegypti* larvae created gaps in the number of larvae with the ability to develop into pupae. Actinobacterial isolates of AJ3 were significantly different from AJ2, AJ4, AJ5, and AJ7, but was not different from AJ1 and AJ6. Furthermore, AJ7 actinobacterial isolates were significantly different from AJ3 and AJ6, but did not vary significantly from AJ1, AJ2, AJ4, and AJ5. This finding indicates that AJ3 had the highest potential to change *Aedes aegypti* larvae into pupae with a percentage of 34.43% or 10.33 individuals. The results also showed that AJ7 had a low potential with a percentage of 9.63% or 2.89 individuals.

The difference in the amount of *Aedes aegypti* larvae that can develop into pupae was presumably caused by the influence of secondary metabolites produced by the actinobacteria. Increased production of these compounds led to a reduction in the larvae that are capable of turning into a pupa in AJ7 due to an increase in exposure. Meanwhile, AJ3 was assumed to produce secondary metabolites that were weak in influencing the growth of the sample, thereby increasing the ability to change into pupa. The failure of pupa formation was suspected because actinobacteria had degraded the larval exoskeleton, and this disrupted the transformation

process. This finding is consistent with Pujiyanto et al. (2008) that bacteria affected the death of larvae as well as their development into pupae. Fitri and Yasmin (2014) revealed that the administration of bacteria to larvae influenced the changing process. Santi and Purnama (2016), also reported that ginger essential oil was an effective compound against *Aedes aegypti* mosquito because it inhibited larval development and has high bioactive components.

Aedes aegypti **Larvae Morphology**

Aedes aegypti mosquito larvae are similar to insects that consist of a head, thorax, and abdomen. Larvae have chiffon and comb teeth in the 8th segment of their body. The samples used in this study were *Aedes aegypti* in the third instar larvae phase. Furthermore, this stage of development was selected because the organisms are easily exposed to actinobacteria, more active in moving and looking for food, and their body structure was perfect compared to the second instar larvae. Based on observation, the second instar larvae actively move to search for food, but their breathing apparatus has not fully formed. The fourth instar has higher body endurance and less active foraging. According to Sanjaya and Safaria (2006), the more mature the larvae, the higher the level of endurance due to the presence of thick skin.

Before the treatment, the larvae appeared to be active, and microscope observation showed that their body seemed black and fresh with the head, siphon (breathing apparatus), and digestive tract appearing normal, as shown in Figure 2. After the administration of actinobacteria, *Aedes aegypti* larvae started to move slowly, and the observation using a microscope indicates differences in the morphology of the body, head, siphon, and digestive tract. This finding is consistent with Yasmin and Fitri (2010), reporting that the movement of the samples decreased after the administration of microorganisms as larvicides.

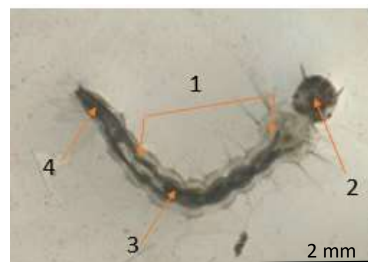

Figure 2. Morphology of *Aedes aegypti* larvae before treatment. 1.Abdomen; 2. Head; 3. Digestive tract; 4. Siphon

Based on the microscopic observation after the actinobacteria treatment, the body, head, and siphon of *Aedes aegypti* larvae became transparent white and the digestive tract was disjointed. The same morphological traits were also observed in all of the larvae given actinobacterial isolates. Some of the isolates can harm the digestive system due to their poisonous nature, while others affect the exoskeletons, as shown in Figure 3. It was assumed that actinobacteria produced a toxin to kill *Aedes aegypti* larvae. The toxin produced was stomach and strong respiratory poison, hence, it can kill the samples and change their morphology. The actinobacteria produced secondary metabolites of flavonoids, which are the same as their host plant. Santos et al. (2019) assumed that the metabolites of flavonoids and saponins were produced by these microbes, causing stomach and digestive poisons.

Holt (1994) revealed that actinobacteria have the same product of metabolism as the host plant. Suadnyani and Sudarmaja (2016) reported that the secondary metabolites contained

in ginger have the potential as bio-larvacides. Furthermore, Lumowa and Nurbayah (2017) stated that flavonoids (kaempferol) are secondary metabolites of ginger that act as potent respiratory inhibitors for insects and can block the respiratory organs in insects. According to Asfi et al. (2015), gingerol is an active compound in the plant that disrupts the physiological processes of larvae.

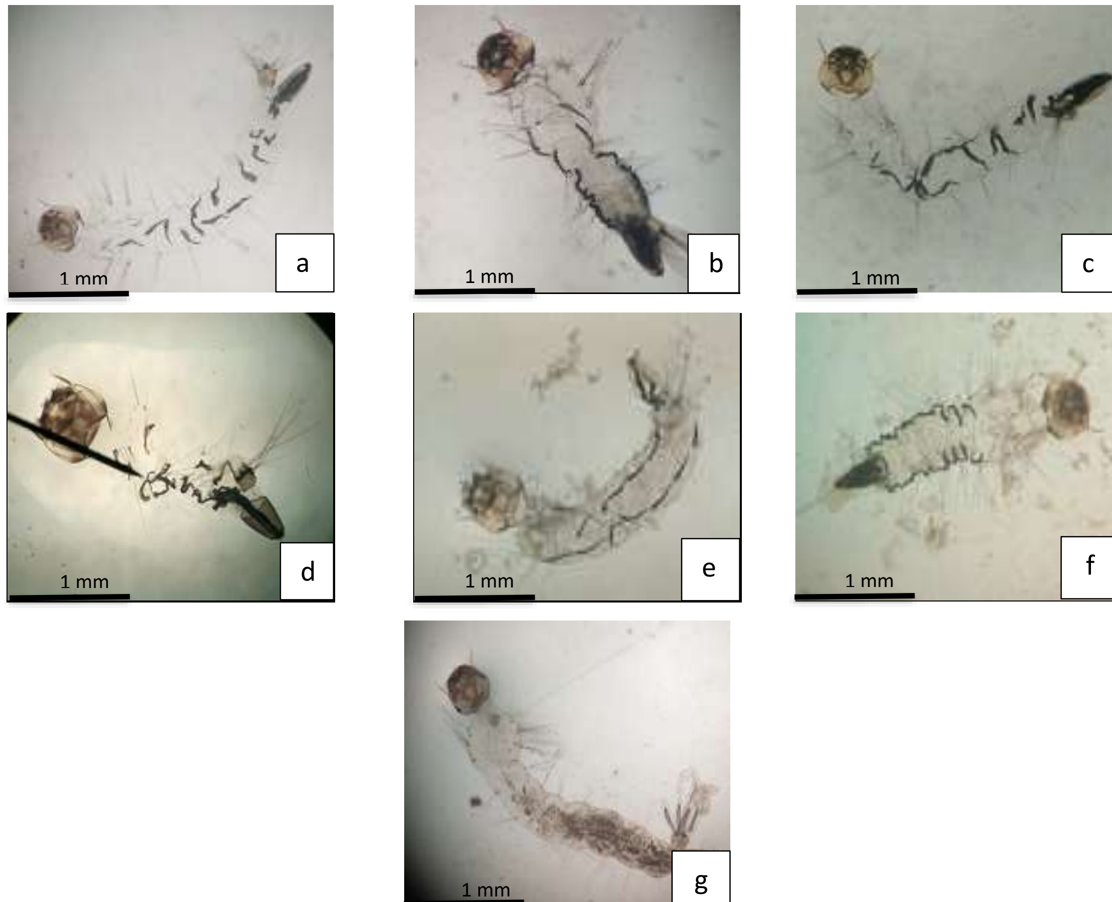

Figure 3. Morphology of *Aedes aegypti* larvae after treatment. (a.) AJ1, (b). AJ2. (c). AJ3, (d). AJ4, (e.) AJ5, (f.) AJ6, and (g.) AJ7 (2x magnification stereo microscope)

Based on Figures 3a and d, the decay of *Aedes aegypti* larvae occurred in the exoskeleton with the administration of AJ1 and AJ4 isolates. This was because the secondary metabolites produced by actinobacteria can degrade chitin, which is a fundamental structure of the larvae exoskeleton. According to Borror et al. (1996), chitin serves as a body protector and the primary mechanism in limiting water loss through the body wall. This finding is consistent with Yunita et al. (2009) that the body walls of insects readily absorb toxic substances. Furthermore, Nasran et al. (2003) stated that bacteria can produce chitinase enzymes that can degrade chitin. Based on Figure 3, the b (AJ2), c (AJ3), e (AJ5), and f (AJ6) samples suffered digestive damage. This was because secondary metabolites produced by actinobacteria were stomach poisons. Arrizqiyani et al. (2019) stated that stomach poison can kill insects by going into the digestive tract through the consumption of food. Additionally, Ahdiyah and Purwani (2015) revealed that flavonoids and saponins can degrade digestive cell membranes as well as change the body color of larvae to transparent. Aziz et al. (2021), in his study, deformed larvae with darker bellies were the most frequently encountered deformities.

CONCLUSION

Based on the results, isolate AJ7 has the potential to be used as a larvicide or biopesticide to control *Aedes aegypti* larvae. However, further studies are needed on the identification, physiology, and molecular tests of ginger endophytic actinobacteria as well as the appropriate concentration for their usage as mosquito larvae control products.

ACKNOWLEDGEMENTS

The authors are grateful to the Microbiology division of the Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Aceh, Indonesia for the facilities and support given in this study.

AUTHORS DECLARATIONS

Funding Statement
This equipment used private funding. Actinobacteria studies are currently supported by research results from Rahayu et al, Universitas Syiah Kuala

Conflict of Interest
The authors declare that they have no conflict of interest.

Ethics Declarations
No ethical issue required for this research

Data Availability Statement
My manuscript has data related to Rahayu et al. research on actinobacterial morphology data

Authors' Contributions
LF and NJ conceived this research and designed experiments; LF, NJ, and YY participated in the design and interpretation of the data; LF, NJ, and YY performed experiments and analysis; LF, NJ, and YY wrote the paper and participated in the revisions of it. All authors read and approved the final manuscript. All authors read and approved the final manuscript

REFERENCES

Ahdiyah, I. & Purwani, K.I. 2015. Effect of Mangkokan (*Nothopanax scutellarium*) leaf extract as larvasida mosquito *Culex* sp. *Journal of Science and Art* 2(4): 2337-3520.

Arrizqiyani, T., Hidana, R. & Agesti, F.F. 2019. Lotion of rind durian (*Durio Zibethinus* Murr's) as natural repellent to *Aedes aegypti* Mosquitoes. *Advances in Health Sciences Research* 26: 12-14.

Asfi, S., Rahayu, Y. & Yuliani. 2015. Bioactivity test of red ginger (*Zingiber officinale*) rhizome filtrate on mortality and inhibition of feeding activity of *Plutella xylostella* larvae in vitro. *Lanterns* 4(1): 50–55.

Aziz, E.I.A., Rahim, N.A.A., Raduan, S.Z. & Safii, R. 2021. A Preliminary study on larvicidal efficacy of *Piper nigrum* L. (Piperaceae) extracts against dengue vector, *Aedes Albopictus* (Diptera: Culicidae). *Serangga* 26(1): 80-94

Borror, D.J., Triplehorn, C.A. & Johnson. 1996. *An Introduction to The Study of Insect*. Yogyakarta: UGM Press.

Castillo. 2002. Munumbicins, wide-spectrum antibiotics produced by *Streptomyces* NRRL 30562, endophytic on *Kennedia nigriscans*. *Microbiology* 148: 2675–2685.

De Sousa, E., Zanatta, L., Seifriz, I., Creczynski-Pasa, T.B., Pizzolatti, M.G., Szpoganicz, B. & Silva, F.R.M.B. 2004. Hypoglycemic effect and antioxidant potential of kaempferol-3,7-O-(alpha)-dirhamnoside from *Bauhinia forficata* leaves. *Journal of Natural Products* 67(5): 829-832.

Ernawati, M., Solihin, D.D. & Lestari, Y. 2016. Analysis of Endophytic endophytic actinobacterial community (*Centella asiatica* L. Urban) based on 16s rRNA gene and its potential as antidiabetic. *International Journal of Pharmacy and Pharmaceutical Sciences* 8(2): 292-297.

Fitri, L. & Yasmin, Y. 2014.The potential of local chitinolitic bacteria isolates as larvacide of *Aedes aegypti* L. *Makara Journal of Health Research* 18(1): 1-6.

Holt. 1994. *Bergey's Manual of Determinative Bacteriology*. 9th Edition. Baltimore: Williams Wilkins.

Kesetyaningsih, T.R. & Suryani, L. 2006. Effectiveness of *Bacillus thuringiensis* bacteria to *Culex quinquefasciatus* mosquito larvae in some kinds of living media. *Mutiara Medical Journal* 6(1): 27-31.

Kumala, S., Utji, R., Sudarmono, P. & Kardono, L.B.S. 2006. Isolation of endophytic fungi from *Brucea javanica* L (Merr). And cytotoxic evaluation of their n-butanol extract from fermentation broth. *Pakistan Journal of Biological Sciences* 9: 825-832.

Lumowa, S.V.T. & Nurbayah. 2017. Combination of Javanese chili extract (*Piper retrofractum* Vahl.) and red ginger (*Zingiber officinale* var. *amarum*) as a vegetable insecticide on mustard (*Brassica juncea* L.). *Bioeducation* 10(1): 65-70.

Nasran, S., Afriyani., F. & Indriat, N. 2003. Production of chitinase and chitin deacetylase from *Vibrio harveyi*. *Indonesian Fisheries Research Journal* 9(5): 33-38.

Pujiati. 2012. Isolation of actinomycetes from garden soils as microbiological practicum instructions materials. *Journal of Florea* 1(2): 42-46.

Pujiyanto, S., Kusdiyantini, E. & Hadi, M. 2008. Isolation and selection of local chitinolytic bacteria isolates potential for controlling *Aedes aegypti* L. mosquito larvae. *Biodiversitas* 9(1): 5-8.

Radji, M. 2005.The role of biotechnology and endophytic microbes in the development of herbal medicines. *Pharmacy Science Magazine* 2(3): 113-126.

Rahayu, S., Fitri, L. & Ismail, Y.S. 2019. Short communication: Endophytic actinobacteria isolated from ginger (*Zingiber officinale*) and its potential as a pancreatic lipase inhibitor and its toxicity. *Biodiversitas* 20(5): 1312-1317.

Rumengan, A.P. 2010. Mosquito larvicide test (*Aedes aegypti*) from ascidian (*Didemnum molle*). *Journal of Fisheries and Maritime Affairs* 6(2): 83-86.

Sanjaya, Y. & Safaria, T. 2006. Toxicity of spiders *Nephila* sp. in larva *Aedes aegypti* L. *Biodiversitas* 7(2): 191-194.

Santi, H.L. & Purnama, S.G. 2016. Pathogenicity test of *Bacillus thuringiensis* var. *israelensis* against *Aedes* sp. as a biocontrol of the causes of dengue hemorrhagic fever in Denpasar year 2014. *Archive of Community Health* 3(1): 14-23.

Santos, L.L., Brandao. L.B., Martins, R.L., Rabelo, E.M., Rodrigues, A.B.L., Araujo, C.M.C.V., Sobral, T.F., Galardo, A.K.R. & Ameida, S.S.M.S. 2019. Evaluation of the larvicidal potential of the essential oil *Pogostemon cablin* (Blanco) Benth in the control of *Aedes aegypti*. *Journal Pharmaceuticals (Basel)* 12(2): 53**.**

Strobel, G. & Daisy, B. 2003. Bioprospecting for microbial endophytes and their natural product. *Microbiology and Molecular Biology Reviews* 67: 491-502.

Suadnyani, A.A.I. & Sudarmaja, I.M. 2016. Effect of ethanol extract of red ginger rhizome (*Zingiber officinale* rosc) on the death of *Aedes aegypti* mosquito larvae. *Medika E-Journal* 5(8): 1-5.

Syukur, K.M.Y., Anwar, C., Dalilah., Salni. & Novrikasari. 2018 The efficacy of red ginger fraction (*Zingiber officinale roscoe* var. rubrum) as insecticidal *Aedes aegypti*. *Bioscientia Medicina* 2(2): 31-41.

Tjitrosoepomo, G. 2007. *Plant Taxonomy (Spermatophyta).* Yogyakarta: UGM Press.

Van Loon, L.C., Baker, P.A.H.M & Pieterse, C.M.J. 1998. Systemic resistance induced by *rhizosphere* bacteria. *Annual Review of Plant pathology* 36: 453-483.

Yasmin, Y. & L. Fitri. 2010. The Effect of *Metharrizium anisopliae* fungi on mortality of *Aedes aegypti* L. Larvae. *Natural* 10(1): 32-35.

Yunita, E.A., Suprapti, N.H. & Hidayat, J.W. 2009. Effect of Teklan leaf extract (*Eupatorium riparium*) on mortality and development of *Aedes aegypti* larvae. *Journal of Biomas* 11(1): 11-17.