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Morphological and ultrastructure alteration of larva *Culex pipiens* exposed from cassava juice extract

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Abstract. *Nindatu M, Jotlely H, Anaktototy Y, Lakoan MR. 2025. Morphological and ultrastructure alteration of larva* Culex pipiens *exposed from cassava juice extract. Biodiversitas 26: 306-314. Culex pipiens* Linnaeus, 1758 is a mosquito commonly often found in residential areas, particularly in puddles of water and storage tanks. This mosquito serves as a vector for lymphatic filariasis in humans, also known as elephantiasis. To eliminate the transmission of elephantiasis, control strategies one are implemented targeting the growth of *C. pipiens* larval population. Cassava tubers have the potential as a biolarvicide to control the population and growth of *C. pipiens* mosquito larvae. This research was aimed to determine the phytochemical content and biolarvicide effectiveness of juice extract cassava (*Manihot esculenta* Crantz), which may cause damage to the head capsule, thorax, and abdomen of *C. pipiens* larvae. Phytochemical test on the cassava tubers juice extract (*M. esculenta*) revealed relatively high levels of flavonoids, ranging from 3 to 3.5 mg. The results of identification using a keyence 3D microscope show the fourth instar larvae of *C. pipiens* in the control group. The body color is pale, and the head and tail are brownish. Squeezed biolarvicide cassava tubers (*M. esculenta*) doses of 0.5, 1.5, and 3.5% caused larval mortality above 90% at the 6th hour for 24 hours. Further characterization using Scanning Electron Microscopy (SEM) indicated that a dosage of 3.5% applied for 24 hours caused significant damage to the head capsule, with the exoskeleton connecting the head and thorax nearly detaching. Higher concentration of cassava juice extract resulted in increased body damage to *C. pipiens* larvae. It can be concluded that the flavonoid compounds in cassava juice extract in this study can effectively damage the bodies of fourth instar *C. pipiens* larvae, as evidenced by the SEM results.

Keywords: Biolarvicide, cassava tubers, *Culex pipiens*, scanning electron microscopy

INTRODUCTION

Mosquitoes are a group of insects that are often found in the environment around residential areas. Stagnant water found around the house is a potential habitat for mosquitoes. The development of mosquito populations in the environment can disrupt health. This is because mosquitoes suck blood and are disease vectors. The mosquito species that can be found in residential areas are *Aedes* sp., *Culex* sp., and *Anopheles* sp. (Novianto et al. 2021). *Culex pipiens* Linnaeus, 1758 mosquito is a vector for filariasis or elephantiasis (Samy et al. 2016; Gyapong et al. 2018).

The World Health Organization (WHO) launched a global filariasis elimination program two decades ago (Global Program to Eliminate Lymphatic Filariasis/GPELF). The aim of this program is to reduce filariasis cases so that they do not become a source of public health problems world for 2024. This has received full support from the Ministry of Health of the Republic of Indonesia through the Decree of the Minister of Health of the Republic of Indonesia Number: 157/Menkes/SK/ Other regulations are also contained in Decree number: 1582/Menkes/SK/XI/2005 concerning Guidelines for Controlling Filariasis (*elephantiasis*) which was later replaced by Minister of Health Regulation No. 94 of 2014 concerning Filariasis Management in 2014 (Meliyanie and Andiarsa 2017; Pantelias et al. 2022; Smith et al. 2023).

As of 2023, Indonesia reported 1,759,142 cases of filariasis, with a chronic case rate of 7,955 (Ministry of Health of the Republic of Indonesia 2023). Maluku Province has a low incidence of filariasis; according to data on chronic filariasis cases in 2024, the Maluku Provincial Health Service reported no new cases detected. Nonetheless, the records indicate that 14 individuals have been recorded as suffering from filariasis and dying from previous incidents in Maluku. High filariasis data is found in Central Maluku and Southwest Maluku districts, with 8 cases each. Data on filariasis cases in Maluku Province reveal the following: South-east Maluku reported 5 cases, West South-east Maluku and West Seram each recorded 4 cases, Ambon City and Tual reported 2 cases, and Aru Islands and Eastern Seram reported 1 case each. Meanwhile, the Buru District in the South reported no filariasis cases (Maluku Provincial Health Service 2024).

To determine the vector population and diseases transmitted by mosquito vectors in the community, it is very necessary to carry out an entomological survey.

Entomological surveys can provide important information regarding vector distribution and vector disease transmission capacity. Scientific information regarding the bionomics of species that act as vectors for disease transmission is very important in vector control efforts in related agencies. Vector control is mainly focused on the use of organophosphate insecticides, growth regulators, and bacterial larvicides. Increasing reports of the development of resistance to these insecticides in vector populations have led to the failure of control efforts (Nenaah et al. 2021; Selim et al. 2022). Control of disease-carrying vectors so far still relies on the extensive and intensive use of chemical insecticide products. The chemicals that make up chemical insecticides are quite successful in stopping the spread of disease by mosquito vectors. However, it can cause side effects from the use of insecticide chemicals (Dhimal et al. 2014; Kioulos et al. 2014; Phillips et al. 2014).

Natural plant extracts have emerged as promising alternatives for mosquito control due to their bioactive compounds, which exhibit insecticidal properties while being environmentally sustainable. Cassava (*Manihot esculenta* Crantz), a staple crop in tropical regions, has garnered attention not only for its nutritional value but also for its phytochemical constituents, including cyanogenic glycosides, flavonoids, and alkaloids, which possess potential insecticidal activity (Manjula et al. 2020). The juice extract from cassava, a byproduct of its processing, is rich in secondary metabolites that may disrupt the normal physiology and development of insects, particularly in the larval stages (Sayono et al. 2019). The morphological and ultrastructural changes induced by exposure to botanical extracts provide insights into their mode of action and potential efficacy as biocontrol agents. Previous studies have demonstrated that natural extracts can cause deformities in the exoskeleton, digestive system, and respiratory siphon of mosquito larvae, highlighting their utility in integrated pest management (Habiba et al. 2022).

Despite these advancements, research focusing specifically on cassava juice extract's impact on *C. pipiens* larvae remains limited. Most studies have concentrated on its toxicity, with little attention to the morphological and ultrastructural alterations that could elucidate the mechanisms behind its insecticidal effects. Understanding these changes is crucial for optimizing the use of cassava-based biocontrol agents and reducing reliance on synthetic insecticides. A study conducted by Velayutham et al. (2016) assessed the larvicidal activity of *M. esculenta* extract against the mortality of two important mosquito species, *Aedes aegypti* and *Culex quinquefasciatus* larvae. *M. esculenta* extract has larvicidal effect against *A. aegypti* and *C. quinquefasciatus* larvae with LC50 dose for *A. aegypti* 66.14 mg/mL and *C. quinquefasciatus* 61.60 mg/mL, which was attributed to phytochemicals such as tannins and saponins. This study suggests that *M. esculenta* extracts could be beneficial for mosquito control, although detailed experiments are needed to confirm their efficacy. The main objective of this research is to explore the application of functional natural plant products as biolarvicides by analyzing the phytochemical properties of cassava tubers (*M. esculenta*), evaluating the biolarvicidal efficacy of pure cassava juice extract at treatment doses of 0.5, 1.5, and 3.5% against fourth instar *C. pipiens* larvae, and assessing the morphological damage caused to the larvae's body surface due to cassava juice extract exposure.

MATERIALS AND METHODS

Study area

The research locations are spread across two location points Tial Village residents and the cassava gardens of Salahutu Sub-district, Central Maluku District, Maluku Province, Indonesia. The map was created using the QGIS application, which is an open source software application (Figure 1) (Aurellia et al. 2023).

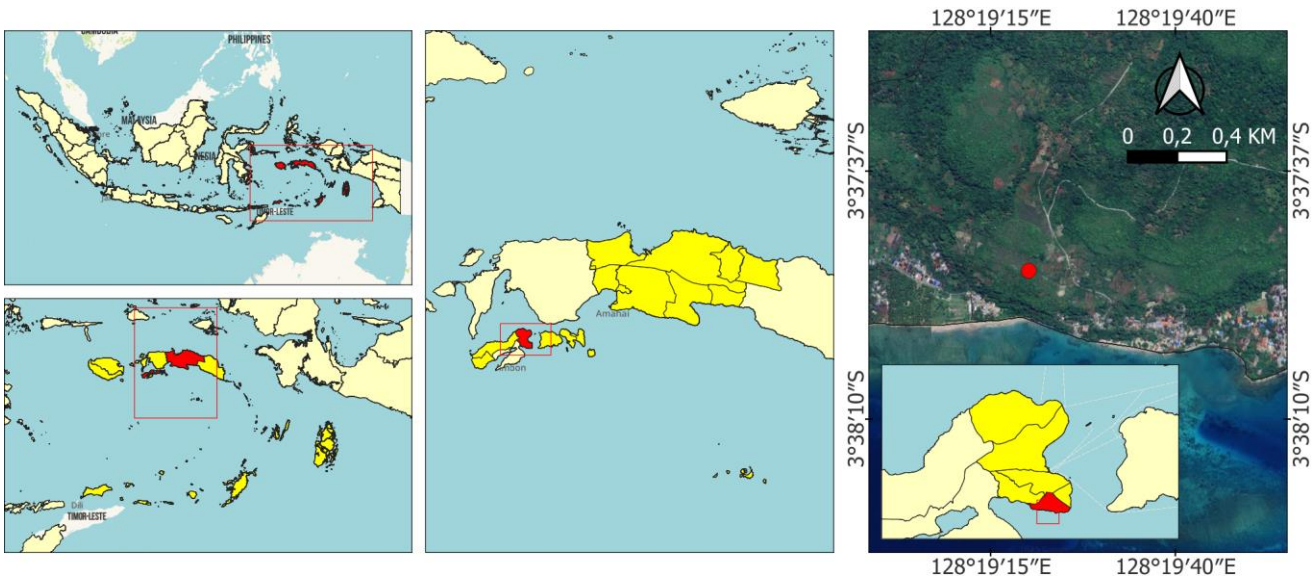

Figure 1. Location map of Tial Village, Salahutu Sub-district, Central Maluku District, Maluku Province, Indonesia

Procedures

Description of larva collection locations and local cassava gardens

All *C. pipiens* fourth instar larvae specimens used in this study were collected from two locations (latitude point: 3°37'50.2"S and longitudinal point: 128°19'20.3"E) Tial Village, Central Maluku District. Larvae samples were collected from two sources, including: a bathtub in the house and a water storage container in an open area. Larvae samples were taken during the day from 11.00 to 13.00. The location of larvae collection was selected based on the better accessibility of procuring cassava ingredients locally. The distance between the cassava plantation and the Tial settlement is 3 Km (Figure 1).

Sample identification

All fourth instar larvae of *C. pipiens* mosquito were taken from residential areas of Tial Village according to the location description above. The total number of larvae is 60 larvae. Larval samples were stored in absolute ethanol for the purpose of observing larval body morphology based on the identification key of the Diptera order of the Culicidae family. All the characteristics of the IV instar larvae specimens of *C. pipiens* mosquitoes in this study were also adjusted according to Hossain et al. (2014) and Qasim et al. (2014).

Preparation of pure Manihot esculenta *Crantz juice extract*

The samples used were cassava juice extract without distinguishing the age of young or old stem tubers. Cassava tubers were taken from a garden located in Tial Village, Salahutu Sub-district, Central Maluku. The cassava tubers were initially washed and grated, then weighed at 1.5 kg, and subsequently squeezed to extract the juice.

Alkaloid test

A total of 1 g of finely squeezed cassava tubers is then dissolved in 10 mL of chloroform. Then 4 drops of NH_4OH were added, then filtered and the filtrate was put into a closed test tube. The chloroform extract in the test tube was shaken with 6 mL of H_2SO_4, 2 M and the acid layer was separated into another test tube. This acid layer is dripped onto a drop plate and Meyer, Wagner and Dragendorf reagents are added which will produce colored precipitates of white, brown and red-orange respectively (Linn and Myint 2018)

Saponin and flavanoid test

For the saponin test, 10 mL of filtrate is put into a closed test tube, shaken for 10 seconds and left for 10 minutes. The formation of stable foam indicates the presence of saponin. For the flavonoid test, another 10 mL of filtrate was added with 0.5 g of Mg powder, 2 mL of chlorhydric alcohol (a mixture of 37% HCl and 95% ethanol in a 1:1 ratio), and 20 mL of alcohol and then shaken vigorously. The formation of red, yellow and orange colours in the amyl alcohol layer indicates the presence of flavonoids (Fachriyah et al. 2023).

Larvicidal test

The cassava juice concentrations extract using 0, 0.5, and 3.5% to larvacidal test. For each treatment dose, 20 *C. pipiens* larvae were placed. Then, placed each larvae group by hand using a plastic pipette into a test tube containing 5 mL of each juice cassava tubers. Larvicidal activity was measured by the number of larvae that died after 24 hours of exposure to cassava tubers juice. The duration of the treatment period in larvacidal test with 4 hours interval (Manait 2023).

Observation of 3-dimensional morphology of Culex pipiens *larvae using a Keyence VHX-6000 Microscope*

Sample preparation begins with making sample preparations. The samples were observed for their morphological characteristics from IV instar larvae of *C. pipiens*. Then, high quality digital images (3 dimensions) are obtained using a High-Performance Zoom Lens VH-Z20T camera (Magnification 20 - 200) equipped with a Dual-Light High-Magnification Zoom Lens VH-Z250T (Magnification 250 - 2500). All the supporting features of the Keyence VHX-6000 Microscope above are a series of facilities Integrated Laboratory of Bioproduct (iLab) at the National Research and Innovation Agency (BRIN-Cibinong).

Characterization using Scanning Electron Microscopy (SEM)

The SEM tests were carried out at the facility Integrated Laboratory of Bioproduct (iLab) at the National Research and Innovation Agency (BRIN-Cibinong). SEM test consists of two testing stages, namely sample preparation and coating. The sample preparation process begins with the samples being soaked in cacodylate buffer for 2 hours. Then put the sample in a 2.5% glutaraldehyde solution for 2 to 48 hours, soak it in 2% tannic acid for 6 hours, wash it with cacodylate buffer which is left for 5 minutes for 4 times, dehydrate it by soaking in graded alcohol 70, 85, and 95% each for 20 minutes, soak in absolute alcohol 10 minutes for 2 times. All processes were carried out at temperature 4°C, except immersion in 95% and absolute alcohol. After the sample preparation is complete, the coating process was conducted using gold-palladium alloy as the coating material, and documentation of the sample photos are carried out. The sample documentation carried out by the Thermo Scientific Aquilos 2 Cryo-FIB.

Observe damage to larval body parts

Observation of the characteristics of the damaged body surface based on the body segment that experienced exoskeleton damage. The characters observed were the head capsule, thorax, siphon and abdominal segments. In addition, the structure and mouth segments of the larvae were also observed (Alba-Tercedor and Vilchez 2023).

Data analysis

Data were analyzed descriptively based on the morphological damage of *C. pipiens* fourth instar larvae treated with cassava tuber juice test solution dosage 0.5, 1.5, and 3.5%.

RESULTS AND DISCUSSION

Phytochemical test of cassava tuber juice

The volume of cassava tubers juice was 1.5 kg weight. The extract was subjected to a qualitative phytochemical test for alkaloids, flavonoids, terpenoids, steroids, phenolics, tannins, saponins and cyanides. The results of these test indicate the presence of alkaloids in the pure juice of cassava tubers. Flavonoids, terpenoids and steroids were also detected in both samples of pure cassava tuber juice. Additionally, saponin and cyanide showed positive results in both samples tested. However, phenolic and tannin compounds showed negative results in all samples (Table 1). The results of phytochemical screening from two types of pure squeezed cassava tuber samples are presented in Table 2. Sample A (concentration 0.5%) and sample B (concentration 3.5%) showed a concentration of flavonoid compounds of 3-3.5 mg. The cyanide test for both samples showed a cyanide compound concentration of 1-1.2 mg.

Larvicidal effect of cassava tuber juice (*Manihot esculenta*) treatment

The results of cassava tubers juice treatment in *C. pipiens* larvae exposed for 4 to 24 hours are presented in Table 3. The motility of fourth instar larvae during the 1st hour of observation (for 4 hours) to the 6th hour (for 24 hours) showed that gradual death of larvae. The results of the research showed clinical symptoms of changes in larval behavior in the test solution showing anxious behavior and moving quickly towards the surface. Next, the larvae die by sinking to the bottom of the test solution. Treatment doses of 0.5, 1.5, and 3.5% showed mortality above 90% at the 6th hour (24 hours) (Table 3).

Identification results using a keyence 3D microscope of *Culex pipiens* IV instar larvae in the control group

The results of morphological observations of the control showed that the external body characteristics of *C. pipiens* instar IV larvae in this study had a pale brownish larval body color. Morphological examination of the digestive tract, the fourth instar *C. pipiens* larvae consisted of the foregut, midgut and hindgut. The abdomen has a cylindrical shape and consists of several segmentations. In segment IV, the abdomen features branching setae (Figure 2). The segmentation of abdominal sections I-VIII is clearly visible in Figures 2 and 3.A.

The body length of the larva is 5.8 mm. The complete body parts of the larvae can be seen in Figure 3. From the results of keyence's microscope photos, different color patterns were found between the larval head segments and other body parts (Figure 3.A-C). The thorax and abdomen display the same pale white coloration. Meanwhile, the dorsal aspect of the head segment is found to have larvae antennae that are shorter than the length of the head measuring 0.02 mm. The length of the larval head measures 0.7 mm. The hair on the edge of the thorax has a hair structure with many branches (Figure 3.C). In the caudal segment of the larva's body there is a siphon, which contains pectin teeth. The color of the chiffon is brownish-white, featuring more than 4 longitudinal stripes (Figure 3.D and 3.E).

Table 1. Phytochemical test results

Parameter	Color	Results
Alkaloids	Yellow	+
Flavonoids	Greenish yellow	+
Terpenoids	Red	+
Steroids	Blue	+
Phenolic	Yellow	-
Tannin	Yellow	-
Saponins	Foam	+
Cyanide	Blue	+

Table 2. Flavonoid and cyanide test results

Sample	Flavonoid test				Cyanide test			
	Sample weight (g)	Absorbance	Concentration (mg)	Content (%)	Sample Weight (g)	Absorbance	Concentration (mg)	Content (%)
Sample A	50.6	0.16	3.3	0.00561	10.3	0.19	1.18	0.1134
Sample B	50.5	0.17	3.4	0.00672	10.2	0.18	1.15	0.1116

Table 3. Larvacidal test of *Culex pipiens* as potential repellent

Treatment	Number (%) of dead larvae based on time						Larval condition after 24 hours
	I (4 hours)	II (8 hours)	III (12 hours)	IV (16 hours)	V (20 hours)	VI (24 hours)	
Control	-	-	-	-	-	-	Normal
Dosage 0.5%	5 (25)	3(15)	3(15)	3(15)	3(15)	3(15)	All larvae drown
Dosage 1.5%	6(40)	4(20)	4(20)	3 (15)	3(15)	-	All larvae drown
Dosage 3.5%	9(45)	5(25)	3(15)	1(5)	2(10)	-	All larvae drown

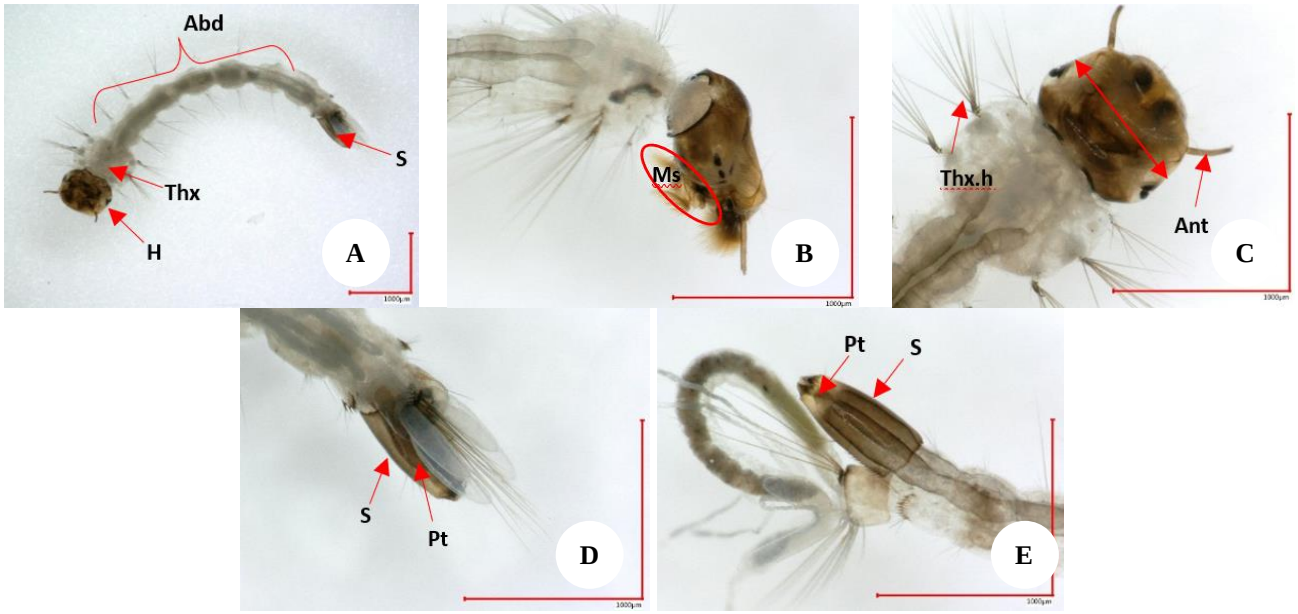

Figure 3. Keyence 3D microscope photo results for body surface morphology of control group fourth instar *Culex pipiens* larvae. The larval body consists of head, thorax and abdominal segments: A. [H: Head; Thx: Thorax; Abd: Abdomen; Mb: Mouth brushes; Thx.h: Thorax hairs; Pt: Pectin teeth; S: Siphon]; B. Dorsal head segment; C. Lateral thorax and head; D. Tail segment; E. Siphon segment section

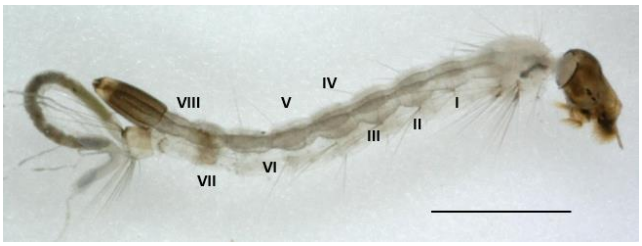

Figure 2. Abdominal segments I-VIII of the body of IV instar larvae of *C. pipiens* in this study

SEM characterization of body damage of fourth instar larvae of *Culex pipiens* given the cassava tubers juice

The results of the body morphology study of *C. pipiens* larvae in the 0.5-1.5% dose treatment group are shown in Figures 4 and 5. Figures 4.A-I depict the SEM results for the body surface of *C. pipiens* larvae treated cassava tuber juice (*M. esculenta*) at three different concentrations, including 0.5, 1.5, and 3.5%. At a concentration of 0.5%, the results of observations of damage to the body morphology of *C. pipiens* larvae showed damage to the head capsule close to the thorax segment. The thorax experienced exoskeleton damage as indicated by the presence of a hole in the thorax segment (Figure 4.A). For a concentration of 1.5%, the results of SEM photos of damage to the body morphology of *C. pipiens* larvae show that the head capsule was initially normal and then became flat. Apart from that, the observation results showed that the abdomen experienced a stiff condition with flattening of the exoskeleton in each abdominal segment (Figure 4.B). In Figure 4.C, it is evident that the level of damage observed is greater than that seen in the larval morphology shown in Figures 4.A and 4.B. A concentration of cassava tuber juice at a dose of 3.5% for 24 hours caused severe damage to the head capsule, with the exoskeleton connecting the head and thorax nearly separating (Figure 4.C and 4.F). Additionally, fatal damage to the body surface of IV instar larvae of *C. pipiens* treated with juice of cassava tubers (*M. esculenta*) dose of 3.5% for 24 hours can be seen in Figure 5. The joint of the head and thorax exoskeletons were peeled and destroyed (Figure 5.A and 5.C).

Discussion

The morphology of IV instar larvae in this study was observed in the larval body state without treatment with cassava tuber juice. The photographs of the normal (control) larvae revealed a smooth cuticle surface on the head and thorax (Figure 4.J and 4.K) outher body smooth cuticle of abdominal segments. Both untread (control) and treated fourth instar *C. pipiens* larvae were examined and photographed using SEM, which revealed various malformation. Additionally, the morphology of *C. pipiens* larvae was assessed after exposure to treatment concentrations of 0.5, 1.5, and 3.5% cassava tuber juice. The larvicidal effect of cassava tuber juice caused the fourth instar larvae of *C. pipiens* to exhibit slowed body movements. This reduction in movement was observed during the third hour of the 12 hour larvicide test across all treatment concentrations. IV instar larvae of *C. pipiens* that died and drowned generally showed damage to the head and thorax. Not all test larvae were examined microscopically, but every image presented in the results section of this study showed that the test larvae exhibited over 50% damage. Specifically, the treatment concentration of 3.5% resulted in 80% damage to the body segments of the fourth instar larvae.

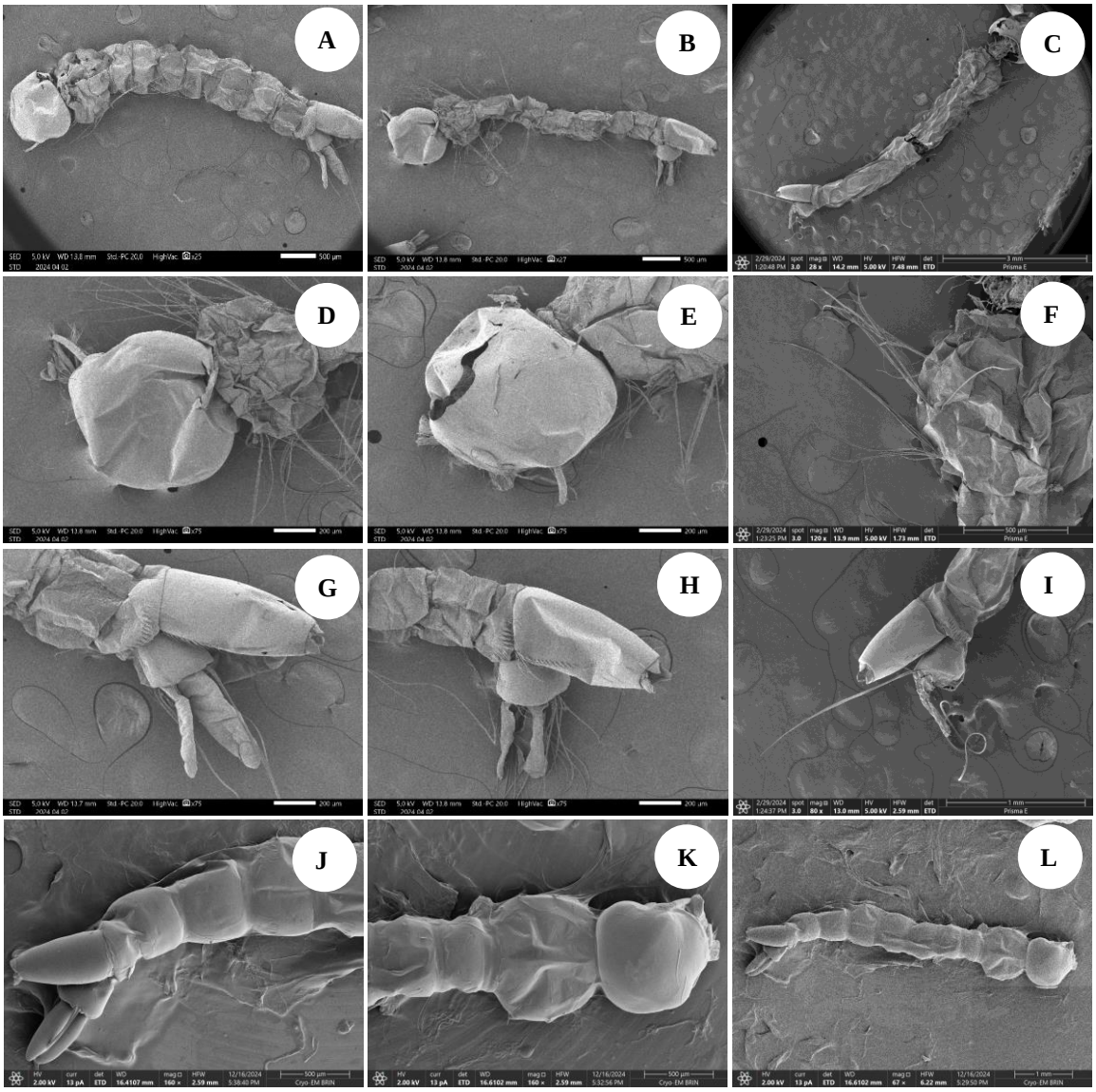

Figure 4. SEM photo image of body surface morphology of IV instar larvae of *Culex pipiens* treated with cassava tuber juice extract (*Manihot esculenta*) for 24 hours. (4A;4D;4G = 0.5% concentration; 4B;4E;4H = 1.5%) concentration;4C;4F;4I = 3.5% concentration;) and (4J =ultrastructure of normal (control) larvae of *C.pipiens* of Siphon.; 4K = Head and thorax., 4L: SEM of normal (control) smooth body surface of IV instar larvae

Based on the results of phytochemical tests conducted in this research, cassava tuber juice was found to contain alkaloids, flavonoids, terpenoids, steroids, saponins and cyanides. The analysis of the chemical compound content in this pure juice extract revealed that a 100 g of sample contained 6.3 mg of flavonoids. A 20 g sample contains 2.4 mg of cyanide compounds. The abdominal color of the control larvae (untreated) appeared transparent. The pale brown color of the larva's body is a characteristic of the *C. pipiens* species. During the initial hour of (with larvae exposed for 4 hours), a total of 20 larvae sank to the bottom of the container across the three concentrations applied (0.5-3.5%). From the second hour (after 8 hours of treatment) to the fifth hour (after 20 hours of treatment), the number of drowned larvae observed were 12, 10, 7, 7, respectively. The submerged larvae displayed no response to external stimuli. However, they did react when their siphons were touched using tweezers.

At a concentration of 0.5%, cassava tuber juice can kill 50% of the larval population within 12 hours of treatment. Meanwhile, a dose of 3.5% cassava tuber juice is a concentration that is effective in killing larvae within 8 hours of treatment. Different doses of cassava tuber juice had a significant effect on larval death. The extract dose that had a significant effect was a concentration of 3.5%. Based on the research results, it is known that cassava tuber juice is effective in killing *C. pipiens* larvae relatively quickly. The higher the dose of cassava tuber juice, the higher the death rate of *C. pipiens* larvae. The high larval death rate is thought to be due to the viscosity of the pure juice extract from cassava tubers. The viscosity of the extract can interfere with the movement of larvae towards the surface to breathe and take in oxygen.

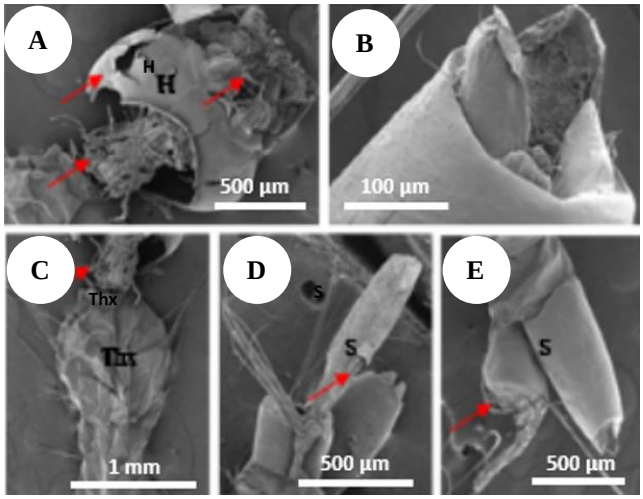

Figure 5. Image of fatal damage body surface of IV instar larvae of *C. pipiens* given 3.5% cassava tuber juice (*M. esculenta*) for 24 hours. A. Head; B. Thorax hairs; C. Thorax, D & E. Siphon

The body damage of *C. pipiens* larvae in the research results was shown by the exoskeleton of the larval body *C. pipiens* the lysed (Figure 5). The lysis of the larval exoskeleton was due to exposure to cassava tuber juice (0.5, 1.5, and 3.5% doses). The body exoskeleton of *C. pipiens* larvae is the part of the body that separates epidermis (one layer of living cells) with a basement membrane. Damage to the exoskeleton will cause the selectively permeable membrane to be disrupted. The transport process in the larval hemolymph canal will be disrupted so that the substances being transported cannot be secreted. The development of the cuticle which is supported by the pore channel network will be hampered (Parle et al. 2017; Kelly et al. 2022). The lysis of larval body cells will change various tissues and organs, as well as changes in hemolymph pH due to the release of gastric juice from the intestines (Alba-Tercedor and Vilchez 2023).

Based on the results of phytochemical tests, it is known that the alkaloid compounds contained in cassava tuber juice can cause death and damage the body surface of *C. pipiens* larvae. Alkaloid compounds are secondary metabolites produced by plants which can suppress mosquito activity. Alkaloid compounds have great potential as larvicides against third instar larvae of *C. pipiens* (Aly et al. 2021). The activity of potential larvicidal compounds obtained from other types of plants containing essential oils is also being studied for control programs for dangerous disease vectors in mosquitoes. Larvicidal effects can also be obtained from *C. aurantifolia* essential oil and can cause damage to the bodies of mosquito larvae. Indicators of larval body damage can be seen from morphological abnormalities in the anus and changes in the ultrastructure of the body of *C. pipiens* larvae. Essential oils from the peel of citrus plants (*Citrus reticulata* Blanco., and *Citrus x sinensis* (L.) Osbeck are very effective against the mortality of Culex larvae and adult mosquitoes. pipiens. *Citrus x sinensis* (L) Osbeck the most effective larvicide because it contains many essential oils (Badawy et al. 2018; Azmy 2021; Baz et al. 2022). Larvacide tests show that the essential oils are plant-like *Citrus x sinensis* (L.) Osbeck and *Mentha xrotundifolia*. (L.) Huds showed insecticidal activity against fourth instar larvae of *C. pipiens* (Kharoubi et al. 2020; Azmy et al. 2021; Gohary et al. 2021).

The Bogor taro plant (*Colocasia esculenta* (L.) Schott) shows potential as a larvicide, with significant effects on *Culex quinquefasciatus* Say, 1823 and *Chironomus* sp. larvae due to reducing substances (Mondal et al. 2019). Methanol extracts of *C. esculenta*, *Eclipta prostrata* (L.) L., and *Wrightia tinctoria* R.Br. demonstrate larvicidal activity against multiple instars and pupae of *C. quinquefasciatus* (Dass and Mariappan 2016). Larvicidal plant compounds, particularly neurotoxins, inhibit acetylcholinesterase (AChE), disrupting hydrolysis, leading to enzyme accumulation, paralysis, and larval death (Hasmiwati et al. 2018; Emam et al. 2022; Rants'o et al. 2022). Essential oils, such as from *Schinus terebinthifolia* Raddi fruit, also exhibit larvicidal effects, causing midgut damage in *C. pipiens* larvae at 100 mg/L concentrations (Dris et al. 2017; El-Sabrout et al. 2019; El-Akhal et al. 2021).

In the results of our research, body damage to IV instar larvae of *C. pipiens* was identified based on the structure of the surface of the larva's body which shrank due to exposure cassava tuber juice. Other research that supports our research results is based on a study from El-Monairy (2015). Histopathological changes in the midgut and gastric caeca of third instar larvae of *C. pipiens* are known to occur due to exposure to *Colocasia esculenta* (L.) Schott leaf extract. The bodies of *C. pipiens* larvae undergo dramatic pathological lesions, especially the malpighian tubules which are severely affected morphologically. Larval midgut cells showed morphological deviations from normal cells. Degeneration occurs on the apical side due to lysis of epithelial cells. Epithelial cells with extensive cellular microvilli experience shrinkage (El-Monairy 2015; Dass 2020; Kumar and Srivastava 2023).

The results of this research can be used as a reference for determining the composition of an effective biolarvacide dose. Choosing the next dose level will greatly support efforts to control the vector of filariasis caused by the *C. pipiens* mosquito. Furthermore, histopathological observations of cells in the intestines of *C. pipiens* larvae need to be carried out in further research.

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