A PRELIMINARY STUDY ON LARVICIDAL EFFICACY OF *Piper nigrum* L. (PIPERACEAE) EXTRACTS AGAINST DENGUE VECTOR, *Aedes albopictus* (DIPTERA: CULICIDAE)

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ABSTRACT

Botanical insecticides have become an alternative biocontrol tool in controlling mosquito population worldwide. Hence, the current study was conducted to determine the efficacy of Sarawak *Piper nigrum* ethanolic extracts as larvicidal potential on its morphological abnormalities against larvae and pupae of *Aedes albopictus.* Plant samples of *P. nigrum* were extracted in 95% ethanol, evaporated and analysed using standard qualitative method. Phytochemical screening revealed the presence of alkaloid, flavonoid, tannin, triterpenes and steroid in the fruit and leaf extracts, while saponins was only found in the leaf extract. Larval bioassays were conducted using the crude extracts following WHO standard for larval susceptibility test. After 24 hours of exposure, the total mortality of larvae was achieved at 10.5 ppm and 450 ppm for *P. nigrum* fruit and leaf extracts, respectively. Fruit extract has shown a more remarkable larvicidal potential with lower LC_{50} (5.07ppm) and LC_{90} (7.85ppm) as compared to the leaf extract which required higher LC_{50} (108.893ppm) and LC_{90} (213.796ppm). Analysis of variance (ANOVA) demonstrated a significant difference in the mean mortality of larvae between each concentration of fruit extract ($F=121.202$, $df=6$, $p<0.05$) and leaf extract ($F=452.875$, $df=6$, $p<0.05$) as compared to the control groups. It was observed that both *P. nigrum* extracts induced several morphological abnormalities at larval and pupal stage that finally led to the individual mortality. Various phytochemical constituents detected in *P. nigrum* extracts may possess the mosquito larvicidal properties and cause the morphological abnormalities in mosquitoes. Hence, the ethanolic extracts of *P. nigrum* from Sarawak may pave the way for the development of an environmentally safe mosquito biopesticide.

Keywords: *Aedes albopictus*, *Piper nigrum*, larvicidal potential, morphological abnormalities, phytochemical constituents

ABSTRAK

Racun serangga berasaskan tumbuhan telah menjadi kawalan biologi alternatif dalam mengawal populasi nyamuk. Oleh itu, kajian ini telah dijalankan untuk menentukan keberkesanan ekstrak tumbuhan *Piper nigrum* yang berpotensi membunuh jentik-jentik dan kesannya terhadap morfologi *Aedes albopictus* yang telah terdedah kepadanya. Komponen fitokimia dalam *P. nigrum* telah dikaji terlebih dahulu. Sampel tumbuhan *P. nigrum* telah diekstrak ke dalam 95% etanol, disejat dan komponen fitokimia dianalisis menggunakan kaedah piawaian kualitatif. Hasil analisis fitokimia menunjukkan kehadiran alkaloid, flavonoid, tanin, triterpin dan steroid di dalam ekstrak buah dan daun, manakala saponin hanya di dalam ekstrak daun. Ujian bioasai jentik-jentik dijalankan menggunakan ekstrak mentah berdasarkan piawaian WHO bagi ujian kerentanan. Selepas 24 jam pendedahan, kematian semua jentik-jentik dicapai pada 10.5 ppm dan 450 ppm bagi ekstrak buah dan daun *P. nigrum.* Ekstrak buah menunjukan larvisid yang lebih baik yang mana kepekatan yang lebih rendah diperlukan untuk menyebabkan 50% (5.07 ppm) dan 90% (7.85 ppm) kematian berbanding ekstrak daun yang memerlukan kepekatan yang lebih tinggi untuk mencapai 50% (108 ppm) dan 90% kematian (213 ppm). Analisis ANOVA menunjukkan perbezaan yang signifikan dalam purata kematian jentik-jentik di antara setiap kepekatan ekstrak buah ($F = 121.202$, $df = 6$, $p<0.05$) dan ekstrak daun berbanding kumpulan kawalan. Kedua-dua ekstrak *P. nigrum* didapati menyebabkan beberapa kesan abnormal dari segi morfologi nyamuk yang akhirnya membawa kepada kematian individu. Komponen fitokimia yang dikesan dalam ekstrak ini mempunyai kesan kepada jentik-jentik dan menyebabkan keadaan morfologi abnormal dalam nyamuk. Oleh itu, ekstrak etanolik daripada tumbuhan *P. nigrum* dari Sarawak sewajarnya dipertimbangkan untuk pembangunan masa hadapan kerana potensinya sebagai biopestisid untuk mengawal populasi nyamuk yang selamat kepada alam sekitar.

Kata kunci: *Aedes albopictus*, kawalan biologi, *Piper nigrum*, potensi larvisid, kecacatan morfologi, komponen fitokimia

INTRODUCTION

The emergence of insecticide resistance in mosquitoes has encouraged the search for natural insecticide from botanical origin with their phytochemical constituents that may have potential larvicidal effects. Botanical insecticides are eco-friendly to the environment and non-target organisms as well as easily biodegradable as compared to the conventional insecticides (Rajkumar & Jebanesan, 2007). In addition, the synergistic effects between the phytochemical constituents in botanical resources may assist in reducing the resistance development in mosquito vectors towards the botanical insecticides (Scott et al., 2008).

Several plant species and their phytochemical constituents had been demonstrated as a general toxicant, insect repellent, growth and reproductive inhibitor as well as in larvicidal, ovicidal and oviposition-deterrent against diverse mosquito vectors (Elango et al., 2009; Govindarajan et al., 2008). Recently, *Piper nigrum* from the family of Piperaceae has drawn an attention to researchers as it comprises diverse phytochemical compounds and biological properties that have been widely contributed to traditional and modern-day applications especially in food additives, pharmaceuticals, agriculture crops and as a mosquito vector and pest control agent (Lara Junior et al., 2012; Marques et al., 2015).

Previous study has demonstrated that different plant parts of *P. nigrum* extract have larvicidal potential against various mosquito species such as *Aedes aegypti*, *Aedes togoi*, *Culex*

pipiens, and *Anopheles gambiae.* However, at present, the efficacy of *P. nigrum* has not been evaluated against the secondary dengue vector, *Ae. albopictus* (Gulzar et al., 2013; Kemabonta et al., 2018; Lee, 2000; Park et al., 2002; Siddiqui et al., 2003). Therefore, our study was conducted to determine the larvicidal potential of Sarawak *P. nigrum* fruit and leaf extracts and to observe morphological abnormalities in exposed *Ae. albopictus.*

MATERIALS AND METHODS

Mosquito Colonies

Mosquito colonies were established at the Entomology Laboratory in Faculty of Medicine and Health Sciences, Universiti Malaysia Sarawak. Mosquito eggs were immersed in dechlorinated water in tray with a pinch of yeast to enhance the eggs hatching. The larvae were fed daily with a small piece of dried beef liver until pupation. The pupae were collected, rinsed and transferred into a bowl containing dechlorinated water. Then, the bowl of pupae was transferred into mosquito cage (30 x 30 x 30cm) where the adults were then emerged. Adults were provided with 10% sucrose solution and the adult female mosquitoes were periodically blood fed on restrained rats for the production of eggs. The mosquito colonies were maintained under laboratory conditions at 25°-30° C and 80-90% relative humidity under a photoperiod of 12:12 (light/dark) in the laboratory.

Plant Collection and Extraction

Piper nigrum fruits and leaves were collected from Kampung Tanjung Bundong, Kota Samarahan, Sarawak (1°27'35.61"N, 110°29'17.74"E) and the species was verified by the Institute of Biodiversity and Environmental Conservation (IBEC), Universiti Malaysia Sarawak (UNIMAS). The extraction process was performed following Somchit et al. (2004) and Zakaria et al. (2007) with slight modifications.

The fresh leaves and fruits were cleaned, dried and powdered using mechanical grinder. The powdered samples were soaked, stirred and extracted with ethanol in a ratio of 1:10 (weight/volume) at room temperature for 48 hours. The extracts were further evaporated and concentrated using rotary evaporator R-III (BUCHI ®) at 60°C until the solvent was completely evaporated and produced dark greenish and brownish crudes. The crude extracts were weighed and preserved in airtight bottles at 4°C for bioassays. The percentage of yield for each sample was determined.

Qualitative Phytochemical Screening

The crude ethanolic extracts of fruit and leaf of *P. nigrum* were subjected to preliminary phytochemical tests. The procedures of phytochemical tests screening for alkaloids, flavonoid, saponins, steroid, tannin and triterpenes of *P. nigrum* fruit and leaf extracts were carried out following the established protocol from Anyasor et al. (2010), Ayoola et al. (2008), Edeoga et al. (2005), and Odebiyi & Sofowora, (1978) (Table 1).

Table 1. Preliminary phytochemical screening for *P. nigrum* fruit and leaf extracts

Phytochemical test	Procedure
Alkaloids	Crude extract of 0.5g was diluted with 10ml of 10% acetic acid in ethanol, boiled and filtered. 2ml of 10% dilute ammonia and 5ml of chloroform was added to 5ml of filtrate. Then, the filtrate was shaken gently to extract the alkaloid base. The chloroform layer was extracted with 5% of hydrocholoric acid (HCl). The filtrate was treated with a few drops of Mayer's reagent. Formation of creamy white precipitate indicated the presence of alkaloids.
Flavonoids	Crude extract of 1g was added with 5ml ethanol, boiled and filtered. A few drops of concentrated HCl and magnesium tape ribbon (1-2cm) were added. Colours ranging from orange to red indicated flavones, red to crimson indicated flavonols and crimson to magenta indicated flavonones.
Saponins	Crude extract of 1g was boiled in 10ml of distilled water in a water bath and filtered. The filtrate was shaken vigorously (1-2 minutes) for a stable persistent froth (at least 15 minutes) regarded as presence of saponins
Steroids	Liebermann-Buchard test: Acetic anhydride (2ml) was added to 0.5g crude extract with 2ml H_2SO_4. The colour changed from violet to blue or green was taken as an evidence for the presence of steroids.
Tannins	Crude extract of 0.5g was boiled in 20ml of water and then filtered. A few drops of 0.1% ferric chloride were added. An intense blue-black colour was taken as an evidence for the presence of hydrolysable tannins, while brownish green indicated that of condensed tannins.
Triterpenes	Salkowski test: An extract of 5ml was mixed in 2ml of chloroform and concentrated H_2SO_4 (3ml) was carefully added to form a layer. A reddish-brown colour was taken as an evidence for the presence of triterpenes.

Larval Bioassay

Larval bioassays were conducted according to the standard procedure by the World Health Organization (World Health Organization, 2005). The efficacy of *P. nigrum* fruit and leaf extracts were evaluated at several concentrations (treatments) yielding a range of 0 to 100% larval mortality at 24 hours intervals. The crude extracts were diluted into concentrations of 2.5 to 10.5ppm and 50 to 450ppm for fruit and leaf extracts, respectively. One percent (1%) of dimethyl sulfoxide (DMSO) and temephos (Liu et al., 2020) were diluted with distilled water and served as control and reference larvicide, respectively. The larval susceptibility test was performed in 300mL plastic containers consisting of 250mL of test medium with 25 early fourth instar larvae. Overall, four replicates per treatment were carried out simultaneously following CRD design with a final total of 100 larvae for each treatment. The larvae were considered as dead if, after 24 hours, they did not show any sign of movements even after gently touched with glass rod (Oliveira et al., 2010). The percentages of larval mortality were recorded after 24 hours of exposure. Three trials of larval bioassay were carried out.

Gross Morphological Abnormalities Observations

All individuals that survived were observed until pupation and adult emergence to determine whether the *P. nigrum* extracts exhibit effect towards the morphological development of *Ae. albopictus.* Dead larvae, pupae, exuviae and adults were transferred into labelled universal bottles containing 70% ethanol. The morphological abnormalities of the dead specimens were observed and photographed using dark field compound microscope. Dead specimens were categorized and described following Ratanatham et al. (1994) and Soonwera & Phasomkusolsil (2016).

Statistical Analysis

The results for larval bioassays were reported as the means ± SEM (Standard error of the mean) from replication data. All the data were tested for normality (Shapiro-Wilk) prior to analysis. One-way analysis of variance (ANOVA) was conducted to determine significant differences among treatments using Kruskal-Wallis H-test and further multiple comparison analysis between groups by Post Hoc Tukey's Test using SPSS 25.0 ($P<0.05$). Probit analysis of concentration-mortality data was performed to compute lethal concentration of LC_{50} and LC_{90} with 95% confidence limits of upper confidence limit and lower confidence limit for each treatment (Finney, 1971).

RESULTS AND DISCUSSION

From the plant extraction performed, the percentage of yield for 25.9g fruit and 87.5g leaf dried powder of *P. nigrum* were 14.67% and 4.23%, respectively (Table 2). Based on the qualitative phytochemical screening, it was revealed that both *P. nigrum* fruit and leaf ethanolic extracts consisted of alkaloid, flavonoid, tannin, triterpenes as well as steroid while saponins was detected only in *P. nigrum* leaf extract. In this study, alkaloid and tannin were the predominant phytochemical compounds in the *P. nigrum* fruit. On the other hand, flavonoids, tannin and triterpenes were the predominant phytochemical compound present in the *P. nigrum* leaf ethanolic extract (Table 3). The current findings are similar to several study conducted whereby detection of bioactive metabolites of plants from genus *Piper* discovered the presence of alkaloids, flavonoids, terpenoids, amides and chromenes which have the potential in economical, medicinal, insecticidal and molluscidal properties (Ahmad et al. 2012).

Table 2. Percentage (%) of dried yield of extract of *Piper nigrum* fruit and leaf.

Plant part	**Dried powder (g)**	**Crude extract (g)**	**Yield (%)**
Piper nigrum fruit	25.9	3.8	14.67
Piper nigrum leaf	87.5	3.7	4.23

Generally, botanical products are comprised of diverse range of phytochemical constituents including alkaloids, flavonoids, tannins, saponins, steroids, coumarins, quinines and triterpenoids that contribute significantly to biological activities and act as a chemical defence against various insect species (Ghosh et al. 2012). Insects that are exposed to these secondary metabolites will encounter toxic effects that resulted in the interruption of insect physiology at various receptor sites (Ghosh et al. 2012). All these phytochemical constituents had demonstrated a lethal effect towards several immature mosquito species including *Ae. aegypti*, *Ae. togoi*, *Cx. pipiens* (Lee 2000; Park et al. 2002; Siddiqui et al. 2003); *Cx. quinquefasciatus* and *Anopheles* species (Farooq et al. 2014; Krishnappa et al. 2012; Scherer et al. 2010).

Table 3: Phytochemical constituents of ethanolic extract of *Piper nigrum* fruit and leaf.

Chemical constituents	***Piper nigrum* fruit**	***Piper nigrum* leaf**
Alkaloids	+++	++
Flavonoids	++	+++
Saponins	-	+
Tannins	+++	+++
Triterpenes	++	+++
Steroids	++	++

Notes: For flavonoids, tannins, triterpenes and steroids: + weak colour, ++ mild colour, +++ strong color. For alkaloids: + negligible precipitate, ++ weak precipitate, +++ strong precipitate. For saponins: + 1-2 cm froth, ++ 2-3 cm froth, +++ >3 cm froth.

In the current study, both *P. nigrum* fruit and leaf extracts produced significant results on larvicidal activity against early fourth instar larvae of *Ae. albopictus.* A significant increase in the percentage of mean mortality of the mosquito larvae were shown after 24 hours exposure. The increasing of *P. nigrum* fruit extracts from 2.5ppm to 10.5ppm resulted in 2.00±0.29 to 100±0.00% larval mortality (Figure 1). Meanwhile, increasing concentrations of *P. nigrum* leaf extracts from 50ppm to 450ppm resulted in 12.00±0.54 to 100±0.000% of larval mortality (Figure 2). Generally, a significant increase in mean mortality percentage of *Ae. albopictus* larvae after 24 hours exposure was observed following the increased concentrations of the crude extract (Table 4).

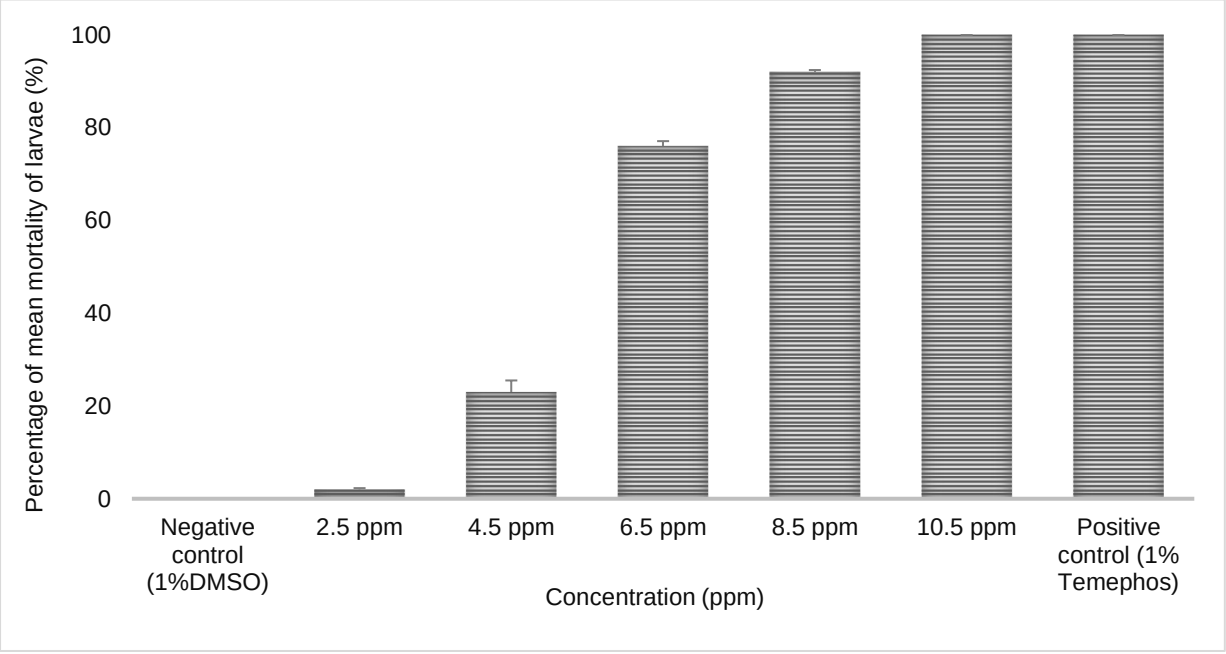

Figure 1. Percentage of mean mortality of *Ae. albopictus* larvae after 24 hours exposure to *P. nigrum* fruit extract with controls

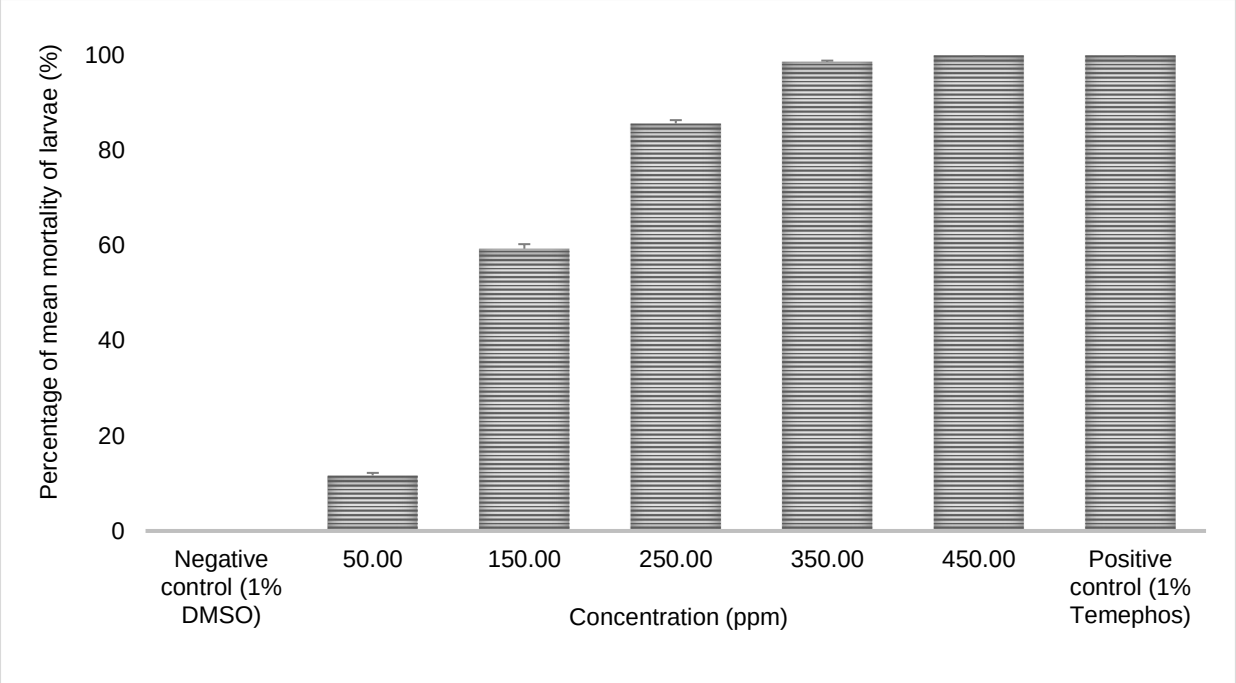

Figure 2. Percentage of mean mortality of *Ae. albopictus* larvae after 24 hours exposure to *P. nigrum* leaf extract with controls

Table 4. Larvicidal activity of *Piper nigrum* fruit and leaf extracts against early fourth-stage instar larvae of *Aedes albopictus*.

***P. nigrum* plant extracts (ppm)**	**% Mortality (Mean ±SEM)**	**Larvicidal activity (95% C.I., ppm)**		**Regression equation**
		LC_{50}	**LC_{90}**	
Fruit		5.07 (4.61-5.54)	7.85 (7.38-8.32)	Y=6.75X+0.24
Negative control (1% DMSO)	0.00±0.00a			
2.5	3.00±0.35a			
4.5	25.00±1.42b			
6.5	78.00±1.42cd			
8.5	92.00±0.58de			
10.5	99.00±0.18e			
Positive control (1% Temephos)	100.00±0.000e			
Leaf		108.89 (108.46-109.33)	213.8 (213.36-214.23)	Y=4.35X-3.86
Negative control (1% DMSO)	0.00±0.00a			
50	12.00±0.54b			
150	59.00±0.97c			
250	86.00±0.71d			
350	99.00±0.23e			
450	100.00±0.00e			
Positive control (1% Temephos)	100.00±0.00e			

Notes:
[1]ppm, parts per million; SEM, standard error of mean values; C.I., confidence intervals; LC_{50}, lethal concentration required to kill 50% of the population exposed; LC_{90}, lethal concentration required to kill 90% of the population exposed.
[2]Mean±SE values followed by different letters within the same column of each concentration of extracts are significantly different (One-way ANOVA followed by Tukey's test, $P<0.05$).

Similar finding was observed in a previous study that showed a decrease in the percentage survival of fourth instar larvae of *Ae. aegypti* when exposed to increased test concentrations of *P. nigrum* leaves methanolic extract solution which resulted in 50% to 90% of larvae mortality at 32.23ppm and 100ppm, respectively (Lija-Escaline et al. 2015). The exposure of *P. nigrum* extract also shown a dosage dependent result in the larval bioassays involving other mosquito species such as *An. gambiae* (Kemabonta et al. 2018) and *Ae. togoi* (Park et al. 2002).

The current study also revealed that the *P. nigrum* fruit extract has shown a remarkable larvicidal potential with lower concentration for LC_{50} (5.07 ppm) and LC_{90} (7.85ppm) as

compared to the leaf extract which required higher concentration for LC_{50} (108.89 ppm) and LC_{90} (213.79 ppm). It is suggested that the present of predominant compound of alkaloid groups such as piperine and several active piperamides in *P. nigrum* fruit ethanolic extract might strongly contributed to the larvicidal efficacy against *Ae. albopictus* larvae as only a lower concentration needed to give 100% larvae mortality compared to the *P. nigrum* leaf ethanolic extract (Scott et al., 2008). Most of the botanical derivatives that owning the mosquito larvicidal properties typically work by shrinking the hemolymph and directly interrupt and damage the nervous system, thus, resulting in death of mosquito larvae and finally disrupting the mosquito's life cycle (Simon-Oke et al. 2015).

The analysis of variance (ANOVA) demonstrated a significant difference in the mean mortality of *Ae. albopictus* between each concentration of *P. nigrum* fruit extract ($F=121.20$, $df=6$, $p<0.05$) and leaf extract ($F=452.87$, $df=6$, $p<0.05$) with the control groups. However, further multiple comparisons analysis revealed that at 8.5ppm and 10.5ppm of the fruit extracts, and 350ppm and 450ppm of the leaf extracts, there were no significant difference in the mean mortality of larvae between all the concentrations with the positive control (1% of Temephos) as both caused 100% larvae mortality after 24 hours exposure. There was no larval mortality in the negative control which indicates that 1% DMSO did not have any larvicidal effect on mosquito larvae.

It is a common phenomenon whereby the variation in the mosquito larvicidal potential is due to various phytochemical constituents at different plant parts (Sukumar et al. 1991). The toxicity level of phytochemical constituents also depends on the geographical distribution, type of solvent, extraction methods as well as plant parts from which they are extracted and plant developmental stages (Sukumar et al. 1991). A recent study conducted by Rajendran et al. (2019) revealed a comparable result where each flower, stem and leaves extract of *Tridax procumbens* showed a different larvicidal efficacy towards both *Ae. aegypti* and *Ae. albopictus*. This variation in larvicidal efficacy of *Tridax procumbens* is common due to different ratio of phytochemicals constituent accumulation in diverse plant parts resulting in differences in mortality rate of *Ae. aegypti* and *Ae. albopictus*. Overall, it is suggested that various phytochemical constituents from different chemical classes and level of toxicity detected in each plant part of the *P. nigrum* extracts may be responsible for the variability in their larvicidal potential against the *Ae. albopictus* larvae (Scott et al. 2008; World Health Organization 2009).

Besides, it was observed that both *P. nigrum* fruit and leaf extracts induced various morphological abnormalities at larval, pupal and adult stages of *Ae. albopictus*. Forms of the morphological abnormalities varied with the concentrations of both extracts. When fourth instars larvae were exposed to higher concentrations of fruit extract (6.5 ppm to 10.5 ppm) and leaf extract (250 ppm to 450 ppm), both extracts caused the greatest mortality at normal larvae (Figure 3), meanwhile, at lower concentrations of fruit extracts (2.5 ppm to 4.5 ppm) and leaf extracts (50 ppm to 150 ppm), the greatest mortality was due to the deformed pupae and deformed adults (Figure 4 and 5). According to Summarwar et al. (2016), the growth regulating effect of botanical extracts not only contributed to prolongation of development period, besides, it also caused the inhibition of morphological development in larvae, pupae and emerging adults and finally reducing the adult emergence.

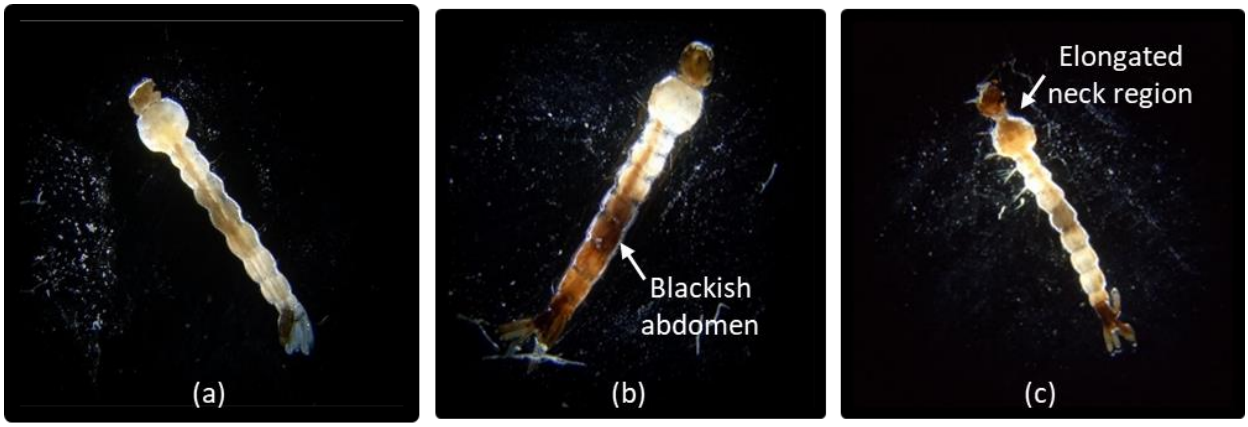

Figure 3. (**a**) Normal forth-instar *Ae. albopictus* larvae, (**b**) Deformed larvae with blackish abdomen, and (**c**) deformed larvae with elongated 'neck' region induced by *P. nigrum* extracts

The abnormalities that were mostly detected in this study include deformed larvae with darkening of abdomen and elongated 'neck' region (Figures 3b-c), deformed and decolorized pupae-adult intermediates with straight abdomen, dwarf pupae with retarded abdomen, partially exuviated pupae remained attached to the skin, as well as deformed pupae with elongated wing pads and distorted digestive tract and posterior abdominal segment (Figure 4a to 4f). At adult stage, deformities observed was a completely extruded main trunk including the head and thorax with the rest of the abdomen enclosed in the pupal exuvium. Several adults that nearly emerged were also found dead with the appendages remained attached to the pupal exuvium (Figures 5a-c). These results indicated a metamorphosis-inhibiting effect of the *P. nigrum* plant extracts, which is possibly based on the disturbance of hormonal control (Grzybowski et al. 2012).

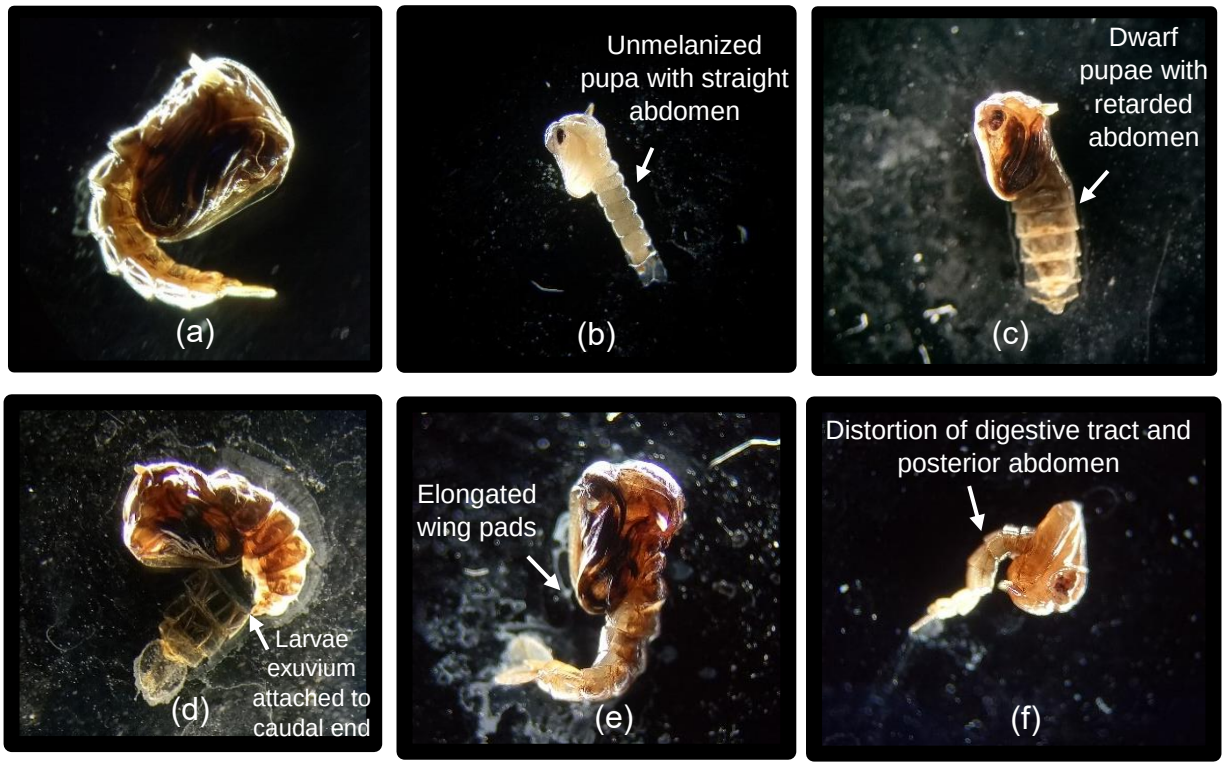

Figure 4. Morphological deformities induced by *P. nigrum* extracts (**a**) normal dead brown pupae, (**b**) the dead pupae remained unmelanized with abdomen is held abnormally in straight position, (**c**) dwarf pupae with retarded abdomen, (**d**) deformed pupae with larval exuvium remained attached at the caudal end, (**e**) deformed pupae with elongated wing pads (**f**) deformed pupae with distortion of digestive tract and posterior abdominal segment

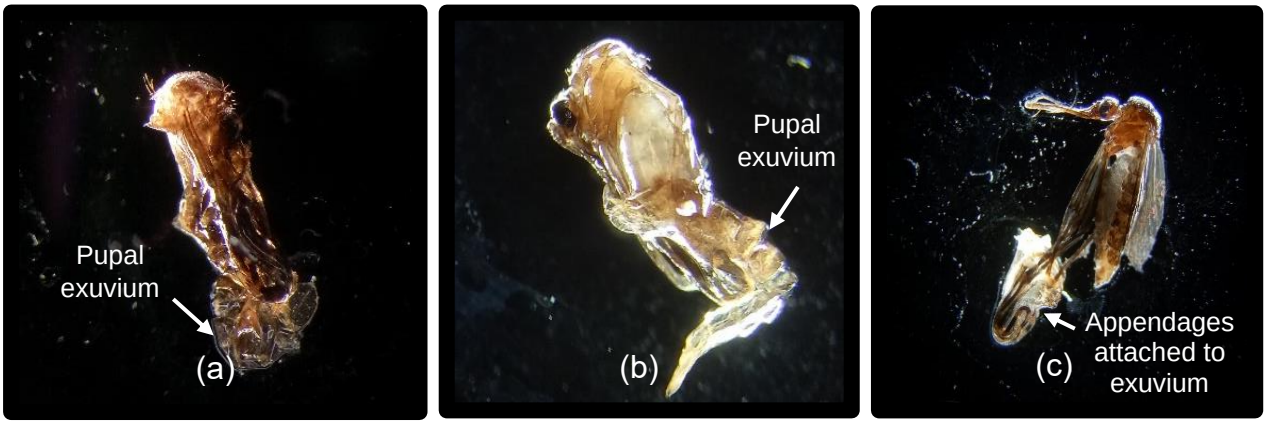

Figure 5. Morphological abnormalities induced by *P. nigrum* extracts where at the adult stage (a) and (b) a completely extruded main trunks including the head and thorax with the rest of the abdomen enclosed in the pupal exuvium, (c) the adults found dead with the appendages remained attached to the pupal exuvium

Similar study was reported by Soonwera & Phasomkusolsil (2016) where various morphological deformities in each developmental stage of *Ae. aegypti* and *An. dirus* were detected when exposed to both *Cymbopogon citratus* and *Syzygium aromaticum* oils that finally led to larval mortality. All these morphological deformities could be due to the interference of hormonal control and interruption in chitin synthesis throughout the molting process by a metamorphosis-inhibiting effect for the botanical extracts. (Soonwera & Phasomkusolsil 2016).

In addition, the exposure of *Aedes* and *Cx. pipiens* larvae to a high concentration of both *Ipomoea cairica* and an aquatic plant, *Echinochloa stagninum* extracts have also resulted in the morphological deformities where darkening and twisting of abdomen as well as producing abnormal pupae and adults that finally caused mortality when compared to normal larvae (Ahbirami et al. 2014; Bream et al. 2010). Other than that, the study on larvicidal efficacy of aqueous leaf extract of *Spathodea campanulata* against *Ae. aegypti* larval morphology have led to the dechitinized of larva with detrimental effect on digestive tracts with exuvium still remained attached to the dead larvae (Saranya et al. 2013). Hence, it is suggested that a variety of phytochemical constituents detected in botanical extracts may possess larvicidal properties, and the synergistic effects between diverse phytochemical constituents may be responsible for morphological abnormalities in different developmental stages of mosquitoes that finally caused individual mortality (Grzybowski et al. 2012; Soonwera & Phasomkusolsil 2017).

CONCLUSION

Both *P. nigrum* fruit and leaf extracts possess larvicidal properties against early fourth instar larvae of *Ae. albopictus.* The fruit extracts were more effective than the leaf extracts. Generally, this study showed that the higher concentrations of the crude extracts caused a significant increase in the percentage of mean mortality of *Ae. albopictus* larvae after 24 hours of exposure. It is believed that diverse phytochemical constituents detected in *P. nigrum* extracts may possess mosquito larvicidal properties as well as contributing to the morphological abnormalities that caused the mortality of the mosquitoes. Therefore, *Piper nigrum* from Sarawak deserved to be considered for further development as a potential biocontrol agent for *Ae. albopictus*.

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