Mosquito Larvicidal Activity of *Eucalyptus deglupta* Crude Extract and Analysis of its Bioactive Compounds

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Abstract---The mean lethal concentration (LC_{50} and LC_{90}) and mean lethal time (LT_{50} and LT_{90}) of *Eucalyptus deglupta* leaf crude extract was evaluated on late third instar laboratory-reared and field-collected larvae of *Aedes sp.* LC_{50} and LC_{90} of aqueous and methanolic extracts showed significant differences at 0.05 level having values of 285.73 ppm to 623.90 ppm and 764.73 to 1398.37 ppm, respectively. LT_{50} and LT_{90} were 389.54 to 314.72 hours for the 40000 ppm extract concentration. Mean mortality percentage in both bioassays revealed its dose-response relationships having a 100% mortality rate on highest extract concentration. Pronounced larvicidal effect and longer efficacy stability were recorded in methanolic extract showing the presence of secondary metabolites such as saponins, phenols, tannins, cardiac glycosides, terpenoids, quinones, anthocyanidin, polyphenols and flavonoids. Flavonoids, saponins and terpenoids are noted for their mosquito larvicidal properties. Likewise, irregular and abnormal movements were eminent after post-treatments in both bioassays. At higher concentration, the larvae showed restless movement for some time then settled at the bottom of the cup and died slowly before the transition into pupal stage. This study suggests the biocidal potential of *E. deglupta* extract against the larvae of *Aedes sp.*

***Keywords*—instar, larvicidal, mean lethal concentration, mean lethal time**

I. Introduction

Dengue ranks among the most important mosquito-borne viral diseases in the world. The number of dengue cases in the Philippines is yearly increasing. Although the Department of Health has already giving free vaccination, developing of new strategies for selective mosquito control is still highlighted. In fact, many researches have reported on the effectiveness of plant extracts against mosquito larvae since, they constitute a rich source of bioactive compounds that are target-specific, ecological safe, non-development of resistance, reduced number of applications, higher acceptability, and suitability for rural areas[1,2,3,4].

In the Philippines, *Eucalyptus deglupta* is widely distributed in the lowland primary forests of Mindanao but has already been introduced to other parts of the country. It is commonly known as the Mindanao gum, rainbow eucalyptus or Bagras tree. They are usually planted for pulp and wood production used for making papers, furniture, moulding, flooring, construction lumber, boat building, veneer and plywood. Such activities give its foliage as residual. And, in spite that the country is heavily infected of dengue virus, no studies have been reported to test its efficacy against the larvae of *Aedes sp.*

Taking into account the high levels of morbidity and mortality of dengue cases, infliction on great economic loss, and social disruption; a necessity of developing mosquito larvicides of being environmentally favorable is the primary goal of this study. As an input, this study aimed to identify the bioactive compounds present in *E. deglupta* and evaluate its larvicidal activity on *Aedes sp.* Specifically, it sought to determine the mean lethal concentration of its leaf crude extract onto laboratory reared mosquito larvae under laboratory condition; determine the mean lethal time of its leaf crude extract onto field collected mosquito larvae under simulated field trials and; describe the changes in the physical behavior of larvae exposed to varying concentrations of the leaf crude extract.

II. Materials And Methods

Study site

The laboratory tests and simulated field trials were conducted at the Science Laboratory of College of Arts and Science, Partido State University, Goa, Camarines, Philippines.

Mosquito culture

Larvae of *Aedes sp.* were collected from the stagnant water in nearby barangays and maintained under laboratory condition which were periodically fed with a diet[5]. Pupae were collected, transferred and placed in a glass modified insectarium (14x20x13 inches) for adult emergence. Adults were continuously provided with a 10% sucrose solution and multivitamins syrup. The mosquitoes were identified using the keys of Rueda (2004). On the 5th day, blood meals were given for blood-feeding female mosquitoes. For the oviposition, filter papers were dipped in each three glass petri dishes filled with 10ml of tap water and placed inside the insectarium. Eggs were then transferred to beaker with tap water for egg hatching under laboratory conditions. Mosquito colony was kept at 27-32°C, 75-85% relative humidity, with a photoperiod of 12:12h light/dark cycle.

Plant collection and extraction

Fully developed fresh leaves of *E. deglupta* were collected in the month of February 2016 at Barangay Sagrada, Pili, Camarines Sur, Bicol Region, Philippines. The plant was authenticated by the Museum on Natural History, University of the Philippines Los Baños, Laguna.

The collected leaves were washed with dechlorinated water, shade dried at room temperature, pulverized using a mechanical grinder and macerated using 90% methanol and distilled water as solvents.

Phytochemical analysis of the plant extract

Crude extracts were qualitatively and phytonutriently screened at the Institute of Chemistry, University of the

Philippines Los Baños, Laguna, Philippines and at Food and Nutrition Research Institute of the Department of Science and Technology, Bicutan, Taguig, Philippines, respectively.

Larvicidal bioassay under laboratory condition

Larvicidal activity was carried out by following the standard procedure of World Health Organization[7] with slight modification. Two hundred milligrams of crude extract was volumetrically diluted separately in 200ml methanol and water to obtain the test concentrations of 200ppm, 400ppm, 600ppm, 800ppm, and 1000ppm which was individually added to 100ml of dechlorinated water. Batches of 25 late third instars larvae were introduced to each of the test concentrations. In the methanolic control group, mosquito larvae were exposed to one milliliter of methanol in 100ml of dechlorinated water. While, for the aqueous control group, untreated distilled water was prepared for the trials. Each concentration was replicated five times and was run three times on different days maintained in 28-32°C and a photoperiod of 12 hour light followed by 12 hour dark (12L:12D). Larval mortality was recorded after 24 hours fasting exposure. Moribund larvae were counted and added to dead larvae for calculating percentage mortality which is equivalent to the number of dead larvae over the number of larvae introduced multiplied to 100.

Larvicidal bioassay under simulated field condition

Field trials were conducted according to the methods recommended by World Health Organization (2005) with slight modification[8,9]. Plastic containers in 17 cm diameter were filled with four liters of collected rainwater and were set outdoors around the vicinity of the study site. Containers were added with larval food and covered with mosquito net to prevent oviposition of field mosquitoes and deposition of debris. Containers were placed under a shade to prevent direct exposure to rain and sunlight then, allowed to stand for 24 hours prior to the experiment. Batches of 25 late third instar field collected mosquito larvae were introduced separately into each containers. After three hours of larval acclimatization, the containers were treated with 1% stock solution of the extract in dilution of 40000ppm, 36000ppm, 32000ppm, 28000ppm and 4ml of methanol for the control set up. The containers were examined after 24 hours and live larvae were counted to score post-treatment larval mortality. Five treatments in three replicates were carried out with residual activities being monitored until no mortality was recorded. Larvae mortality was recorded at 48 hours post treatment after each introduction period. The trial for each formulation was terminated when no mortality was observed.

Statistical analysis

The percentage of larval mortality was corrected by Abbott's formula:

$$Mortality\ (\%) = \frac{X - Y}{X}\ 100$$

where, X = percentage survival in the untreated control
Y = percentage survival in the treated sample

Results were analyzed using log-probit method of Finney and probit software of SPSS. This software estimates the slope of regression lines and 50% and 90% lethal concentration (LC_{50} and LC_{90}) with 95% confidence intervals (CIs). Chi-square values were considered significant at $P<0.05$ level.

In the simulated field trials, the mean number of dead larvae from all replicates of each treatment and the control was calculated for each day of observation and also corrected with Abbott's formula. The percentage of mortality was computed. The mean lethal time (LT_{50} and LT_{90}) and its 95% CI were determined using logistic or probit regression analyses. The post-treatment day up to which 50% reduction is observed for each treatment was compared to determine the residual effect and optimum application dosage.

III. Results And Discussion

Bioactive compounds present in *E. deglupta*

The results of preliminary qualitative phytochemical analysis of tested leaf crude extract of *E. deglupta* revealed the presence of some secondary metabolites (Table I).

TABLE I: Phytochemical present in methanolic and aqueous extracts of *E. deglupta* leaves

Phytochemicals	Solvent	
	90% Methanol	Aqueous
Saponins	+	+
Phenols/Tannins	+	+
Flavonoids	-	-
Cardiac Glycosides	+	+
Terpenoids	+	+
Phytosterols	-	-
Quinones	+	-

Saponins, phenols, tannins, cardiac glycosides, terpenoids were both present in the extracts. However, quinones was only noted in 90% methanolic extract.

The methanolic extract was further subjected for phytonutrient analysis since more secondary compounds were obtained from it. From the test, a 6.78±0.00 g/100g of flavonoids was traced; while, a minute amount of anthocyanidin, 2.15±0.04 g/100g sample, was detected. Presence of phenols was recorded in phytochemical test and with the phytonutrients test, an amount of 9.46±0.09 g/100g sample was noted (Table II).

TABLE II: Phytonutrients test of methanolic crude leaf extract of *E. deglupta*

Analysis (Unit)	Method Used	Results
Total Anthocyanidin (g catechin equiv./100g sample)	Colorimetric	2.15±0.04
Total Polyphenols (g gallic acid equiv./100g sample)	Colorimetric	9.46±0.09
Total Flavonoids (g catechin equiv./100g sample)	Colorimetric	6.78±0.00

Mean lethal concentration of *E. deglupta* crude extract under laboratory condition

The larvicidal activity of the methanolic and aqueous extracts of the *E. deglupta* against the larvae *Aedes sp.* was determined through mosquito larval bioassay. Mortality was noted in 200 ppm, 400 ppm, 600 ppm, 800 ppm, and 1000 ppm concentrations of extracts after 24 hours of exposure (Table III).

TABLE III: Mean percentage mortality of *Aedes sp* larvae in the laboratory bioassay after 24 hours exposure

Concentration (Mean Percent of Mortality ± SD)	Solvent	
	90% methanol	Aqueous
200 ppm	35.5 ± 2.6^{Da}	1.9 ± 0.7^{Db}
400 ppm	58.4 ± 2.9^{Ca}	6.7 ± 2.7^{Db}
600 ppm	85.3 ± 2.7^{Ba}	27.2 ± 0.7^{Cb}
800 ppm	100.0 ± 0.0^{Aa}	46.9 ± 3.5^{Bb}
1000 ppm	100.0 ± 0.0^{Aa}	80.3 ± 3.0^{Ab}
Control	0.0 ± 0.0^{Ea}	0.0 ± 0.0^{Ea}

Means followed by the same uppercase letter(s) within a column are not significantly different at the 0.05 level (ANOVA-Scheffe).

Means followed by the same lowercase letter(s) within a row are not significantly different at the 0.05 level (t-test).

Table III shows no mortality was recorded in the control group both in methanolic and aqueous solvents. However, the least percentage mortality was observed in 200 ppm in both solvents used. The larval mortality in methanolic extract provided a 100% death as compared in aqueous extract with only 80.3±3.0 %. In 800 ppm concentration, the methanolic extract can already kill 100% population of the tested mosquito larvae. Statistically, in the methanolic extract, it showed that the concentration of 1000 ppm was not significantly different at 0.05 level to 800 ppm. Meanwhile, the 1000 ppm concentration was significantly different to 600, 400, 200 ppm and to the control group. However, in the aqueous extract, the 1000 ppm concentration was significantly different from 800, 600, 400, 200 ppm and to the control group. Likewise, in aqueous extract, the 400 and 200 ppm were not significantly different to each other at the 0.05 level. Using the T-test in comparing the efficacy of the two solvents used, results showed that they are significantly different for all the tested concentrations.

Based on the results of percentage mortality, it can deduce that methanolic extracts can cause death at lower concentration as compared to aqueous extracts. It is further noted that the leaf extracts exhibited a concentration dependent activities against mosquito larvae since the percentage mortality were observed to increase with increasing concentrations of the plant extracts.

TABLE IV: Larvicidal effect of methanol leaf extract of *E. deglupta* against the late third instar larvae of *Aedes sp* treated in 24 hours

Solvents	Conc. (ppm)	% mortality ± SD	LC_{50} (ppm) [LCL-UCL]	LC_{90} (ppm) [LCL-UCL]	Regression	Chi-Square x^2
90% Methanol	Control	0.0±0.0	285.73 (219.88 - 342.71)	623.90 (512.61-851.61)	$y = -9.28 + 3.78x$	9.630 (df= 3, p=0.201)
	200	35.5±2.6				
	400	58.4±2.9				
	600	85.3±2.7				
	800	100.0±0.				
	1000	100.0±0.				
Aqueous	Control	0.0±0.00	764.73 (672.71 - 897.65)	1398.37 (1121.61 - 2187.35)	$y = -14.10 + 4.89x$	4.714 (df= 3, p=0.194)
	200	1.9±0.7				
	400	6.7±2.7				
	600	27.2±0.7				
	800	46.9±3.5				
	1000	80.3±3.0				

LC_{50} – Lethal concentration that kills $_{50}$% of the exposed larvae, LC_{90} – Lethal concentration that kills $_{90}$% of the exposed larvae, LCL – lower 95% confidence limit, UCL – upper 95% confidence limit, Regression Equation was obtained from the Probit model where y refers to the percent of mortality and x refers to the concentration (ppm) transformed using the base 10 logarithm, SD – standard deviation, df – degrees of freedom, p – p-value (Sig.).

Table IV summarized the mean lethal concentration (LC_{50} and LC_{90}) values of two extracting solvents on *Aedes sp.* larvae after 24 hours exposure. It shows that the aqueous extract found to be less effective as compared to methanol extract since, it has higher LC_{50} and LC_{90} values of 764.73 ppm and 1398.37 ppm, respectively. Also, the aqueous extract has higher lower 95% confidence limit (LCL) and upper 95% confidence limit (UCL) giving values of 672.71 to 897.65 ppm and 1121.61 to 2187.35 ppm, respectively. Conversely, results showed that in methanolic solvent, it gave a LC_{50} of 285.73 ppm and LC_{90} of 623.90 ppm. Its 95% LCL and UCL values of 219.88 to 342.71 and 512.61 to 851.61, respectively, were significantly more effective than to the aqueous solvent in terms of larvicidal activity.

Mean lethal time of *E. deglupta* crude extract under simulated field condition

Since the larvicidal activity was found to be more efficient in methanolic extract, it was further evaluated in semi field trials. Results of mean percentage mortality of *Aedes sp.* treated with crude leaf extract of *E. deglupta* in semi field trials with different concentrations at different exposure time periods are presented in Table V.

TABLE V: Mean percentage mortality of *Aedes sp* larvae in semi field trials after 48 hours exposure

Hours	Concentration (Mean Percent of Mortality)				
	28000 ppm	32000 ppm	36000 ppm	40000 ppm	Control
48	100.0	100.0	100.0	100.0	0.0
96	100.0	100.0	100.0	100.0	0.0
144	100.0	100.0	100.0	100.0	0.0
192	97.32	100.0	100.0	100.0	0.0
240	45.32	100.0	100.0	100.0	0.0
288	0.0	56.0	100.0	100.0	0.0
336		12.0	46.68	69.32	0.0
384		0.0	33.32	57.32	0.0
432			14.68	32.0	0.0
480			0.0	13.2	0.0
528				0.0	0.0

The results in Table V indicate that methanolic extract at different concentrations had caused different levels of mortality and residual activities. As shown in the table, it can be interpreted that the higher the concentration, the longer is the residual activity of the extracts. All of the concentrations attained 100% mortality at different time interval. However, the 50% residual activity of each concentrations only lasted for 240, 288, 432, and 480 hours for 28000 ppm, 32000 ppm, 36000 ppm, and 40000 ppm, respectively. No mortality was recorded on the control group.

TABLE VI: Mean lethal time of *Aedes sp* in *E. deglupta* leaf crude extract under simulated field trial

Conc. (ppm)	LT_{50} (hrs) (LCL-UCL)	LT_{90} (hrs) (LCL-UCL)	Regression Equation	Chi-Square x^2
40000	389.54 (372.41-406.50)	314.72 (286.74-334.37)	$y = 35.84 - 13.84x$	4.880 (df=9, p=0.845)
36000	355.28 (339.77-369.98)	286.14 (270.02-313.75)	$y = 41.34 - 16.21x$	7.660 (df=8, p=0.467)
32000	295.85 (283.92-306.93)	262.04 (240.07-274.88)	$y = 60.08 - 24.31x$	0.985 (df=6, 0.986)
20000	265.27 (251.37-279.66)	216.22 (192.19-231.46)	$y = 34.99 - 14.47x$	4.948 (df=5, p=0.422)

LT_{50} – Lethal time that kills 50% of the exposed larvae, LT_{90} – Lethal time that kills 90% of the exposed larvae, LCL – lower 95% confidence limit, UCL – upper 95% confidence limit, Regression Equation was obtained from the Probit model where y refers to the percent of mortality and x refers to the concentration (ppm) transformed using the base 10 logarithm, SD – standard deviation, df – degrees of freedom, p – p-value (Sig.).

Data on the mean lethal time of the late third instar larvae of *Aedes* sp. treated with different concentrations of the leaf crude extract of *E. deglupta* under simulated field conditions at the end of 528 hour are presented at Table VI. The 50% and 90% lethal time of the extracts on *Aedes sp.* showed its relationship with different concentrations. The residual activity of the lower concentration lasted lesser hours as compared to those of extracts in high concentrations. The LT_{50} of 40000 ppm, 36000 ppm, 32000 ppm, and 28000 ppm showed an average of 389.54, 355.28, 295.85, and 265.27 hours, respectively. Whereas, the LT_{90} of 314.72, 28614, 262.04, and 216.22 hours were recorded in 40000 ppm, 36000 ppm, 32000 ppm, and 28000 ppm, respectively. As time increased, the effectiveness of the tested extract depreciated thus, resulting to lower rate of mortality. Similarly, the lower the concentration of extract, the shorter the period of its efficacy in the mortality of *Aedes sp.*

Table VI also shows the LCL and UCL values of the 50% and 90% lethal time against the larvae of *Aedes* sp. in the simulated field condition. In LT_{50} and LT_{90}, of the 40000 ppm extract concentration, the LCL and UCL were 372.41 to 406.50 hours and 286.74 to 334.37 hours, respectively. This is significantly different to the rest concentrations tested on trials. The lowest range of 251.37-279.66 and 192.19 to 31.46 hours for LT_{50} and LT_{90} were recorded in 20000 ppm concentration of the *E. deglupta* methanolic crude leaf extract.

One of the strategies of WHO in contending tropical diseases is to destroy their vectors or intermediate hosts through applying insecticides into their larval habitats since, they could be easily manipulated in this stage due to their immobility. Chemical pesticides are usually used for several decades now for its control as they have a quick detrimental effect. However, their unselective use resulted in several problems such as resistance and revival of pests, elimination of natural enemies, toxic residues in food, water, air and soil which affect human health and disrupt the ecosystem, leading to the threat that their continued use may further harm the environment[10].

The plant tested in the present study is grown widely as wood plant in the island of Mindanao, Philippines. The result of the present study showed that the crude leaf extract of *E. deglupta* have significant larvicidal property against *Aedes* sp. Such results may offer tremendous advantage as controlling agents which is economically viable, eco-friendly approach, and harmless to beneficial insects when adopted on a large scale. The plant's larvicidal activity is supported by the presence of phytochemicals such as saponin, phenols, tannins, cadiac glycosides, terpenoids, quinones, anthocyanidin, polyphenols, and flanovoids which serve as huge storage of compounds that have biological action[11].

Phytochemicals of plants possess a broad scope of bio-control potential[12] such as plant's defense mechanisms against microorganisms, insects and herbivores[13]. However, as reported by Shalaan et al.[14], bioactivity of phytochemicals against mosquito larvae can significantly vary depending on plant species, plant parts, age of plant, and mosquito species. Also, according to Raymond[15], total mortality was consistent positively correlated with insecticide concentrations and duration of exposure. The methanol leaf extract of *Clitoria ternatea* L. showed a dose-dependent larvicidal activity against *An. Stephensi*[16]. Similarly, in the study it showed that as the concentration of methanolic extract of *E. deglupta* increased from 200 ppm to 1000 ppm, the percentage mortality were also increased. The mortality of methanolic extract at 800 ppm and 1000 ppm were 100% after 24 hours exposure under laboratory condition. Whereas, only 80.3% mortality was achieved in the aqueous extract. The aqueous extract's slow mortality effect on mosquito larvae could be ascribed to the lower concentration of saponins, phenols, tannins, cardiac glycosides, and terpenoids. Further, the efficacy of plant extracts as larvicide may be due to the use of solvent as extracting media because different polarity of the solvents could attribute to the phytochemicals that it can extract. In the present study, it showed that quinones compound was extracted only using the methanol as the solvent. The absence of quinones could be a factor for having less detrimental effect in aqueous extract, for it is the compound responsible for the aromatic smell of the extract which believe to be of great factor in larvicidal activity. Conversely, methanol is widely used as extracting agent mainly because of the many polar and non-polar compounds dissolved on it with great freedom. Moreover, it evaporates at lower temperature so, it can easily be separated from the extract and, it brings out trace amounts of various substances which is less toxic than other extracting solvents[17].

The larvicidal efficacy of the extract is not as promising as that of synthetic insecticides commonly use today but, the present results are comparable to those of earlier authors who

worked on various plant extracts as larvicides against different mosquito species. Even if *E. deglupta* species remains unexplored on its larvicidal activity, some species of eucalyptus are already well studied on the same purpose. Essential oils of *Eucalyptus camaldulensis* and *E. tereticornis* demonstrated larvicidal activity against *Anopheles stephensi*[18]. The ρ–cymene of *E. pellita* showed a repellent activity on *Aedes aegypti* mosquitos[19]. Larvicidal bioassay showed that *E. grandis* essential oil has a LC_{50} of 32.4 ppm, while both pinenes presented higher toxicity (LC_{50}: 15.4 and 12.1 ppm, respectively), but 1,8-cineole presented a lower activity (LC_{50}: 57.2 ppm)[20]. According to GC-MS analyses, the major constituents of the leaf essential oils were α-pinene, ρ–cymene, and α-phellandrene form *E. camaldulensis* and 1,8-cineole from *E. urophylla*. Results obtained from the larvicidal tests, using the leaf essential oil from *E. camaldulensis* had excellent inhibitory effects against both *Ae. egypti* and *Ae. albopictus* larvae[21]. Watanabe et al. [22] isolated a new compound, eucamol and 4-isopropylbenzyl alcohol from *E. camaldulensis*. These new compounds were compared with DEET and proved to be highly active against *Ae. aegypti*.

Saponins and tannins are known to possess medicinal and pesticidal properties and are responsible for the insecticidal and toxicity to other animals[22,24,25,26]. Saponins isolated from *Achyranthes aspera*[26], *Sapindus emarginatus*[27] and, *Balanites aegyptica*[28,29] possessed larvicidal efficacy against *Ae. Aegypti*, *Cx. quinquefasciatus* and *Stegomyia aegypti*. A small fraction proved to be sufficient to kill 50% of the larvae before the formation of adults thus, suggesting that saponins is a cheap way to reduce and control mosquito population.

Moreover, saponins are known to have various biological properties. They have membrane-permeabilising, haemolytic, antioxidant, anti-inflammatory, immunostimulant, and anticarcinogenic activities. They are also responsible in lowering food intake, reducing weight, cause retardation and disturbances in development, and decrease reproduction in pest insects[30,31,32,33]. The mechanism underlying these actions is still largely unknown. Saponins are freely soluble that can be extracted in both aqueous and organic solvents.

It was also noted that the methanolic extract of *E. deglupta* leaves emitted a distinct aromatic scent which may be attributed to its essential oils (EOs) property. The family Myrtaceae, where the tested plant belongs, is considered to possess different EOs. EOs are the by-products of plant metabolites that are present as droplets of fluid in the leaves, stem, flowers and fruits, bark, or roots of plants[34]. In nature, the aromatic characteristics of EOs serve various functions for the plants including defense materials against pests and/or microorganisms. Its component include two groups of distinct bio-synthetically origin[35]. The main group is composed of terpenes and terpenoids, and the other of aromatic and aliphatic constituents. In the phytochemical screening of the tested plant in this study, presence of terpenoids both in methanol and aqueous extract were detected. Although, the aromatic scent was more pronounced in the methanolic extract compared to aqueous extract.

Changes in the physical behavior of larvae treated with *E. deglupta* crude extract

Compared to control group, physical changes were noticed in *E. deglupta* leaf extract treated groups. This may due to the presence of neurotoxins in the plant extract. After 24 hours post-treatment of extracts under the laboratory bioassay, larvae become abnormal and irregular in movement. At higher concentration, the larvae showed restless movement for some time then settled at the bottom of the plastic cup and died slowly before the transition into pupal stage. Similar thing mode of actions were observed under the simulated field trials. There is susceptibility of mosquito larvae to surface the water, observing that the extracts increased the tendency of tracheal flooding and chemical toxicity in the tracheal system[36].

The mode of action of this leaf extract on mosquito larvae are not known, but previous studies demonstrated that phytochemicals interfered with the proper functioning of mitochondria more specifically at the proton transferring sites. Other studies found that phytochemicals primarily affect the midgut epithelium, the gastric caeca and the malphigian tubules of mosquito larvae which is responsible for its malfunctions[21]. Furthermore, the phytochemical components of the crude extracts maybe more effective compared to the individual active compounds due to natural synergism that discourages the development of resistance in the vectors[37].

Plants extracts can affect pest behavior which includes repelling the pest or prohibiting its feeding activity. It can also affect the pest physiology through moulting and respiration inhibition, growth and fecundity reduction, and cuticle disruption[38]. Moreover, exposure to varying mixtures of the biosynthetically different compounds found in plant extracts can delay the evolution of resistance[39].

Another possible cause of death to treated insects according to Al-Sharook et al.[39], is may be due to the inability of the molting bodies to swallow sufficient volume of air to split the old cuticle and expand the new one during ecdysis or metamorphosis inhibiting effect of the plant extract which is possibly based on the disturbance of the hormonal regulation. Also, the 100% mortality might be due to the chemical constituents present in the methanol leaf extract of *E. deglupta* that arrest the metabolic activity of the larvae, causing its high percentage of mortality.

ACKNOWLEDGMENT

A research grant from the Philippine Council for Health Research and Development- Department of Science and Technology through Bicol University in behalf of the Bicol Consortium for Health Research and Development and Partido State University is gratefully acknowledge.

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