GLOBAL ACADEMIC RESEARCH INSTITUTE

COLOMBO, SRI LANKA

GARI International Journal of Multidisciplinary Research

ISSN 2659-2193

Volume: 02 | Issue: 02

On 30th June 2016

http://www.research.lk

Author: Thimali Rathnayaka, Koshala De Silva

BMS, Sri Lanka

GARI Publisher | Medicinal Plants | Volume: 02 | Issue: 02

Article ID: IN/GARI/ICHM/2016/272 | Pages: 153-175 (23)

ISSN 2659-2193 | Edit: GARI Editorial Team

Received: 20.03.2016 | Publish: 30.06.2016

LARVICIDAL EFFECTS OF *LANTANA CAMARA* ETHANOL LEAF EXTRACT ON *ANOPHELES TESSELLATUS*

[1] Thimali Rathnayaka , [2] Koshala De Silva

[2]*cell.bio@bms.lk*

ABSTRACT

Mosquitoes transmit thoughtful human diseases, causing millions of deaths every year and the development of resistance to chemical insecticides resultant in recovering vectorial capacity. Plants can alternative sources of mosquito control agents. The present study assessed the role of natural products of plant origin with insecticidal properties for control of insect vectors. The résistance to chemical insecticides among mosquito species have been considered as a setback in vector control. Botanical larvicides are including conspicuously as alternative to synthetic chemical insecticides which are less degradable and toxic to non-target organisms. The aim of this study was larvicidal effects of Lantana camara plant extract on Anopheles tessellatus. The larvicidal activity and phytochemical showing of ethanol extract of leaves of Lantana camara were investigated in the laboratory against third instar larvae of Anopheles tessellatus for 24 and 48 hours and Lantana camara extracted were prepared and four different concentrations. Statistical analysis showed significant differences between the higher mortality for larvae. The highest (100%) mortality in the larvae occurred on treatment with 2000 ppm extract of L.camara. The maximum adult mortality was detected in the leaf extract (LC_{50} 1000 ppm for 24 hours and LC_{50} 199.526 ppm for 48 hours) while no mortality was noticed in the control groups. The L.camara plant extracts effect on larvae with introduction of apoptosis or effect of larvae DNA. Therefore, done in a DNA fragment analysis and observed histological changes in tissue of larvae. The extracts of Lantana camara plant showed potent larvicidal efficacy. This study suggests that, the leaf extracts of the Lantana camara can considered as promising larvicide against Anopheles tessellates third stage larvae.
Keywords: Anopheles tessellatus, Lantana camara, Larvae, Mosquitoes, Malaria

INTRODUCTION

Vector-borne diseases

A vector is a living organism that transfers an infectious agent from an infected animal to a human or another animal. Vector-borne diseases are transmitted by the bite of infected arthropod species, such as mosquitoes, ticks, triatomine bugs, sandflies, and blackflies (Confalonieri *et al.*, 2007). Arthropod vectors are ectothermic and sensitive to climatic factors. However, climate is only one of several factors influencing vector distribution, such as habitat destruction, using land, pesticide application, and host density (Roberts, Schmidt, and John, 2008).

Mosquitoes are best known vector for disease and major public health problem throughout the world (World Health Organization, 2016). Among the 3000 species of mosquitoes noted worldwide, more than a hundred species are accomplished of transmitting various diseases in human and other vertebrates. Vector-borne infectious diseases are malaria, dengue fever, yellow fever, and plague, cause a significant fraction of the worldwide infectious disease burden; indeed, half of the world's population is infected with at least one type of vector-borne pathogen (Ricci *et al.*, 2012).

Over the years, synthetic insecticides were introduced. Even though, these are effective; insect tends to develop resistance to such products. These problems have required the essential for search and progress of environmentally safe, biodegradable, low cost, native methods for malaria vector controlling which can be used as an alternative to synthetic insecticides (Jirakanjanakit *et al.*, 2007).

Malaria

Malaria is caused by one of four species of the *Plasmodium* species parasite transmitted by female *Anopheles spp*. mosquitoes (Kuhn, Campbell-Lendrum & Davies, 2002). Historically malaria was endemic in Europe, but it was finally eliminated in 1975 through a number of factors connected to socioeconomic development. Any role that climate played in malaria reduction would have been small. However, malaria transmission is intricately connected to meteorological conditions such as temperature and precipitation (Guerra *et al.*, 2008).

Epidemiology of Malaria

The World Health Organization (WHO) has published global estimates of the number of people that die from malaria. In these 15 years the global death toll has been cut in half: from 839, 000 deaths in 2000 to 438,000 in 2015. Among these diseases, malaria remains the most thoughtful vector-borne disease affecting some 300-500 million people and 1.4 to 2.6 million deaths yearly throughout the world. More than 40% of the world population lives in areas prone to malaria (Figure 01) (Moss *et al.*, 2015). The World Health Organization has certified that Sri Lanka is a malaria-free nation, in what it called a truly extraordinary achievement. But, the WHO said, the country had begun an anti-malaria campaign that successfully targeted the mosquito-borne parasite that causes the disease (WHO, 2015).

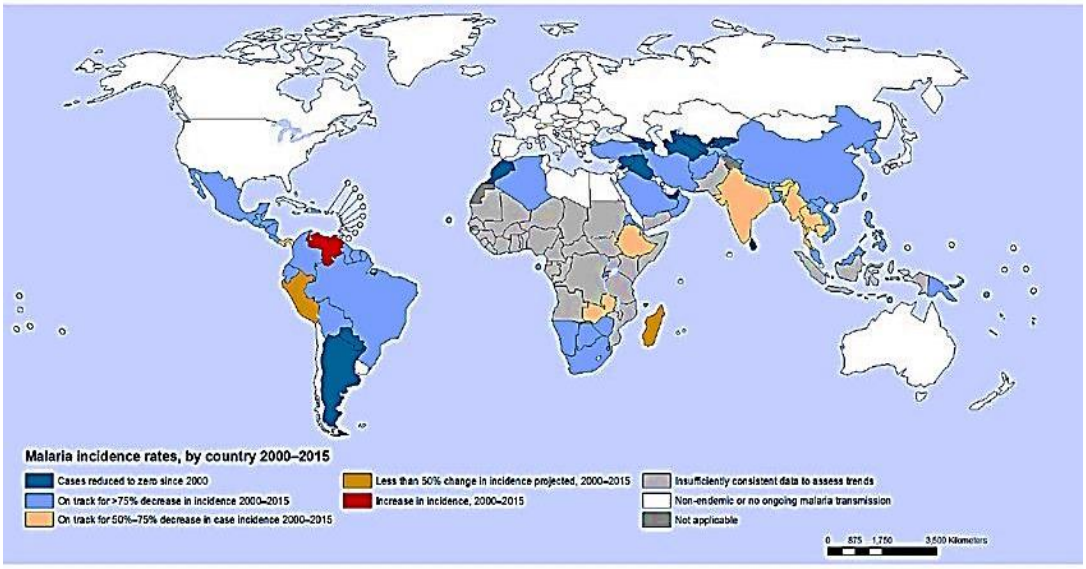


Figure 01. Malaria incidence rates by countries 2000-2015 (World Health Organization, 2015)

ANOPHELES MOSQUITOES

Anopheles species mosquitoes occur almost worldwide, with the four species of human pathogenic *Plasmodium* spp. are transmitted significantly in nature by only some 30 species of *Anopheles* (Figure 02). Of the vectorial species, a handful is important in stable malaria, while others only become involved in epidemic spread of unstable malaria (Vinayagam, Senthilkumar and Umamaheswari, 2008).

Sri Lanka is a Tropical Island located close to the southern tip of India. The most common species of *Anopheles* in Sri Lanka such as *A. culicifacies*, *A. fluviatilis*, *A. stephensi* and *A. tessellates* (Kannathasan *et al.*, 2008). Anopheles life cycle is showed in Figure 03.

ANOPHELES LIFE CYCLE

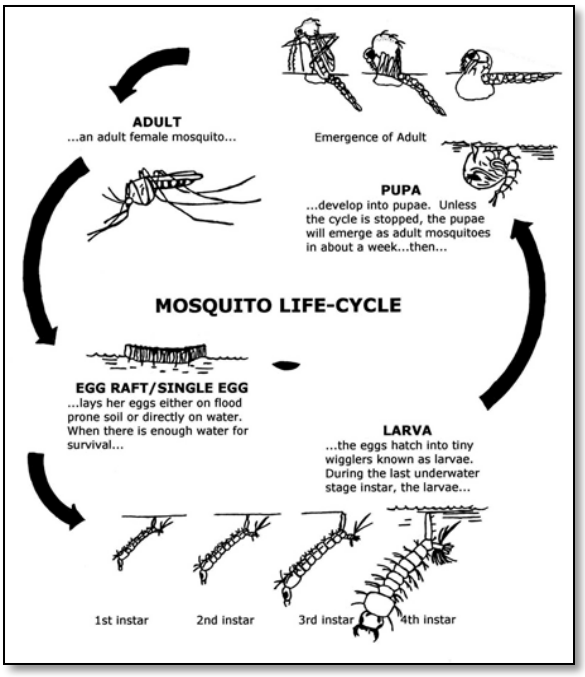


Figure 02. The life cycle of mosquitoes. Stages within 24 to 48 hours, the eggs will hatch into larvae. The larvae will grow approximately 5mm in length within a span of seven to 10 days; the larvae will enter the stage of a pupa. When a mosquito is fully developed, it will emerge from its pupal

case and will become a mosquito. The new adult, at this time, will stand upon the water to dry its wings and prepare for its flight (Eckhoff, 2011).

THE MALARIA PARASITE LIFE CYCLE

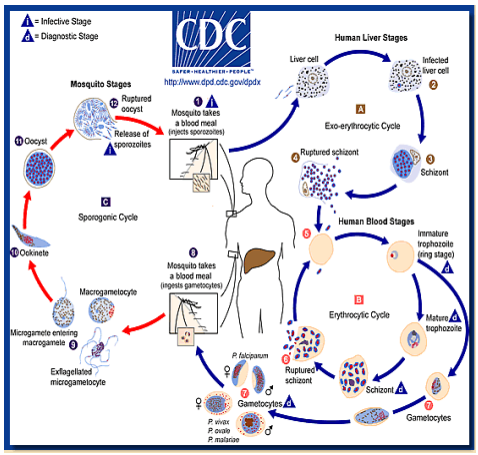


Figure 03. The malaria parasite life cycle involves two hosts. During a blood meal, a malaria-infected female Anopheles mosquito inoculates sporozoites into the human host. After this initial replication in the liver, the parasites undergo asexual multiplication in the erythrocytes. The gametocytes, male and female are ingested by an Anopheles mosquito during a blood meal. The parasites' multiplication in the mosquito is known as the sporogonic cycle. The zygotes in turn become motile and elongated which invade the midgut wall of the mosquito, which make their way to the mosquito's salivary glands. Inoculation of the sporozoites into a new human host perpetuates the malaria life cycle (Centers for disease Control and Prevention, 2016)

MALARIA CONTROL BY USING NATURAL PRODUCTS

Methods to controlled mosquito a necessary component of disease prevention programs in developing countries. Mosquitoes of *Anopheles* in the larval stage are attractive target for control process due to their low mobility in the breeding habitats and the ease to control in these habitats (Howard, Zhou & Omlin, 2007). According to study of Lima *et al.*, (2006), focused interest on alternative compounds for mosquito control due to increasing resistance of mosquitoes to the insecticides. Plants, being a natural source of various compounds are known to contain larvicidal agents (Govindarajan *et al.*, 2011).

Natural products are best option because they are less harmful to environment and non-target organisms. Several compounds from different plants families have been evaluated for new and promising larvicides (Hemalath *et al.*, 2015). In recent years, the top priority in finding a nature based insecticide is that, they must be of plant origin and should not have any ill effects on the ecosystem. Isolated compounds of plants provided alternative source of mosquito repellents agents (Yang *et al.*, 2004). *Lantana camara* an important medicinal plant with several medicinal uses in traditional medication system.

Lantana camara

Lantana camara is a species of flowering plant within the family Verbenacea. It is a hefty extensive evergreen shrub which can grow up to 3 m in height and has a strong scent. It is a perpetual shrub found growing up to 2000m altitude in tropical, subtropical parts of the world. All parts of this plant have been used conventionally for numerous illnesses all through the world (Vardien, *et al.*, 2012).

Lantana camara also known as Ganda-Pana or Rata Hinguru in Sri Lanka. An invasive plant is introduced to Sri Lanka in 1926 and currently has spread across the island to a significant extent (Gunatnillake and Ranasinghe, 2001). Morphology of *L. camara* is showed in Figure 04.

Figure 04. Lantana camara plant with its pink-yellow flower

Whole plant parts have been thoroughly studies for their chemical constituents. The major constitutes of leaf and flower essential oils are showed in Table 01.

Table 01. Major compounds of Lantana camara leafs (Tesch et al., 2011)

Chemical compounds	
Terpenoids steroids alkaloids β-caryophyllene zingiberene δ-humulene	bicyclogermacrene germacrene D carvone phellandrene limonene cineole bisabolene

Studies showed that *L. camara* has antimicrobial, fungicidal band insecticidal activity against stored grain insect pests, vegetable crops pest, mosquito larvae, and other biological activities. *L. camara* has also been used in traditional herbal medicines for treatment of ailments, including cancer, skin itches, rabies, asthma and ulcers (Sousa and Costa, 2012).

This study will assumed to assess in the present communication, an attempt has been made to evaluate the larvicidal efficacy of the ethanol extracts from the *Lantana camara* leaves against medically important species of *Anopheles spp*.

METHODOLOGY

Collection of Lantana camara plant

The mature leaves of *Lantana camara* were collected from Homagama area in western province, Sri Lanka. The area around 6° 59' 36" N, 79° 58' 29" showed Figure 05. The *Lantana camara* plant and leaves showed Figure 06.

Figure 05. Lantana camara plant leaves collecting from Homagama area in Sri Lanka

Figure 06. Lantana camara plants

Fresh leaves (Figure 07 A) of 300.0g were washed tap water and air dried on polythene layer and it covered from polythene layer in 7 days in a shaded environment at room temperature. After 7 days, the dried leaves were grinder and prepared the 200g powder. Then powder was packed in a polythene bag (Figure 07 B) and it was covered in aluminum foil. The powder was refrigerated at 4^{o} C.

A B

Figure 07. A) Lantana camara fresh mature leaf B) Lantana camara leaf powder packet

REARING MOSQUITO LARVAE

The mosquito larvae were obtained from the Department of Parasitology, Faculty of medicine, University of Colombo. Three larvae (n=3) were allocated into a petri dishes, each containing 10.0 ml of de-chlorinated water (Figure 08). Larvae feeding with appropriate provide a food. Physiological parameters of the de-chlorinated water including temperature and pH were measured. Container were

covered by using nylon net with small pore sizes and allowed to adapt to the environmental conditions for 24 hours.

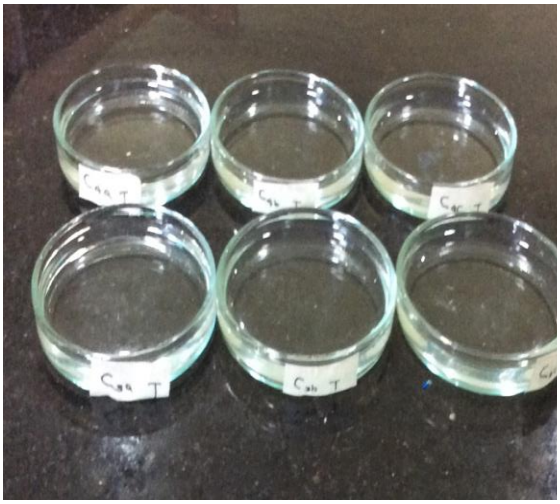


Figure 08. Three larvae were allocated into petri dishes.

LARVAL IDENTIFICATION

Stage III larvae were placed individually in petri dishes and observed under an inverted light microscope (LABOMED - TCM 400) with an objective (100x and 400x). Using standard larval keys developed for Sri Lankan anophelines. They were identified to the species level using Gunarathna, 2017 Anopheles larvae key. Key larval characters were recorded for each species. Further, larval species identification was reconfirmed through adult identification.

ADULT MOSQUITO IDENTIFICATION

The adult mosquito immersed in 70% ethanol and refrigerated overnight. Thereafter, the mosquito was dissected and parts were mounted by using Canada balsam. Prepared permanent slides were observed and identified under the light microscope (CXL) with objective 400x using standard adult mosquito keys developed for Sri Lankan *Anophelines*.

PLANT EXTRACTS PREPARATION

The ethanol extract of the plant was prepared by dissolving 50.0g of dried powder in 150.0 ml of solvent. The mixture was left in the dark at room temperature for 24 hours with shaking at regular intervals. Thereafter, the mixture was subjected to stirring at 500 rpm for 20 minutes in a magnetic stire. The solution was filtered using a glass funnel and Whatman no. 1 filter paper. Filtrates were dried in the fume hood. The evaporated *Lantarna camera* extract was collected to an air tight container. Covered with aluminum foil and stored at 4^{o} C refrigerated until further use.

A 20 000 ppm stock solution was prepared by dissolving a measured 160.0 mg of *Lantana camera* extract in 100.0µL DMSO. Once the residue was completely dissolved, de-chlorinated water until the 8.0 ml. (The ultimate concentration of DMSO in the stock solution was 0.1%). The stock solution was vigorously vortex to obtain a homogenized mixture.

LARVICIDAL BIOASSAY TEST

Four batches of concentrations were prepared each containing three sets of larvae triplicates (n=3) treated with different concentration *Lantana camara* plant extract. The larvicidal activity was assessed by the procedure (World Health Organization, 2005). Each concentration was prepared by replacing, an appropriate volume of de-chlorinated water with the stock solution. The control set was prepared by adding 0.1% DMSO. The larval behavior was noted at regular time intervals and the mortality was recorded

following 24 and 48 hours of exposure (Table 03).

Table 03. Concentration with stock and deionized water

Treatment	Concentration (ppm)
C0 [control]	0
C1	100
C2	500
C3	1000
C4	2000

DNA FRAGMENTATION ANALYSIS

The concentration of 2000 ppm larvae obtained 24 hours post-exposure was in 5 ml of ice cold PBS. For the DNA extraction procedure, PBS buffer was removed from the micro centrifuge tube. Thereafter, the larvae was homogenized in Liffton's buffer and kept for 30 minutes. Subsequently, Proteinase K was added to the homogenized sample and vortex. The mixture was incubated for 1 hour at 55°C.

This was followed by the addition of phenol: choloroform: Isoamyl alcohol to the sample. The sample was vortex and centrifuged at 12 000 rpm for 15 minutes. The aqueous layer of the sample was transferred to a new micro centrifuge tube and equal volume of Ice cold Isopropanol was added. The extract was stored in -20°C for 30 minutes. Thereafter, the extract was centrifuged at 12 000 rpm for 15 minutes. The supernatant was removed from the Eppendorf and the pellet was dried. Subsequently, nucleus free water was added to micro centrifuge tube.

1.0% Agarose gel was placed on the electrophoresis chamber in a horizontal position and TBE buffer was added to the chamber until it covered the gel. Then 8.0µL ethidium bromide was added to the chamber. Then 10 µL of DNA extract were mixed with 5µL of gel loading buffer and loaded into the wells. The lid of the chamber was closed and gel was let to run at 100V for 1 hour till the dye. The gel was placed in the UV Trans-illuminator and bands were observed using the software and the monitor.

HISTOLOGICAL PROCEDURE

For histological studies, 2000 ppm live larvae after 24 and 48 hours exposure to the *Lantarna camera* extract were fixed in 5 ml of 10% neutral buffered formalin and refrigerated. After dehydration (see Appendix 02) in a graded ethanol series, the larvae were subjected to two washes in xylene. Thereafter, larvae were immersed in two paraffin wax baths at 58-60° C before infiltration by using a paraffin dispenser (TEC 9000). For the paraffin block preparation, the mould was filled with wax in to which the larvae were oriented and the cassette was placed. Once solidified, paraffin block was kept at 4°C until use. The paraffin block was sectioned using rotary microtome (SHANDON FINESSE 325) at 7 µm. Sections were mounted on to glass slides which were homogenized coated with egg albumin. The slides were placed on the slide dryer (SD 2800) at 38° C for 30 minutes. The slides were observed under the light microscope (CXL) at objective 100x to ensure the presence of larval tissue.

The larval tissues were stained using Hematoxyllin and Eosin staining procedure (See Appendix 02) and observed under the light microscope (CXL) at objective 400x to identify histological changes in the

cuticle after the exposure to the plant extract.

It was noted the death of larvae exposed to high concentrations. Therefore, to study histological changes in Anopheles larvae, the experiment was extended. A new set of second instar larvae were exposed to C3 C4, control (n=3) in triplicates.

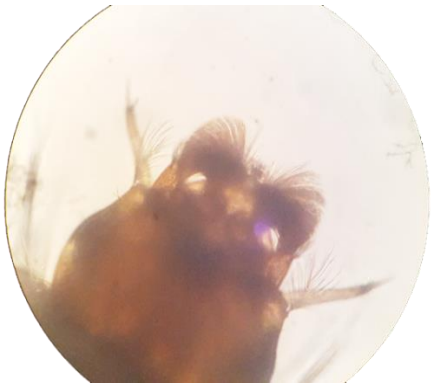
STATISTICAL ANALYSIS

Statistics at 95% confidence limits of upper confidence limit, lower confidence limit Morphological features of head, thorax, and abdomen three body regions were noted.

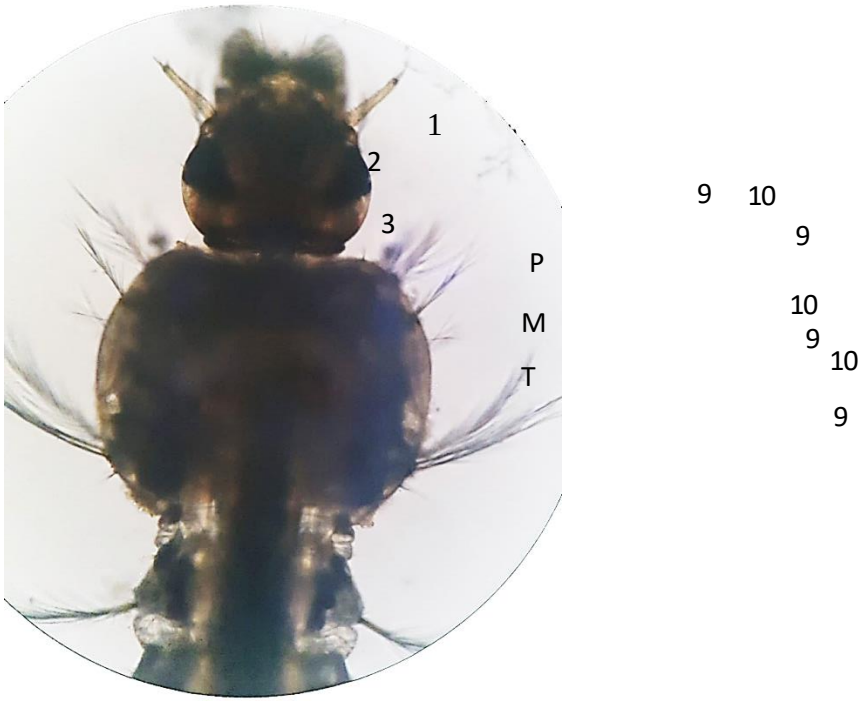
were calculated using the SPSS 11.5 (Statistical Package of Social Sciences) software and Excel. Results with $P < 0.05$ were considered to be statistically significant used two-way ANOVA. The mean mortality percentage of larvae mortality data was subjected to probit analysis for calculating $LC_{50.}$

RESULTS

Larvae Identification



A **B**



C

Figure 09. A) Microscopic of view of mosquito larvae (100x) B) Figure 2: Seta 1-A simple; 2-C inserted at least as far apart as the distance between 2-C and 3-C on one side C) Long thoracic pleural setae 9, 10, 12-P, 9, 10-M and 9, 10-T simple. Seta 1-P weak, 2-5 branched; 1, 2-P arising from separate basal tubercles, only tubercle of 2-P prominent and sclerotized (400x)

According to the morphological features of the dosal view of head (Figure 7 A), the subgenus Cellia was disguised from the subgenus *Anopheles* (Amarasinghe, 1997). The Sri Lankan anophelines of this subgenus belong to four taxonomic series: the Myzomyia, Pyretophorous, Neocellia, and Neomyzomyia series. The subgenus *Cellia* includes several species; *culicifacies, karwari, subpictus, tessellatus, vagus, varuna* (Gunathilaka, 2017).

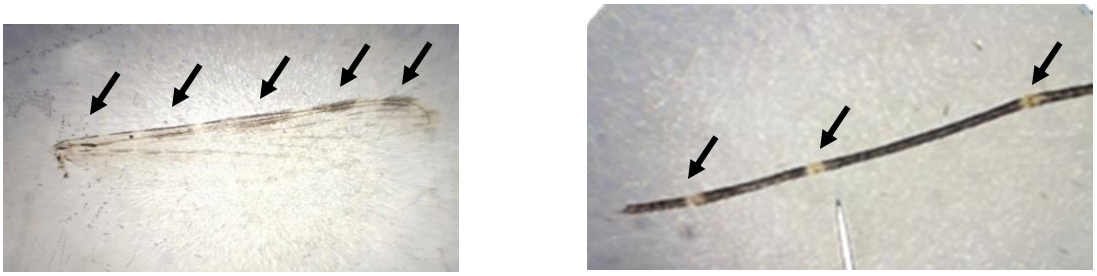
The features observed ventral view (Figure 09, C) distinguished the taxonomic series as Neomyzomyia. Two species, *A. tessellatus* and *A. elegans* have been reported under Neomyzomyia series in Sri Lanka.

Based on the characteristics observed in the dosal view of prothorax (Figure 09) distinguished the spices as *A. tessellatus* from *A. elegans*. The species identification was further confirmed by observing the morphological features of the adult mosquito.

ADULT MOSQUITO IDENTIFICATION

In the adult mosquito, following morphological features distinguished species of subgenus *Cellia* from those of the subgenus *Anopheles*: wing with 4 or more darks marks involving both costa and veins R-R1 accessory sector pale (ASP) spot present on costa. The subgenus *Cellia* includes several

species; *culicifacies, pseudojamesi, subpictus, tessellatus, vagus, varuna* (Gunathilaka, 2017).



A B

Figure 10. Features of adult mosquito A) Femur and tibia speckled B) Apical half of the proboscis pale scale

According to these features such as speckled femur and tibia as shown in Figure 08. Based on the following characteristics distinguished the *A. tessellatus* (Gunathilaka, 2017).

Mortality rate of *Anopheles tessellatus*

The following table showed in a mortality rate of *Anopheles tessellatus* within 24 and 48 hours.

Table 04. Mean percentage mortality of Anopheles tessellatus caused Lantana camara

Sample	**Concentration (ppm)**	**Mortality Mean±SE (%)**	
		24hrs	**48hrs**
N/C	0	0	0
C1	100	11.11 ± 11.11	55.56 ± 11.11
C2	500	22.22 ± 11.11	33.33 ± 0
C3	1000	44.44 ± 11.11	88.89 ± 22.22
C4	2000	77.78 ± 11.11	100 ± 0

The highest rate of mortality in larvae was detected where the highest concentration of extraction was exposed ($P<0.05$, Tukey's pairwise tests after one way ANOVA). Highest mortality rate was observed in the highest concentration of the extract. According to statistical test results, it was

evident that the mortality of larvae exposed to 2000ppm was significantly different from control, 100ppm, 500 ppm and 1000ppm in 24h exposure($P<0.05$, Tukey's pairwise tests after one way ANOVA). And also the mortality rate was increased with the exposure period ($P<0.05$, Turkey's pairwise tests after one way ANOVA).

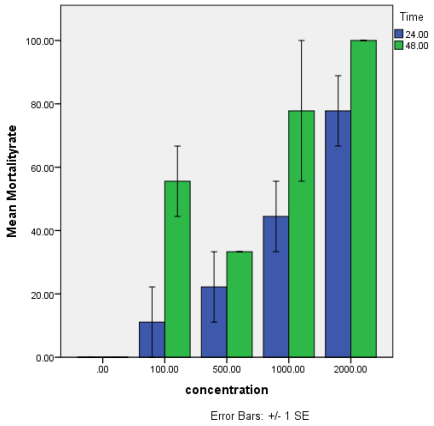


Figure 11. Mean percentage mortality rates of A. tessellatus larvae at different concentrations of different time periods of Lantana camara (±SE)

Table 05. Summary of the two-way ANOVA (balanced design) between the Lantana camara extract concentrations and the time of exposure upon the percentage mortality of Anopheles tessellatus larvae

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
concentration	27630.482	4	6907.620	20.721	.000
Time	3703.630	1	3703.630	11.110	.003
concentration * Time	1852.037	4	463.009	1.389	.274
Error	6667.111	20	333.356		
Total	93333.334	30			
Corrected Total	39853.259	29			

MEDIUM LETHAL CONCENTRATION LC_{50}

According to results of the probit analysis at a 95% confidence level showed that the LC_{50} values gradually decreased with the exposure time from 24 to 48 hours.

Table 06. Probit regression line parameters response of Anopheles tessellatus larvae to Lantana camara plant extracts.

Time period	LC50	Regression equation	R value
24 hours	1000ppm	y=1.66x+0.072	0.98261
48 hours	199.526ppm	y=2.17x+0.036	0.90939

Behavioral changes of A. tessellatus larvae, after exposing to the ethanol leaf extract Lantana camara

Physical characteristics of water used for the study, medium temperature 28^0C and pH 6.6 were within the permissible limits throughout the study period.

Mosquito larvae exposed to the *L.camara* leaf extract showed significant behavioral changes during 30 minutes of exposure. The most clear behavioral change observed in *A. tessellatus* was inability to come on the surface. According to the 1 hour exposure results, all the larvae of control showed movements. All the larvae of highest concentration (2000 ppm) came to the surface of the solution. Also, 55.56% larvae in 1000 ppm concentration came to the surface and others were showed some movements. All the larvae of 500ppm and 100ppm concentration showed lethargic.

After the exposing 24 hours, all the larvae of control showed less movement. 55.56% larvae were dead and 22.22% were in lethargic and 2 were in showed fewer movements in 2000ppm concentration. 33.33% larvae were dead and 33.33% were in lethargic and 33.33% were showed some movements in 1000ppm concentration. 11.11% larvae dead and 33.33% larvae were lethargic and others were showed some movements in 500 ppm. 22.22% larvae were lethargic and others were showed movements of 100 ppm concentration. No such behavioral changes were obtained in control group. Metamorphosis was not occurred in larvae during the 48 hours.

DNA FRAGMENTATION ANALYSIS

The quality of genomic DNA using phenol:chloroform extraction methods was visualized on 1% Agarose gel (Figure 12). Clear bands were observed control and sample (2000 ppm concentration), indicating no degradation of genomic DNA.

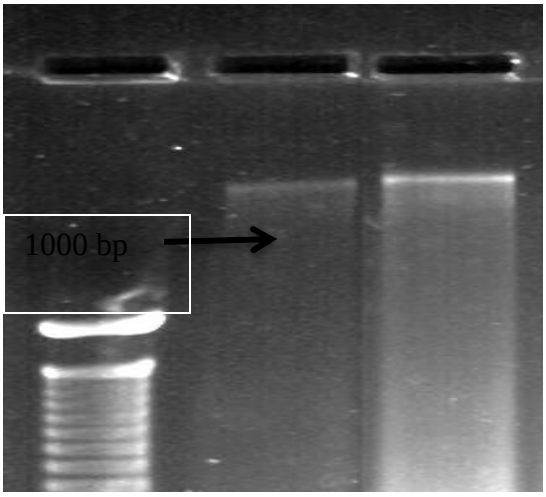


Figure 12. Gel image of DNA from larvae obtain agarose gel electrophoresis at 1 hour. Lane M contains molecular weight marker (50 bp DNA ladder). Lane 1 for control and Lane 2 for extracted DNA in Anopheles tessellatus larvae treated with Lantana camara extracts

HISTOLOGY

Anopheles tessellates larvae treated with *Lantana camara* extract were compared with untreated larvae. The results of the histological studies confirmed that *Lantana camara* extract caused distinguished damaged to the cuticle leading to complementary destroyed. When treated with *Lantana camara*, the mid gut epithelium and caeca of the larval tissue was affected, as showed in figure 13.

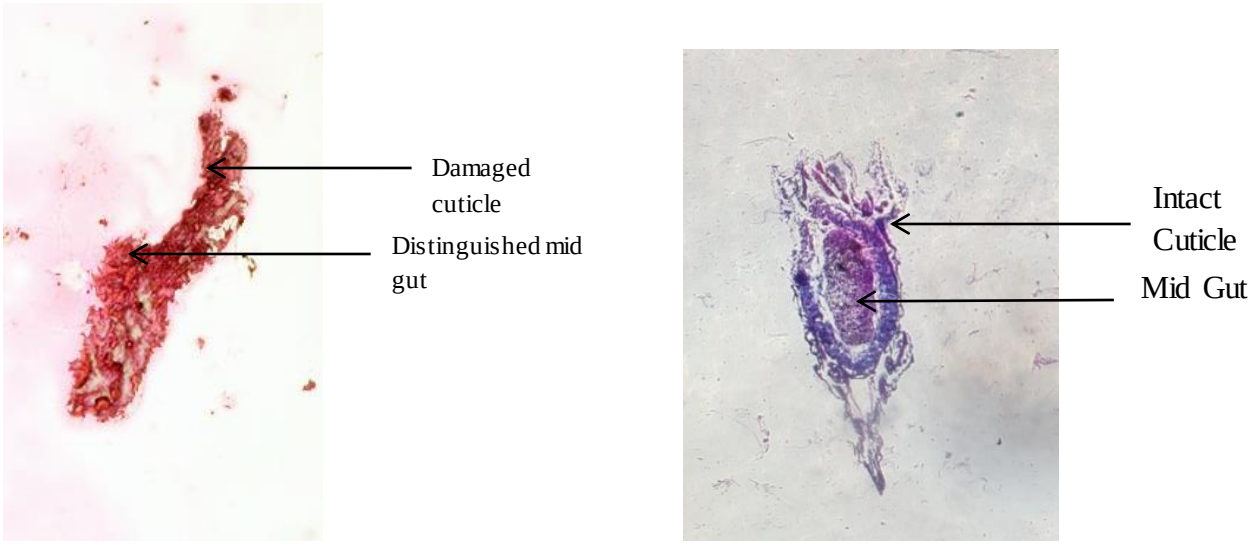


Figure 13. Histological alterations on Anopheles tessellatus larvae. (A) Larvae exposed to the Lantana camara extract. The larvae tissue was showed degradated tissue B). Control larvae stained with HE. The close association among rope-like cuticle. Cuticle was interact and showed mid gut

DISCUSSION

Mosquito larval control using larvicidal agents is a major component in the control of vector borne diseases. Plant as larvicide is considered as practical and chosen alternative in the control of the mosquito species at the community level (Hemalatha *et al.*, 2015). Therefore, alternative control methods are in useful; plant sources possess a wide range of pharmaceutical and insecticidal assets. Botanical insecticides serve as appropriate alternatives to synthetic insecticides in future as they are relatively safe, degradable, and are readily available in many areas of the world (Sivagnaname and Kalyanasundaram, 2004). Many plant extracts are known to be toxic to different species of mosquitoes and could be used to control the diseases they transmit (Willcox et *al.*, 2004).

A large number of plant extracts have been reported to shave mosquitocidal or repellent activities against mosquito vectors, but few plant products have shown practical utility for mosquito control (Sun *et al.*, 2006). In the present study ehanolic extract of *Lantana camara* showed 100% larvicidal activity against the third instar larvae of *Anopheles tessellatus*.

A point to be highlighted here is that synthetic pesticides have been used to kill mosquito larvae. Similarly, the present study purpose of this work was larvicidal effects of *Lantana camara* plant extract on *Anopheles* species. The larvicidal properties of *Lantana camara* have been investigated in a number of studies (Remia and Logaswamy 2010). Kumar *et al*., (2013) found that the ethanol extract of *L. camara* leaves showed maximum mortality towards *Aedes aegypyti* and *Culex quinquefasciatus* mosquito larvae. The present study was carried out to study the effects of the *L.camara* leaf extract on the mortality of *Anopheles* larvae which may be helpful in the control of *Anopheles* mosquitoes.

Lantana camara is reported to possess insecticidal activity against stored grain pest, vegetable crops pest, mosquito larvae and antifungal, repellent, and other biological activities (Barreto *et al*., 2010). In the present study the powder extracted from *L. camara* leaves showed adulticidal activity against various mosquitoes against *A. tessellatus* using 100% extracts respectively. This is in agreement with Dua *et al.,* (2010) who found the adulticidal activity of the *L. camara* to be most toxic to *An. fluviatilis* followed by *A. culicifacies* and *A. tessellatus* and (Kemabonta *et al*., 2014). Senthilnathan, (2010) studied the ethanol xtract of *L. camara* which shows high larvicidal activity against *Ae. aegypti* and *Culex quinquefasciatus.*

As opposed to the comparative study of Sathish and Maneemegalai (2008) wherein they utilized different parts of *L. camara*, the present study only bused the leaf part which is the abundant part of the plant. In their study, methanolic flower extract yields 100% mortality rate on the concentration achieving the same results with ethanolic extract of the flower.

The effects of extract were studied win a dose dependent manner (Yuan and Hu, 2010). The ethanol extract of *L. camara* was found to have a higher rate of mortality on *Anopheles tessellatus*. The third-instar stage of *A. tessellatus* larvae was treated with different concentrations. And also, the mortality rate increased with increase in concentration and exposure of time.

Furthermore, ethanol is safe to man, not as highly toxic as methanol which is known to produce skin irritations and even blindness when ingested (Alothman *et al*., 2009; Durling *et al*., 2007). Then again, in a study conducted by Rajasekaran & Duraikannan (2012), ethanol extract *Lantana camara* showed 100% mortality after 24hrs of incubation.

Also, the results of the study showed that there were significant differences between the higher concentrations of each plant extract and the larvicide against *An. tessellatus* larvae. The highest rate of mortality in larvae was detected where the highest concentration of extraction was exposed. Therefore done by a statistical analysis to these data according to ANOVA, Kolmogorov smirnov test. This showed significance level less than the 0.05 that mortalities increased with concentration ($0.002 < 0.05$), these results confirm the report of (Pelah *et al*, 2005) that there was a positive correlation between concentration and the percentage of the larval mortality (Rahuman *et al*., 2009). The data confirmed in normally distributed and post hoc tests equal variance assumed by Tukey and it was showed results in significance level then done by a Two-way ANOVA test.

The present study also shows that there is significant difference among the treatments and the concentrations of all yields *L. camara* ethanolic leaf extract as an ineffective larvicide as different to the study made by Sathish and Maneemegalai (2008). The extraction solvent and the part of the plant utilized greatly affect the result of the experiment recently done.

However, on the individual basis, *L. camara* exhibited a higher larvicidal effect and observed with their LC_{50}. Ethanol leaf extract of *L. camara* showed least LC_{50} value of 1000 ppm for 24 hours and 199.526 ppm for 48 hours at 95% confidence interval confirming the presence of bioactive compounds in the extract. These results show the drastically decreased LC_{50} values with the exposure time from 24 to 48 hours. Low concentrations (1000 ppm) which makes them preferable to synthetic insecticides. However, combined effects or synergistic effects of various control agents have proved very advantageous in the control of various pests (Seyoum *et al.*, 2002) reviewed different mosquito larvicidal plant species with growth retarding, reproduction inhibiting, ovicides, synergistic, additive and antagonistic ction of botanical mixtures (Shaalan *et al*., 2002).

The results of *lantana* leaves extract of ethanol proved that they have larvicidal properties against *A. tessellatus* larvae. These findings agreed with Nath *et al*., (2006) who found that leaf extract of *L. camara* showed larvicidal (LC_{50}) activity against *Aedes albopictus*. Another study by Innocent *et al.*, (2008) showed the effect of the root barks extracts of *Lantana camara* against late third instar larvae *of Anopheles gambiae*.

The mosquito larvae exposed under *L.camara* plant extract showed significant behavioral changes observed within 30 minutes, 1 hour and 24 hours of exposure. The most obvious sign of behavioral changes observed in *A. tessellatus* was inability to come on the surface. The larvae also showed restlessness, loss of equilibrium and finally led to death. These behavioral effects were more pronounced in case of *L.camara* extracts after exposure. These effects may be due the presence of neurotoxic compounds in plant (Remia and Logaswamy, 2010). These larvae showed different levels of susceptibility demonstrated by the time required to larvae lose their agility after exposed to pling extract. This was probably because larvae lost their capacity to maintain at the water surface to get to the surface for breathing.

Results of the experiment conducted for evaluating the larvicidal efficacy of *Lantana camara* showed that was toxic to the *A. tessellatus*. According to the study of park *et al*., (2002) determined the LC_{50} and observed behavioral changes and mortality in the larvae. Similar observations were noticed in the present study.

According to all these results, *L.camara* plant extract may be effect on larvae tissues of the organism with induction of apoptosis or effect of larvae DNA. Therefore, done in a DNA fragmentation analysis and observed histological changes in tissue of larvae.

Based on our results of DNA analysis, had no *Lantana camara* ethanol extract effect in *A. tessellatus* DNA. It can be due to the time period and exposed concentration of the plant. These all larvae were exposed in 24 hours therefore, the time period may not enough to effect larvae DNA. Although, *L.*

camara plant compounds were slowly broken down enzymatically (Juen and Traugott, 2005). This could be either due to the fact that plant compounds were harder to digest or caused by the physiology and metabolic rates of these larvae.

Phenol chloroform extraction is used a lysed cells or homogenised tissue and this procedure was often performed multiple times to increase the purity of the DNA. This procedure takes advantage of the fact that deproteinization is more efficient when two different organic solvents fare used instead of one. Furthermore, phenol denatures proteins efficiently; it does not completely inhibit RNase activity. The phenol chloroform isolation method is not efficient, because many samples can be processed in parallel and hazardous chemicals are used (Sambrook and Russell, 2017). However, DNA generated by the phenol chloroform isolation method is sheared band often contains a huge amount of contaminating compounds, which make this DNA unsuitable for requests susceptible to sheared or contaminated DNA.

In the histological study the whole larva processing method can be easily and quickly performed in a Histology laboratory, with only minimal precautions to achieve an optimal fixation and inclusion. However, the exposed *A. tessellatus* larvae were degreagated compared to the control. This can be due to some errors of tissue fixing and sectioning. Fixative fixation protocol may depend on the additional processing steps and final analyses that are planned. In this case, quick fix method using cold formalin for around 24 hours is typically mused and that time can vary depending on the biological material.

The recent study is guided by the following objectives all throughout the research, to determine the larvicidal effects of *Lantana camara* leaf in ethanol extract against *Anopheles* sp. Larvae of mosquitoes found within the Sri Lanka, to assess the effectiveness of the treatments in a dose-dependent manner which were used different concentrations of *Lntana camara* extract and to figure out if there is a significant difference in the mortality among the treatments used.

The recent study is guided by the following objectives all throughout the research, to determine the larvicidal potential of *L. camara* leaf extract against larvae of common species of mosquitoes, to assess the effectiveness of the treatments in a dose-dependent manner which are 100, 500, 1000 and 2000 ppm and to figure out if there was a significant difference in the mortality among the treatments used. The *L. camara* ethanolic leaf extracts was found to have larvicidal potential against *A. tessellatus* mosquitoes. Results showed that it had 100% percentage of mortality on the larvae tested for 48 hours.

FUTURE WORK

For future work, to consider using bother parts of the *Lantana camara* such as the flowers, because it has been proven to have higher mortality rate than the leaves that had used in this experiment. Utilize a higher treatment dosage to check the potency of the extract. Also, extend the observation of larvae for about 72 hours after pouring the extract because the efficacy of the extract takes longer hours. Last, to identify the *A. tessellatus* mosquito larval and the exact instar they are going to utilize to know if the *Lantana camara* has

a different effect based on a specific *A. tessellatus* and instar.

Recently, a novel method has become available for identifying the *A. tessellatus* of mosquito larvae. This method compares DNA sequences from field-collected, unidentified larval samples with those of identified adult samples. Using this molecular species identification technique, studies have revealed previously unknown morphological and ecological traits of many *A. tessellatus* during larval stages.

The *Lantana camara* leaf extract effect of the *A. tessellatus* larvae, DNA extraction can be used directly for molecular analyses such as PCR. This is an added advantage, especially where small initial samples have DNA of low concentration due to the loss of some of the DNA in the process of extraction and elution.

A partially purified toxin fraction and lantadene can be obtained from *Lantana camara* leaves by batch extraction, column chromatography and fractional crystallization. Further scientific investigation for the development of effective therapeutic compounds will be found in *Lantana camara*.

ACKNOWLEDGEMENT

I would like to grant my sincere gratitude to my supervisor, Ms. Koshala de Silva, the lecturer of BMS for her guidance, advices and extreme support throughout this project. I would also like to thank all the staff of Parasitology Department, Faculty of medicine, University of Colombo and also thank Ms. Vajirapani de Silva and the team of Genetech for their support. I am also obliged to BMS, School of science, University of Northumbria for this opportunity they gave me to conduct this study.

REFERENCES

Alothman, M., Bhat, R. & Karim, A.A. (2009), "Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents", Food Chemistry, Vol. 115, No. 3, pp. 785–788.

Amarasinghe, F.P. (1992), "A Guide to the identification of the Anolheline or Mosquitoes (Diptheria:Culicidae) of Sri Lanka, Larvae", Ceylon Journal of Science, Vol. 22, No. 1, pp: 1-13.

Barreto, F., Sousa, E., Rodrigues, F., Costa, J. & Campos, A. (2017), "Antibacterial Activity Of Lantana Camara Linn Lantana Montevidensis Brig Extracts From Cariri-Ceara, Brazil", Journal of Young Pharmacists, Vol. 2, No. 1, pp: 42-44.

Confalonieri, U., Menne, B., Akhtar. R., Ebi, K.L., Hauengue, M., Kovats, R.S., Revich, B. & Woodward, A. (2007), Human Health. In: Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, Cambridge, U.K.

Durling, N. E., Catchpole, O. J., Grey, J. B., Webby, R. F., Mitchell, K. A., Foo, L. Y. & Perry, N. B. (2007), "Extraction of phenolics and essential oil from dried sage (Salvia officinalis) using

ethanol-water mixtures", Food Chemistry, Vol. 101, No. 4, pp: 1417-1424.

Eckhoff, P.A. (2011), "A Malaria Transmission-Directed Model Of Mosquito Life Cycle And Ecology", Malaria Journal, Vol. 10, pp: 303.

Govindarajan, M., Mathivanan, T., Elumalai, K., Krishnappa, K. & Anandan, A. (2011), "Mosquito Larvicidal, Ovicidal, and Repellent Properties Of Botanical Extracts Against Anopheles Stephensi, Aedes Aegypti, And Culex Quinquefasciatus (Diptera: Culicidae)", Parasitology Research, Vol. 109, No. 2, pp: 353-367.

Green, M. R. & Sambrook, J. (2017), "Isolation of High-Molecular-Weight DNA Using Organic Solvents", Cold Spring Harbor Protocols, Vol. 4, pp: pdb.prot093450.

Guerra, C.A., Gikandi, P.W., Tatem, A.J., Noor, A.M., Smith, D.L., Hay, S.I. & Snow, R.W. (2008), "The limits and intensity of Plasmodium falciparum transmission: implications for malaria control and elimination worldwide", PLoS Med, Vol. 5, No. 2, pp:e38

Gunathilaka, N. (2016), "Illustrated Key to The Adult Female Anopheles (Diptera: Culicidae) Mosquitoes Of Sri Lanka", Applied Entomology and Zoology, Vol. 52, No. 1, pp: 69-77.

Gunatnillake, N. & Ranasinghe, D.M.S.H.K. (2001), "Habitat utilization pattern of Lantana camara in udwalawa national park", Proceedings of the Seventh Annu'll Forestry and Environment Symposium 2001. At: http://journals.sjp.ac.lk/index.php/fesympo/article/viewFile/1625/792, Visited: 01 May 2017

Hemalatha, P., Elumalai, D., Janaki, A., Babu, M., Velu, K., Velayutham, K. & Kaleena, P.K (2015), Larvicidal activity of Lantana camara aculeate against three important mosquito species", Journal of Entomology and Zoology Studies, Vol. 3, No. 1, pp: 174-181.

Howard, A. F., Zhou, G. & Omlin, F. X. (2007), "Malaria Mosquito Control Using Edible Fish In Western Kenya: Preliminary Findings of a Controlled Study", BMC Public Health, Vol. 7, No. 9, pp: 199.

Innocent, E., Cosam, J., Gikonyo, N., Nkunya, M. & Hassanali, A. (2009), "Larvicidal Properties Of Some Tanzanian Plant Species Against Anopheles Gambiae S.S. Gile (Diptera:Culicidae) Mosquitoes", International Journal of Biological and Chemical Sciences, Vol. 3, N0. 2, pp: 266-270.

Jirakanjanakit, N., Saengtharatip, S., Rongnoparut, P., Duchon, S., Bellec, C. & Yoksan, S. (2007), "Trend of temephos resistance in Aedes (Stegomyia) mosquitoes in Thailand during 2003-2005", Environmental entomology, Vol. 36, No. 3, pp:506-11.

Juen, A. & M. Traugott, M. (2005)Detecting predation and scavenging by DNA gut-content analysis: a case study

using a soil insect predator-prey system, Oecologia, Vol. 142, No. 3, pp. 344–352.

Kalita, S., Kumar, G., Karthik, L., Venkata, K. & Rao, B (2012), "A Review on Medicinal Properties of Lantana camara Linn", Research Journal of pharmacology and technology, Vol. 5, No. 6, pp:711-715.

Kannathasan, S., Antonyrajan, A., Karunaweera, N., Anno, S. & Surendran, S. (2008), "Identification of Potential Malaria Risk Areas Of The Jaffna District Of Northern Sri Lanka: A GIS Approach", Journal of the National Science Foundation of Sri Lanka, Vol. 37, No. 3, pp: 231-239.

Kemabonta, K. A., Anikwe, J. C. & Adaezeobiora, I. (2013), "Bioefficacy of skeetar in Anopheles gambiae and Aedes aegypti mosquitoes from insecticides resistance areas in Lagos and Oyo State", Biology Agriculture HealthCare, Vol. 3, No. 2, pp: 122–135.

Kuhn, K.G., Campbell-Lendrum, D.H. & Davies, C.R. (2002), "A continental risk map for malaria mosquito (Diptera: Culicidae) vectors in Europe", Journal of Medicine Entomology, Vol. 39, No. 4, pp:621-30.

Kumar, S., Bajwa, B.S., Kuldeep, S. & Kalia, A.N. (2013), "Anti-inflammatory activity of herbal plants: A review", International Journal of advances in Pharmacy, Biology and Chemistry, Vol. 2, No. 2, pp: 272-281.

Lima, E.P., Oliveira, Filho, A.M., Lima, J.W.O., Ramos-Junior, A.N., Cavalcanti, L.P.G. & Pontes, R.J.S. (2006), "Resistencia do Aedes aegypti ao temefos em municipios do Estado do ceara", Journal of the Brazilian Society of Tropical Medicine, Vol. 39, No. 3, pp:259-263.

Mayes, M.A. &.Barron, M.G. (1991), Aquatic Toxicology and Risk Assessment, ASTM international.

Moss, W. J., Dorsey, G., Mueller, I., Laufer, M. K., Krogstad, D. J., Vinetz, J. M., Guzman, M., Rosas-Aguirre, A. M., Herrera, S., Arevalo-Herrera, M., Chery, L., Kumar, A., Mohapatra, P. K., Ramanathapuram, L., Srivastava, H. C., Cui, L., Zhou, G., Parker, D. M., Nankabirwa, J. & Kazura, J. W. (2015), "Malaria Epidemiology And Control Within The International Centers Of Excellence For Malaria Research", American Journal of Tropical Medicine and Hygiene, Vol. 93, No. 3, pp: 5-15.

Nath, D., Bhuyan, M. and Goswami, S. (2006), "Botanicals as Mosquito Larvicides", Defence Science Journal, Vol. 56, No. 4, pp: 507-511.

Park, D. C., Lautenschlager, G., Hedden, T., Davidson, N. S., Smith, A. D. and Smith, P. K. (2002), "Models Of Visuospatial And Verbal Memory Across The Adult Life Span", Psychology and Aging, Vol. 17, No. 2, pp: 299-320.

Pelah, D., Abromovich, Z., Markus, A . and Wiesman, Z. (2005), "The use of

commercial saponin from Quallaja saponaria bark as a natural larvicidal agent against Aedes aegypti and Culex pipiens", Journal of Ethnopharmacology, Vol. 81, No. 3, pp: 407-409.

Rahuman, A. A., Bagavan, A., Kamaraj, C., Saravanan, E., Zahir A. A. and Elango G. (2009), "Efficacy of larvicidal botanical extracts against Culex quinquefasciatus Say (Diptera: Culicidae)", Parasitology Research, Vol. 104, No. 6, pp: 1365-1372.

Rajasekaraan, A. and Duraikannan, G. (2012), "Larvicidal activity of plants extracts an Aedes aegypti", Asian Pacific Journal tropical biomedicine, Vol. 2, pp: S1578-S1582.

Remia, K.M. and Logaswamy, S.L. (2010), "Larvicidal efficacy of two botanicals against mosquito vector Aedes ageptyi", Indian Journal of Natural products and Recourses, Vol. 1, No. 2, pp: 208-212.

Ricci, I., Valzano, M., Ulissi, U., Epis, S., Cappelli, A. and Favia, G. (2012), "Symbiotic Control Of Mosquito Borne Disease", Pathogens and Global Health, Vol. 106, No. 7, pp: 380-385.

Roberts, L., S., Schmidt, G.D. and John, J. (2008), Foundations of Parasitology. 8th edn. McGraw Hill.

Sathish, M.K. and Maneemegalai, S. (2008), "Evaluation of larvicidal effect of Lantana Camara Linn againstmosquito species Aedes aegypti and Culexquinquefasciatus", Advances in Biological Research, Vol. 8, No. 2, pp: 39-43

Senthilnathan S. (2007), "The use of Eucalyptus tereticornis Sm. (Myrtaceae) oil (Leaf extract) as a natural larvicidal agent against the malaria vector Anopheles stephensi Liston (Diptera Culicidae)", Bioresource Technology, Vol. 98, No. 9, pp:1856-1860.

Seyoun, A., Palsson, K., Kng'a, S., Karbiru, E.W., Lwande, W., Kllen, G.F., Hassanali, A. and Knols, B.G.J., (2002), "Traditional use of mosquito repellent plants in western Kenya and their evaluation in semi-field experimental huts against Anopheles gambiae: ethnobotanical studies and application by thermal expulsion and direct burning", Transactional Royal Society Tropical Medicine &. Hygiene, Vol. 96, No. 3, pp: 225-231.

Shaalan, E.H.S., Canyonb, D., Younese, M.W.F., Wahab, A. and Mansoura, A.H., (2005), "A review on botanical phytochemicals with mosquitocidal potential", Environment International, Vol. 3, No. 8, pp: 1149-1166.

Sinka, M.E. (2013), "Global Distribution Of The Dominant Vector Species Of Malaria", Oxford, UK

Sivagnaname, N. and Kalyanasundaram, M. (2014), "Laboratory Evaluation Of Methanolic Extract Of Atlantia Monophylla (Family: Rutaceae) Against Immature Stages Of Mosquitoes And Non-Target Organisms", Memórias do Instituto

Oswaldo Cruz, Vol. 99, No. 1, pp: 115-118.

Sousa, E. O. and Costa, J. G. (2012), "Genus Lantana: Chemical Aspects And Biological Activities", Revista Brasileira de Farmacognosia, Vol. 22, No. 5.

Sun. R., Sacalis, J.N., Chin, C.K. and Still, C.C. (2001), "Bioactive aromatic compounds from leaves and stems of Vanilla fragrans", Journal of Agricultural and Food Chemistry, Vol. 49, No. 11, pp: 51- 61.

Tesch, N.R., Mora, F., Rojas, L., Diaz, T., Velasco, J., Yanez, C., Rios, N., Carmona, J. and Pasquale, S. (2011), "Chemical composition and antibacterial activity of essential oil of Latana camara var. moritziana", Natural Product Communications, Vol. 6, pp: 1031-1034.

Vardien, W., Richardson, D., Foxcroft, L., Thompson, G., Wilson, J. and Le Roux, J. (2012), "Invasion Dynamics Of Lantana Camara L. (Sensu Lato) In South Africa", South African Journal of Botany, Vol. 81, pp: 81-94.

Vinayagam, A., Senthilkumar, N. and Umamaheswari, A. (2008), "Larvicidal Activity of Some Medicinal Plant Extracts against Malaria Vector Anopheles stephensi", Research Journal of Parasitology, Vol. 3, pp: 50-58.

Wilcox, A.J. (2002), "On the Frequency Of Intercourse Around Ovulation: Evidence For Biological Influences", Human Reproduction, Vol. 19, No. 7, pp: 1539-1543.

Yang, Y.C., Le, E.H., Lee, H.S., Lee, D.K. and Ahn, Y.J. (2004), "Repellency of aromatic medicinal plant extracts Aedes aegypti", Journal of American Mosquito Control Association, Vol. 20, No. 2, pp: 146-149.

Yuan, Z. & Hu, X.P. (2012), "Repellent, antifeedant, and toxic activities of Lantana camara leaf extract against Reticulitermes flavipes (Isoptera: Rhinotermitidae)", Journal of Econ Entomology, Vol. 105, No. 6, pp: 2115-21.

APPENDIX 01 – HISTOLOGY PROCEDURE

Hematoxylin and Eosin staining procedure (H & E Staining)

The mounted larval tissue slides were stained with using following H & E Staining Procedure.

Xylene I - 5 minutes

Xylene II - 5 minutes

100% Ethanol I - 3 minutes

100% Ethanol II - 3 minutes

95% Ethanol - 3 minutes

70% Ethanol -3 minutes

Distilled Water - 30 Seconds

Hematoxylin- 45 Seconds

Tap water - 2 -3 minutes

Distilled water - 1-2 minutes

Acid Alcohol - 15 Seconds

70% Ethanol -3 minutes

Eosin - 5-10 Seconds

95% Ethanol - 30 Seconds

100% Ethanol I - 3 minutes

100% Ethanol II - 3 minutes

Xylene I - 3 minutes

Xylene II -3 minutes

After the staining procedure, a drop of Canada balsam was put on the section and placed a cover slip, press to remove air bubbles. Let it dry for 5-10 minutes.