

MEDICAL ENTOMOLOGY RESEARCH DIVISION

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The Division undertook research projects on vectors of malaria and Dengue Haemorrhagic Fever (DHF). Insecticide susceptibility status of vectors and suspected vectors of malaria were continued at the selected study sites in Kayah and Mon States. The identification of vectors and suspected vectors of malaria from the field study sites were also carried out during the reporting period. Two research projects were carried out on Dengue vectors. The primary vector of malaria, *An dirus* mosquitoes from Thanbyu Zayat Township were reared and maintained in the insectary and now they are in F 3 generation. *Ae. aegypti* and *Culex quinquefasciatus* mosquitoes from Yangon Region, Mandalay Region and Mon State have also been colonized in the insectary.

RESEARCH PROJECTS

1. COMMUNICABLE DISEASES

1.1 MALARIA

1.1.1 Assessment of the insecticide susceptibility status of Anopheles mosquitoes in Myanmar (2016)

Monitoring of insecticide susceptibility status in some *Anopheles* were conducted in Loikaw Township, Kayah State, Thanbyu Zayat and Beelin Township Mon State and Taungoo Township Bago Region using WHO test kits. Batches of Anopheles were exposed to standard impregnated papers for standard exposure time of one hour. These studies were conducted to monitor the susceptibility of Anopheles mosquito to various insecticides in selected areas of Myanmar. There was no insecticide resistance detected in Loikaw Township, Kayah State, Thanbyu Zayat and Beelin Township Mon State and Taungoo Township Bago Region. Mosquito species and insecticides used were shown in the table below. All mosquitoes were susceptible to the insecticides tested.

Township	<i>Anopheles</i> species	No.	Tested Insecticides	Remark
Loikaw Township, (Kayah State)	<i>An. minimus</i>	60		
	<i>An. tessellatus</i>	170	DDT 4%, Deltamethrin 0.05%,	
	<i>An. philippinensis</i>	60	Permethrin 0.75% and	Susceptible
	<i>An. vagus</i>	100	Cyfluthrin 0.15%	
<i>An. annularis</i>	60			
Thanbyu Zayat Township, Mon State	<i>An. tessellatus</i>	60		
	<i>An. vagus</i>	60	DDT 4%, Deltamethrin 0.05%,	
	<i>An. supitus</i>	60	Permethrin 0.75% and	Susceptible
	<i>An. Barbirostris</i>	60	Cyfluthrin 0.15%	
Beelin Township, Mon State	<i>An. minimus</i>	60		
	<i>An. aconitus</i>	60	DDT 4%, Deltamethrin 0.05%,	
	<i>An. philippinensis</i>	60	Permethrin 0.75% and	Susceptible
	<i>An. annularis</i>	60	Cyfluthrin 0.15%	
Taungoo Township, Bago Region	<i>An. minimus</i>	60		
	<i>An. maculatus</i>	60		
	<i>An. culicifacies</i>	60	DDT 4%, Deltamethrin 0.05%,	
	<i>An. philippensis</i>	60	Permethrin 0.75% and	Susceptible
	<i>An. jamesi</i>	60	Cyfluthrin 0.15%	
<i>An. Vagus</i>	60			

1.1.2 Incrimination of suspected anopheline vectors using sporozoite ELISA and cytogenetic methods (2016)

Anopheles mosquitoes including malaria vectors and suspected vectors of malaria were collected from Loikaw Township Kayah State, Thanbyu Zayat and Beelin Township, Mon State and Tangoo Township, Bago Region. Morphological identification was done according to standard Anopheles mosquito keys. A total of 1237 Anopheles mosquitoes comprising 111 *An. minimus*, 78 *An. annularis*, 102 *An. maculatus* and 141 *An. culicifacies*, 70 *An. tessellatus*, 65 *An. supitus*, 75 *An. aconitus*, 196 *An. philippinensis*, 198 *An. jamesi* and 201 *An. vagus* were collected and tested with sporozoite ELISA test kit in the laboratory. No malaria parasites were detected from mosquito samples from all study areas. Cytogenetic studies showed that all collected *An. culicifacies* were sibling species B and all *An. minimus* were observed sibling species A in these areas.

1.1.3 Vector bionomic and efficacy of insecticides in malaria endemic areas

The study on vector bionomics and Susceptibility of Anopheles mosquito vectors for malaria was studied at Yenikan and Thatabalu villages in Loikaw Township, Kayah State. About 1000 people in Yenikan and 800 people in Thatabalu villages were resided in 134 and 112 houses during 2016. *Anopheles* mosquitoes were collected from fixed mosquito catching stations using animal baited Kanda net (K-net). Light traps were also used for indoor, outdoor collections. Mosquitoes were caught with WHO sucking tubes from 18:00 hour in the evening to 06:00 hours of the next morning. For identification of breeding sites, larval surveys were conducted in and around three kilo-meters from the study villages. Collected

adult *Anopheles* mosquitoes and adult emerged from larva survey were identified for species according to different identification keys. Susceptibility of collected mosquitoes were tested with WHO impregnated paper with WHO test kits. Head and thorax of mosquitoes were dissected to find out *Plasmodium* sporozoites in salivary gland by ELISA test for conforming potential vectors. A total of 1559 *Anopheles* mosquitoes belonging to 9 species were collected from both villages. The major vector *An. minimus* (128) and secondary vectors *An. aconitus* (6), *An. vagus* (280), *An. Jamesi* (7), *An. tessellatus* (18), *An. philippinensis* (32), *An. annularis* (45), *An. barbirostris* (318) were collected from Yenikan village. *An. minimus* (85) and secondary vectors *An. aconitus* (8), *An. vagus* (165), *An. Jamesi* (5), *An. tessellatus* (14), *An. philippinensis* (35), *An. annularis* (30), *An. barbirostris* (191) were collected respectively from Thatabalu village. In the biting outdoor collection of *Anopheles* mosquitoes, 8 *An. hyrcanus*, 78 *An. barbirostris*, 32 *An. vagus*, 2 *An. minimus*, 3 *An. aconitus*, 1 *An. philippinensis* and 1 *An. jamesi* were collected and main vector *An. minimus* was collected in 20:00-21:00 hour in biting outdoor collection. *An. minimus* larvae were collected in rice fields and slowly running water, *An. annularis* were collected in rock pools and *An. vagus* larvae were found in foot prints and small muddy water pools of the bank of the Lawpita creek. All collected *Anopheles* mosquitoes were found sporozoites negative by ELISA method and susceptible to WHO recommended deltamethrin 0.05%, Permethrin 0.75%, Cyfuthrin 0.15% and DDT4% impregnated paper.

1.1.4 Bio-efficacy of long lasting insecticidal mosquito nets (LLINs) on *Anopheles* mosquitoes under field conditions

The present study aimed to determine the bio-efficacy of long lasting insecticidal mosquito nets (LLINs) on malaria vector *Anopheles* mosquitoes in malaria endemic areas. Therefore Hnitkine village Thanbyuzayat and Khaemouk village Beelin Townships from Mon state and Kyauk Lone Gyi village, Taungoo Township Bago Region were selected to test the Bio-efficacy of used Long Lasting Insecticide Nets (LLINs). *Anopheles* mosquitoes were collected from each Township by animal bait K-net method for 5 days. A total of used 30 long lasting Insecticide Nets (10 each) were collected from each Township. Ten households were randomly selected from each Township and a used LLINs net each was collected from these houses of the Townships. Before bio-efficacy test, collected *Anopheles* mosquitoes were tested susceptibility with WHO test kits and WHO recommended impregnated papers as Deltamethrin 0.05%, Permethrin 0.75%, Cyfuthrin 0.15% and DDT4%. Susceptible strain of *Anopheles* mosquitoes were used for Bio-efficacy of distributed used long lasting insecticide treated nets. If not effective (reduced bio-efficacy nets) LLINs nets were re-impregnated with Deltamethrin at the dose of 86 mg/meter square. Result found that a total of 371 and 767 *Anopheles* mosquitoes were collected from Thanbyu Zayet and Beelin Township within 5 days. Of this 117 *An. tessellatus*, 76 *An. vagus*, 113 *An. supictus* and 65 *An. barbirostris* were collected from Thanbyu Zayet and 72 *An. minimus*, 115 *An. aconitus*, 114 *An. philippinensis*, 124 *An. annularis*, 153 *An. jamesi*, 123 *An. vagus* and 66 *An. barbirostris* were collected from Beelin Township respectively. In Kyauk Lone Gyi village, Taungoo Township, a total of 877 *Anopheles* mosquitoes were collected. Of this 118 *An. minimus*, 113 *An. jamsi*, 148 *An. maculatus*, 141 *An. culicifacies*, 128 *An. philippensis*, 141 *An. vagus* and 88 *An. barbirostris* were collected respectively. All mosquitoes were susceptible to Deltamethrin 0.05%, Permethrin 0.75%, Cyfuthrin 0.15% and DDT4%. A total of 30 LLIN nets (10 each) were collected from Thanbyu Zayet, Beelin and Taungoo Township for testing bio-efficacy of LLIN nets. Of this 2 PermaNet 2.0 (Vestergard) nets, 6 DAWA TANA (Pakistan) nets and 2 Yorkool (China) nets from Thanbyuzayat and 2 PermaNet 2.0 nets, 4 DAWA TANA nets, 3 BASF (Thailand) nets and one Net Protect (India) (3DF) net were collected from Beelin Townships and 3 Permanet 2.0, 2 DAWA

TANA, 2 BASF, One Net Protect and 2 Yorkool nets were collected from Kyauk Lone Gyi village Taungoo Township. All collected nets were washed 1-5 times with soap powder. Bio-efficacy of LLIN nets were found to be PermaNet 2.0 100%, DAWA TANA nets 83.33%, Yorkool nets 100% mortality of *Anopheles* mosquitoes in Thanbyzayat Township and PermaNet 2.0, DAWA TANA, BASF, and Net Protect nets were found to be 100% sensitive (100% mortality) in Beelin and In Taungoo Township PermaNet 2.0, DAWA TANA, BASF, Net protect and Yorkool nets were found 100% sensitive (100% mortality). All tested LLIN nets were highly sensitive against *Anopheles* mosquitoes. Only one DAWA TANA net from Thanbyu Zayat was gave 30% mortality. Therefore this mosquito net was re-impregnated with deltamethrin insecticide at the rate of 86mg/meter square in the survey.

1.2 DENGUE HAEMORRHAGIC FEVER

1.2.1 Vector surveillance and mapping of insecticide resistance in Dengue vector *Aedes aegypti*

Field survey was carried out in Shwe Myin Tin village, Pathein Township, Ayeyarwady Region during December 2016. Hundred households from the study site were selected and all types of water containers within the premise of each household were examined for the presence of *Aedes* larvae and pupae. All positive containers were noted and larvae were collected. Point data were collected using GPS receiver for mapping. Data from the GPS receiver were downloaded using Map Source software, viewed in Google Earth and image registration was processed using ERDAS imaging software. Identification of mosquitoes was made by using standard mosquito keys. Collected mosquitoes were reared in the insectary for further insecticide susceptibility test. The most common outdoor water container type was small earthen Bago jar (27.5%) and indoor water container was flower vase (38.6%) in Shwe Myin Tin village, Pathein Township, Ayeyarwady Region. Container index (CI) and house index (HI) were 5.4% and 24% respectively and Pupae Per Person index (PPP) was 0.1 in this area. The mosquito density was quite low in the study area due to the periodic inspection of local health staff and dry season.

1.2.2 Dengue vector control in school using newly modified pyriproxyfen in Yangon, Myanmar

Schools were considered to be an important place for dengue virus transmission among children in Myanmar; however, little information was available about aquatic habitats of dengue virus vectors in schools. As a school compound usually consists of school building area and staff housing area, the habitats may be unique. The larvicide, one of the most common control measures, currently used in Myanmar is effective for three months. On the other hand, newly developed pyriproxyfen “Matrix Release” formation was expected to have long-term effect. This study characterized aquatic habitats of dengue virus vectors in 12 school compounds in Hlaing Thar Yar, one of the most populated townships in Yangon, and examined the effectiveness of newly developed pyriproxyfen. In total, 1311 potential aquatic habitats were found in the schools between June and November 2016. Of them, 265 (20.2%) habitats had *Aedes* larvae. Staff housing area had significantly more positive *Aedes* positive habitats than school building area (OR: 2.08, CI: 1.42-3.09, $P < 0.001$). At the end of November, newly developed pyriproxyfen was placed in the potential habitats of randomly selected 6 schools and the rest of 6 schools were considered as control. The number of positive habitats was significantly reduced during the post intervention period when compared to the pre intervention period (OR: 0.29, CI: 0.15-0.56, $P < 0.001$). On the other hand, the difference was not significant for the control schools between the two periods.

Moreover, we collected 8 month used newly developed pyriproxyfen discs from the field and tested again in laboratory and found that it was still 100% fully prevented mosquito adult emergence from larva. These findings will be useful for dengue vector control in schools and the future vector control activities.

1.3 OTHERS

1.3.1 Larvicidal and ovicidal effect of *Cymbopogon nardus* (L) Rendle Lemon grass and root essential oil against *Aedes aegypti* larvae

Mosquito-borne diseases are mostly harmful to children being and it is a public health problem in Myanmar. Laboratory reared Dagon Myothit North Township strain of *Aedes aegypti* mosquito larvae were used for testing larvicidal and ovicidal properties of leave and root essential oil of *Cymbopogon nardus* (L) Rendle which plant was collected from Kalama Mountain, Paung Township Mon State. Separately shade dried ground leaves and roots 100 gram each of powder were extracted with 800ml distilled water by steam distillation method at 100°C for 3 hour separately. After complete removal of the water resulting 0.92gm and 0.87gm of essential oil were obtained from 100gm each of dried leave and root powder. Different concentration of leaves and roots essential oil of *Cymbopogon nardus* were prepared freshly in 100ml each of distilled water in 150 ml plastic cups. Detail testing was done on 3rd and 4th instar larvae and gravid *Aedes* mosquitoes according to WHO standard method. Result revealed that the highest dose 0.01g of leaves and root essential oil produced 100% knockdown within 60minutes and 98.22% and 96.67% mortality within 24 hours followed by 95.56%, 95.00% knockdown and 86.11, 81.11% mortality respectively in 0.005g concentration. The effective lethal concentrations LC₅₀ for 50% mortality and LC₉₀ for 90% mortality against *Aedes* larvae were found to be 0.0012g and 0.0054 for leaves, 0.0015g and 0.0066g for roots essential oil of *Cymbopogon nardus*. LC₅₀ and LC₉₀ values were not significantly difference between leaves and roots essential oil. Although leaves essential oil found slightly higher efficacy than root essential oil against *Aedes* larvae within 24 hours. Very high ovicidal efficacy was found to be 100% recovery at 0.0025g concentration for both essential oils. The leaves and roots essential oil of *Cymbopogon nardus* exhibited strongly larvicidal and ovicidal activities against *Aedes aegypti* larvae and gravid mosquitoes.

1.3.2 Efficacy of ovitrap colours for attracting *Aedes* mosquitoes at field sites in Pakokku Township, Magway Region

Wild female *Aedes aegypti* and *Ae. albopictus* were allowed to lay eggs in ovitraps with different coloured ovistraps as black, blue, green, orange, red, white, yellow and control (without colouted) 7 coloured bowls were used in indoor and outdoor for oviposition preference in Pakokku Township Magway Region. Oviposition cycle was studied 14days/1cycle trial in different places for 84 days. The number of larvae and frequency were observed daily. Experiments were conducted to determine egg laying preference for any specific colour of the ovitrap. Result found that for oviposition preference *Ae. aegypti* gravid laid their eggs in all coloured bowls in both indoor and outdoor. The combined result of indoor and outdoor was observed that the maximum number (402) of larvae was observed in red coloured bowl followed by black coloured bowl (300 larvae) in containing purified water. Although white coloured bowl in indoor and red coloured bowl in outdoor were found most preferred by *Ae. aegypti* and black coloured ovitrap was found to be most preferred by *Ae. albopictus* in outdoor. And also black coloured bowls found to be preferred both *Aedes* species in outdoor. In addition, during the study period *Aedes* mosquitoes laid more frequency in red bowl (23frequencies) and followed by White and Yellow coloured bowl (20

frequencies each), lowest frequencies was found in orange bowls (9 frequencies). In the Black bowl was found 18 frequencies of oviposition by female *Ae. aegypti* and *Ae. albopictus* was found 5 frequencies in outdoor within 84 days. In the present studies *Ae. aegypti* laid the maximum number of eggs in red coloured bowls and *Ae. albopictus* laid the maximum number only in black bowl in outdoor and *Ae. aegypti* was found maximum number in white bowl in indoor. The study revealed that the colour of the ovitraps plays an important role in attracting the oviposition of females *Aedes* mosquito species and due attention can be given while considering the colour of the ovitraps, to be used for different objectives of the investigations. The ovitraps of red, yellow, black and white colour were found to be appropriate for *Aedes* eggs and larvae collection.

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Medical Entomology Research Division conducted a number of studies on dengue vector and their control. The studies included detection of metabolic-based resistance against pyrethroids in *Aedes aegypti*, screening of cross resistance on temephos (abate) selected *Ae. aegypti* with certain insecticides, detection of resistant on malathion induced *Ae. aegypti* in the laboratory condition and the activities of larvicide and ovicidal of plant-based extracts conducted on vectors (*Aedes*, *Culex* & *Anopheles*).

RESEARCH PROJECTS

1. COMMUNICABLE DISEASES

1.1 DENGUE HAEMORRHAGIC FEVER

1.1.1 Detection of metabolic-based resistance against pyrethroids in *Aedes aegypti* from selected areas in Mandalay

The development of vector resistance to insecticides is a main problem for mosquito borne diseases control. The metabolic detoxification through enhanced level of enzyme activities (oxidases, esterases and glutathione S-transferases) involved in insecticide resistance. Based on the resistance data, appropriate alternative insecticides can be conducted for effective vector control. This study was conducted to determine pyrethroids (0.05% deltamethrin and 0.75% permethrin) resistance in *Aedes aegypti* by WHO standard procedure and to detect the level of enzyme activities (oxidases and esterases) in mosquitoes by biochemical assay. *Aedes aegypti* were collected from three selected areas (Min Te Ekin, Maha Myaing, Keik Sana Mahe wards) in Mandalay, from August 2014 to December 2015. Results showed that the adult of *Ae. aegypti* from selected areas was resistant to deltamethrin and permethrin. The mean optical density values of oxidases in Maha Myaing, Keik Sana Mahe, Min Te Ekin and laboratory populations were 0.87 ± 0.2 , 0.64 ± 0.1 , 0.54 ± 0.1 and 0.53 ± 0.1 at 630 nm, respectively. The level of oxidases activity was significantly higher in Maha Myaing and Keik Sana Mahe populations when compared to the laboratory population ($p < 0.05$). Furthermore, the mean optical density values of esterases in Min Te Ekin, Maha Myaing, Keik Sana Mahe and laboratory populations were 0.58 ± 0.1 , 0.39 ± 0.1 , 0.38 ± 0.03 and 0.37 ± 0.03 at 450 nm, respectively. The level of esterases was significantly higher in *Ae. aegypti* in Min Te Ekin compared to laboratory population ($p < 0.05$). The enhanced enzyme activities (oxidases and esterases) contribute to pyrethroids (deltamethrin and permethrin) resistance in *Ae. Aegypti* from selected areas.

1.1.2 Screening of cross resistance on temephos (abate) selected *Aedes aegypti* with certain insecticides

Insecticide resistant in mosquitoes is an increasing problem worldwide. Cross-resistance occurs when resistance to one insecticide confers resistance to another insecticide, even where the insect has not been exposed to the latter product. The present study aimed to evaluate cross-resistance of temephos (abate) treated generation against three insecticides (4% DDT, 5% malathion, 0.05% deltamethrin). Samples of *Ae. aegypti* from Pyin Oo Lwin, Mandalay Region were subjected to temephos selection at the larval stage for twenty eight consecutive generations. Cross-resistance towards the same and different groups of insecticides was determined using temephos-selected adult females. Result revealed that *Ae. aegypti* can survive WHO diagnostic dosage DDT4%. Fully resistant was observed in test with DDT 4% in all test group. Little or no cross-resistance was observed to the organophosphates, malathion 5%, however, high cross-resistance was observed for the pyrethroid group, deltamethrin 0.05%. The cross resistance to deltamethrin from temephos selection could limit the use of both insecticides for *Ae. aegypti* control.

1.1.3 Determination of insecticide resistance in dengue vector upon selection pressure with malathion

Dengue is a major public health problem globally and *Aedes aegypti* plays a major role of dengue transmission. Chemical control is an effective way to reduce vector population. Frequent usage of the same insecticide will select for those individuals in a population, which will favor to survive in the presence of recommended dose of the compounds. Over time this selection pressure will lead to a resistant population becoming established. This study was performed to investigate insecticide resistance of dengue vector against malathion. Field strains of *Ae. aegypti* were collected from five different townships in Mandalay Region: Pyin Oo Lwin, Chan Mya Thar Zi, Pyi Gyi Dagon, Pa Thein Gyi and Pyaw Bwe. Bioassays were conducted in both larval and adult stages following WHO standard procedures. Pyin Oo Lwin strain was selected to treat malathion every generation at larval stages. Result revealed that Chan Mya Thar Zi strain was most susceptible and Pyin Oo Lwin strain was least susceptible in the baseline susceptibility test in both larval and adult stages. High resistance development was observed after subjection of malathion to *Ae. aegypti* generation 17 at larval stage, however, the resistance development was not prominent in the adult stage.

1.1.4 Oviposition altering and ovicidal activity of ethanolic leaf extract of *Lantana camara* L. (စိန့်နားဝန်) against the dengue vector *Aedes aegypti*

Mosquitoes are the prominent vectors of human diseases viz., malaria, yellow fever, dengue, filariasis, Japanese Encephalitis including Zika. Among the different genus of mosquitoes, individuals of genus *Aedes* are considered highly dangerous because these show more dependency on human blood and breed in artificial containers. *Ae. aegypti* is the potential vector of dengue, yellow fever including Zika. Various plant based products are safe and biodegradable alternatives to synthetic chemicals for use against mosquitoes. This study aimed to evaluate oviposition altering and ovicidal efficacy of leaf extract of *Lantana camara* L. against the dengue vector *Ae. aegypti*. The *L. camara* L. leaves were collected from Pyin Oo Lwin District and the leaves were shade dried, powdered and extracted by using ethanol. For oviposition altering and ovicidal activity tests, twenty gravid female mosquitoes of *Ae. aegypti* and eggs of *Ae. aegypti* mosquito (100) were exposed to various concentrations. Oviposition altering and ovicidal activity were observed after 24 hrs and 96 hrs exposure.

Each test concentration was repeated to 4 times in each experiment. Results indicated that, oviposition deterrent activity of ethanolic extract of *L. camara* L leaves against *Ae. aegypti*, it showed 61.54%, 68.44%, 76.21%, 88.06% and 93.15% at 50 ppm, 100 ppm, 200 ppm, 300 ppm and 400 ppm after 24 hrs exposures, respectively. In the ovicidal test, egg hatchability showed 94.12%, 86.17%, 77.50%, 68.18%, 64.13%, 59.04%, 56.25%, 51.39%, 44.44% and 30.67% with leaf ethanolic extract of *L. camara* L. at 100 ppm, 200 ppm, 300 ppm, 400 ppm, 500 ppm, 600 ppm, 700 ppm, 800 ppm, 900 ppm and 1000 ppm after 96 hrs exposures, respectively. The study indicated that the leaf ethanolic extract of this plant have showed the oviposition deterrent and ovicidal properties.

1.2 MALARIA

1.2.1 Larvicidal efficacy of *Morindacitrifolia* L. (ရဲဝိ) leaf extract against *Anopheles minimus*, *Aedes aegypti* and *Culex quinquefasciatus*

Morindacitrifolia leaf extract was tested for larvicidal efficacy against three medically important mosquito vectors such as malaria vector *Anopheles minimus*, dengue vector *Aedes aegypti* and filarial vector *Culex quinquefasciatus*. *M. citrifolia* leaves were washed with tap water and shade dried at room temperature ($28\pm 2^{\circ}\text{C}$). The air-dried plant materials (leaves) were powdered by an electrical blender. From the leaf, one kg powder was macerated with 3.0 L of methanol for a period of 72 hours and filtered. The solvent from the extract was removed using a vacuum evaporator and stored at a temperature of 4°C . Standard stock solution was prepared at 1% by dissolving the residues in acetone, which was used for the larvicidal bioassay. Twenty five late third instar larvae or early fourth instar larvae were introduced into a 250 ml beaker containing 199 ml of tap water and 1 ml of different experimental concentration was added. The concentrations of plant leaf extract were 10 ppm, 20 ppm, 30 ppm, 40 ppm and 50 ppm, respectively. The control was set up by mixing 1 ml of acetone with 199 ml of tap water. Five replicates were kept for each concentration along with the control. Mortality was recorded after 24 hours of treatment. Fifty percent lethal concentration (LC_{50}) and ninety percent lethal concentration (LC_{90}) were calculated by using the LdP line software. The results showed that the LC_{50} and LC_{90} values of *An. Minimus* were 12.63 ppm, 39.75 ppm and *Ae. Aegypti* were 15.33 ppm, 47.74 ppm and *Cx. quinquefasciatus* were 17.17 ppm and 49.58 ppm, respectively. The highest larval mortalities of *An. minimus*, *Ae. aegypti* and *Cx. quinquefasciatus* were found 100%, 93.60% and 91.73% when treated with 50 ppm of plant leaf extract. In the present study, methanol extract of *M. citrifolia* leaf can be used effectively to control the larvae of vector mosquitoes; *An. minimus*, *Ae. Aegypti* and *Cx. quinquefasciatus*.