

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 Bago Region. 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. Establishment of Anopheles mosquito colonies were continued with *An. dirus* mosquitoes from Mudon Township and now they are in F 55 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 (2015)

Monitoring of insecticide susceptibility status in some *Anopheles* was conducted in Phyu 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 Phyu Township, Bago Region. Mosquito species and insecticides used were shown in the table below.

Township	<i>Anopheles</i> species	No.	Tested Insecticides	Remark
Phyu Township, (Bago Region)	<i>An.minimus</i>	77		Susceptible
	<i>An. maculatus</i>	170	DDT 4%, Deltamethrin 0.05%,	
	<i>An.culicifacies</i>	30	Permethrin 0.75% and	
	<i>An.vagus</i>	30	Cyfluthrin 0.15%	
	<i>An. annularis</i>	30		

1.1.2. Incrimination of suspected anopheline vectors using sporozoite ELISA methods (2015)

Anopheles mosquitoes including malaria vectors and suspected vectors of malaria were collected from Ann Township and Taung Goke Township, Rakhine State and Thayetchaung Township, Tanintharyi Region. Morphological identification was done according to standard *Anopheles* mosquito keys. A total of 429 *Anopheles* mosquitoes comprising 21 *An. dirus*, 57 *An. minimus*, 5 *An. annularis*, 157 *An. maculatus* and 3 *An. culicifacies*, 103 *An. kochi*, and 83 *An. vagus* were collected and tested with sporozoite ELISA test kit in the laboratory. Only 1 *An. culicifacies* from Ann Township was positive with Pf circumsporozoite antigen. No positive reaction was detected from mosquito samples from Taung Goke and Thayatchaung Townships.

1.1.3 Vector bionomic and efficacy of insecticides in malaria endemic areas

The study on vector bionomics and identification of potential vector for malaria was studied at Kywemakuu village, Phyu Township, Bago Region. About 500 people were resided in 130 houses in Kywemakuu village during 2015. *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 kilometers from the study villages. Collected adult *Anopheles* mosquitoes and adult emerged from larva survey were identified for species according to different identification keys. 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 639 *Anopheles* mosquitoes belonging to 11 species were collected. The major vector *An. minimus* (n=92) and secondary vectors *An. maculatus* (n=188), *An. culicifacies* (n=48), *An. vagus* (n=58), *An. annularis* (n=41), *An. jamesii* (n=12), *An. tessellatus* (n=5), *An. stephensi* (n=5), *An. aconitus* (n=9) and *An. barbiprosis* (n=175) were collected. *An. minimus* and *An. culicifacies* larvae were collected in sand pools and slowly running water, *An. maculatus* and *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 Phyu creek. All collected *Anopheles* mosquitoes were found sporozoite negative by ELISA method.

1.1.4. Prevalence of sibling species complex of *Anopheles minimus* using ovarian nurse cell chromosome method in two different areas in Myanmar

Malaria remains a major health problem in Myanmar. *An. dirus* and *An. minimus* are the main vectors of malaria in Myanmar. The present study aimed to detect the prevalence of sibling species complex of *Anopheles minimus* using ovarian nurse cell polytene chromosome method and to determine the potential vector using vector incrimination. Therefore, the study was conducted in Pyin Oo Lwin Township, Mandalay Region and Kamamaung Township, Kayin State during 2015. *Anopheles* mosquitoes were collected by animal bait K-net, human bait indoor and outdoor and the light trap collection. After species identification, all blood fed *An. minimus* were kept in paper cups with glucose. When the ovary development is reached to semi-gravid stage (Christophe stage), ovary specimens were dissected and preserved in Cornoy's fixative in the screw type bottle and ovaries were tapered to obtain polytene chromosome arms. Collected Anopheline mosquitoes head and thorax were homogenized and tested for circumsporozoite antigen by ELISA method. Results found that all collected *An. minimus* were observed sibling species A in both areas. Circumsporozoite

ELISA test found that only one *An. minimus* was sporozoite positive in Kamamaung Township 0.54% (1/185) and no found in *An.minimus* collected from Pyin Oo Lwin Township (0/553).

1.2. DENGUE HAEMORRHAGIC FEVER

1.2.1. Study on dengue vector population and insecticide susceptibility status of *Aedes* mosquitoes to Temephos (Abate) in Cyclone Nargis affected area in Myanmar (Yangon, Ayeyawady and Mon)

Aedes aegypti is the major vector of Dengue and DHF in Myanmar. The transmission of dengue is largely influenced by vector abundance, survival, bionomics, type and abundance of water containers and housing conditions. One hundred households from each study site was selected using multistage sampling method. All types of water containers within the premises of each household were examined for the presence of *Aedes* larvae and pupae. All positive containers were noted and larvae were collected. Identification of mosquitoes was made by using standard mosquito keys. Collected mosquitoes were reared in the insectary for further insecticide susceptibility tests. Field and laboratory based larva susceptibility tests were carried out according to WHO guidelines. Field surveys were conducted in Patheingyi Township, Ayeyawady Region, Hlaing Thar Yar Township, Yangon Region and Thanbyuzayat Township, Mon State during 2014-2015. House index (HI) were 20%, 13% and 12%, container index (CI) was 7%, 2.4% and 1.4%, pupae per person index (PPI) was 0.55, 0.35 and 0.19 in Patheingyi, Hlaing Thar Yar and Thanbyuzayat respectively. Since pupal mortality is very low, PPI can serve as a proxy for adult mosquito population. For an average temperature of 27°C with seroprevalence of 33%, the estimate of the transmission threshold is approximately 0.71 PPI. Therefore, our PPI findings suggest that there is a low risk of transmission in these areas. 100% larva mortality was found in the field test with Abate 0.1 gm/L (recommended field dose).

1.2.2. Knowledge on DHF and Larva survey in Urban area of Thanbyuzayat Township

A community based cross sectional study of knowledge on Dengue Haemorrhagic Fever and larva survey in selected 2 urban wards (Aung Chan Thar and Aung Khu Tho) of Thanbyuzayat Township, Mon State was carried out during September 2015. A total of 150 respondents were interviewed with structured interview questionnaire and larva survey was performed at their houses. Most of respondents had passed primary school level and can read and write only. Nearly all of the respondents were dependent. Per capita income of respondents was 36452 kyats (Mean), 8333.33 kyats (Minimum) and 125000 kyats (Maximum) per month. There was statistically significant association between education, income of the respondents, history of DHF in their children, family members and neighbors with knowledge on DHF in univariate analysis. But there was 73 of the respondents still had poor knowledge about DHF. Although most of the respondents had good knowledge, they could not sustain larva control measure and high larval indices were seen. Out of 150 houses, 68 houses were larva infested (HI). There were total 1468 containers; 602 containers located inside and 866 containers located outside of the households. Among these containers, 157 containers were larva infested (CI). There was 104.67 positive containers per 100 houses inspected (BI) and (PPI) was 0.086. Education level of respondents significantly influenced the knowledge. The highest knowledge score was obtained by young age. It was also found that the presence of history of DHF among the family members had higher knowledge. The results of our study pointed out that promoting socio-economic status of respondents and enhancing health education is necessary to sustain proper knowledge for preventive activities

and practicable methods for removal. Household members still required adequate support to perform destruction of larva without wasting domestic water. It is necessary to encourage and motivate communities, especially ladies, to participate actively in the preventive activities.

1.2.3. Insecticidal properties of *Citrus hystrix* DC (တောရှောက်ခါးသီး) essential oil against *Aedes aegypti*

The present study aimed to evaluate the larvicidal activity of ethanol extracts of dry fruit, internal fruit materials and peels of *Citrus hystrix* DC against *Aedes aegypti*. The 3rd and 4th stage larvae of *Aedes aegypti* collected from Hlaing Thar Yar, Shwe Pyi Thar and North Dagon Townships, Yangon Region and Thanbyuzayet were exposed for 24 hours to various concentrations (0.15, 0.075, 0.0375, 0.01875 and 0.009375gm/100mL) of ethanol extracts of different parts of the *Citrus hystrix* fruit. The dry fruit extract resulted in significantly higher (96-100%) mortality ($P < 0.05$) when compared to the mortality (80-90%) caused by internal material of *Citrus hystrix* fruit at the concentration of 0.15gm/100mL against *Aedes* larvae of Hlaing Thar Yar, Shwe Pyi Thar and North Dagon. The mortality of *Aedes* larvae in peel extract was found (92-96%). Although all kinds of *Citrus hystrix* DC extracts were very sensitive to *Aedes* larvae collected from Thanbyuzayet 100% mortality was found at 0.1gm/100mL on peel and fruit extract. The LC_{50} and LC_{90} values were 0.0142, 0.0276 and 0.0138, and 0.0522, 0.1045 and 0.0515 g for peel, internal material and fruit extract. The findings of the present study revealed that the ethanol extract of the fruit of *Citrus hystrix* DC as a good source of preparations for pest control especially mosquito control.

Table 1. LC_{50} and LC_{90} values of dried fruit, peel and internal material extracts against *Aedes* larvae from three different Townships

Townships	Dried fruit extract		Dried peel extract		Dried internal fruit material extract	
	LC_{50}	LC_{90}	LC_{50}	LC_{90}	LC_{50}	LC_{90}
Shwe Pyi Thar	0.0226	0.0729	0.0332	0.1124	0.0392	0.1533
North Dagon	0.0231	0.0742	0.0343	0.1256	0.0415	0.1584
Hlaing Thar Yar	0.0280	0.1	0.0396	0.1408	0.0552	0.2322

SERVICES PROVIDED

ACADEMIC

Sr. No.	Name	Course	Responsibility
1	Dr. Yan Naung Maung Maung	MMedSc (Microbiology) Workshop on Research Methodology (2015)	Teaching Facilitator
2	Dr. Maung Maung Mya	MMedSc (Microbiology) Workshop on Research Methodology (2015)	Teaching Facilitator

MEDICAL ENTOMOLOGY RESEARCH DIVISION (POL)

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	...	Daw Naw Hnin Myint BSc(Hons)(Mawlamyine University), MSc(Credit)(Zoology)(YU)
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	...	Daw Si Si Aung BA(History)(UDE)

Medical Entomology Research Division conducted a number of studies on dengue vector and their control. The studies included susceptibility of dengue vectors to insecticides and the activities of larvicide, pupicide and repellency of plant-based extracts conducted on dengue vector.

RESEARCH PROJECTS

1. COMMUNICABLE DISEASES

1.1 DENGUE HEMORRHAGIC FEVER

1.1.1 Susceptibility of *Aedes aegypti* and *Aedes albopictus* to insecticides in Mahar Aung Myae Township, Mandalay City

The level of insecticide susceptibility of mosquitoes is one of the important factors for success of mosquito-borne diseases control programme. The study was carried out to investigate the susceptibility of *Ae. aegypti* and *Ae. albopictus* to insecticides (5% malathion, 0.05% deltamethrin and 0.75% permethrin), following the WHO susceptibility procedures and conducted in Mahar Aung Myae Township. The mortality of *Ae. aegypti* was 100% after 24 hours exposure period to 5% malathion. The mortalities of *Ae. aegypti* were 35.23% against 0.05% deltamethrin and 11.42% to permethrin. The study revealed that *Ae. aegypti* from Mahar Aung Myae was susceptible to malathion but highly resistant to deltamethrin and permethrin. The mortality of *Ae. albopictus* was 100% to insecticides (malathion, deltamethrin and permethrin) in Mahar Aung Myae. *Ae. albopictus* was still susceptible to insecticides (malathion, deltamethrin and permethrin). This study indicated that synthetic pyrethroid (0.05% deltamethrin and 0.75% permethrin) is not effective for controlling the primary vector, *Ae. aegypti*.

1.1.2. Laboratory evaluation of plant-based repellent cream against *Aedes aegypti* mosquito

Mosquito borne diseases are public health problems in worldwide. Mosquito repellent is commonly used method to prevent mosquitoes bite. The most widely marketed synthetic-based insect repellent is N, N-diethyl-3-methylbenzamide (DEET), however, it has been reported that the synthetic repellents is not safe for the public use. Essential oils of plant are needed to replace the synthetic repellents as an alternative approach for personal protection. The aim of this study was to evaluate the repellent potential of plant-based cream on *Ae. aegypti* mosquito. The main constituents of plant-based cream were olive, lavender, lemon

grass, eucalyptus and citronella oils. The cream was evaluated at three formulations; 45% (v/w), 50% (v/w) and 60% (v/w) concentrations in olive oil base was prepared by mixing different amount of essential oils, binder (triethanolamin, cera alba, stearic acid) and distilled water. The mean complete protections of biting times were 110 minutes for 45% formulation, 176 minutes for 50% formulation and 341 minutes for 60% formulation, respectively. The present study indicates the formulated plant-based repellent cream can prevent effectively from biting of mosquitoes.

1.1.3. Determination of insecticide resistance in dengue vector upon selection pressure with malathion (Phase I)

Dengue is a major public health problem globally and *Aedes aegypti* play 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 favour 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* larvae were collected from four townships, Mandalay Region by larval collection method. Bioassays were conducted by using the WHO standard susceptibility procedures. Result for both larval and adult bioassays test showed that Pyi Gyi Da Gon strain was the most susceptible and Pyin Oo Lwin strain was the least susceptible in study populations. Every generation of Pyin Oo Lwin strain was chosen for malathion inducing. Adult bioassays will be carried out on phase II after 25 generations of malathion inducing.

1.1.4. Larvicidal and pupacidal efficacy of 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 species 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 larvicidal and pupacidal efficacy of leaf extract of *Lantana camara* L. against the dengue vector *Ae. aegypti*. The *Lantana camara* L. leaves were collected from Pyin Oo Lwin District and the leaves were shade-dried, powdered and extracted by using ethanol. Among them, 25 late third and early fourth instars of *Ae. aegypti* larvae and 25 pupae were exposed to various concentration and the larvae mortality were observed after 24hr, 48hr exposure and pupae mortality was observed after 48hr exposure. Each test concentration was repeated to 6 times in each experiment. Results indicated that larvicidal efficacy of ethanol extract of *Lantana camara* L. leaves against *Ae. aegypti*, LC₅₀ and LC₉₀ values were 335 ppm , 1718 ppm after 24 hr exposure and 277 ppm, 1692 ppm after 48hr exposure respectively. In the pupacidal test, it showed 8%, 13.3 %, 18.67 % and 22.67 % with ethanol leaves extract of *Lantana camara* L. at 1500 ppm, 2000 ppm, 2500 ppm and 3000 ppm after 48hr exposure respectively. The study indicated that the leaf extract of this plant showed the larvicidal and pupacidal properties.

SERVICES PROVIDED

ACADEMIC

Sr. No.	Name	Course	Responsibility
1.	Daw Yi Yi Mya	Research Finding 2015 Dissemination: Facility Assessment of Reproductive Health Commodity and Services	Presenter

LABORATORY

Sr. No.	Laboratory test	Tested samples
1.	Repellency test	3 samples