



SEASONAL DISTRIBUTION OF *Aedes* LARVAE IN VARIOUS WATER STORAGE CONTAINERS IN LAYMYETHNAR TOWNSHIP, HINTHADA DISTRICT, AYEYAWADY REGION

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ABSTRACT

Seasonal study was conducted in Lal Tan Ngal village, Laymyethnar Township during the study period from October 2017 to September 2018. All potential breeding sites were examined in order to carry out the systematic study. Larval positive containers of different container categories as major, minor, miscellaneous containers and types were recorded. Key containers and Key premises were recorded and compared with each other. In wet season (2017), cool season (2018) and dry season (2018), 54%, 62% and 44% of households were found *Aedes* larval positivity. Season larval positivity in major, minor and miscellaneous containers were found 42.64%, 6.77%, 8.57% in wet; 18.67%, 75%, 23.76% in cool and 62.5%, 36.36%, 10.14% in dry seasons respectively. Key containers and Key premises in village were found 41.43%, 18% in wet; 24.07%, 16% in cool and 8.57%, 2% in dry respectively. The larval indices such as House Index (HI), Container Index (CI) and Breteau Index (BI) were observed highest in cool season 62, 31.03, 108 than 54, 21.08, 140 in wet and 44, 19.13, 70 in dry season. In Lal Tan Ngal village, pupal indices in positive containers and percentage of Key containers and Key premises were significantly declined from wet (2017) to dry (2018). Larval positivity was high in major (concrete jars, big bago jars), minor (small bago jars, earthen pots) and miscellaneous (broken bago jars and earthen pots, bamboo stamps) containers. It will become high-risk area of DF and DHF due to high infestation level of *Aedes* larvae. Therefore, proper control methods are needed to clean out the *Aedes* larvae in water storage containers and cover with lids completely the various water storage containers to reduce transmission of DF and DHF in children.

KEYWORDS: Major, Minor, Miscellaneous, containers, HI, BI, CI, Key containers, Key premises.

INTRODUCTION

Aedes aegypti is the primary vector involved in Dengue Fever, Yellow Fever, Zika virus infection, and Chikungunya. *Aedes aegypti* and *Aedes albopictus* are belonging to the subgenus *Stegomyia* and they are closely associated with peridomestic environments (Balasubramanian *et al.*, 2015). They are distributed especially between latitude 45° north and latitude 40° south in the tropical regions. In dengue endemic regions such as Southeast Asia, even though *Aedes albopictus* is incriminated in dengue transmission, *Aedes aegypti* remains so far the major vector. Especially in recent years, the distribution space of both mosquitoes and mosquito-borne disease have been changing and expanding for reasons such as increasing rates of environmental changes, climate changes, vector and pathogen resistance to insecticides and drugs,

progressive urbanization and population movement (Aziz *et al.*, 2012).

Dengue is the most important mosquito-borne virus disease in the world, with nearly 2500 million people at risk worldwide (WHO, 2017). The abundance of dengue is closely associated with the abundance of vectors and environmental factors (rainfall, temperature and relative humidity (Simmon *et al.*, 2012). *Aedes aegypti* was higher in number during the dry season and *Aedes albopictus* was higher in the wet season (Mogi *et al.*, 1988).

Aedes density, as well as the number of dengue cases, increased in the wet season in Malaysia, India, Sri Lanka, Myanmar, Indonesia, Philippines and Thailand (Wai *et al.*, 2012). About 50 million people are at risk in dengue endemic countries. South-East Asia region and

Western Pacific region, which bear nearly 75% of the current global disease burden due to dengue.

Aedes aegypti is a vector of dengue in urban areas but now the species are distributed in rural areas in Myanmar. DF and DHF are increasingly becoming serious public health problems in Myanmar especially among the 5-10 and 11-15 years old age groups. The vast majority of the DHF cases occur in 5-8 years old age group. Recently, DMR research team reported that the adult age DHF cases were found from sample of adult patients of Yangon General Hospital. A severe outbreak of DHF occurred for the first time in Yangon in 1970. In Myanmar, the highest numbers of DHF cases were reported from Ayeyawady, Kachin, Magway, Mandalay, Mon, Rakhine, Sagaing, Tanintharyi and Yangon Regions. According to Ministry of Health and Sports (2018), 11,750 people were infected with the DHF and there were 59 fatalities against more than 100 corresponding last year. DHF mainly affects children under 15 years old, occurred mostly in Ayeyawady and Yangon Regions and Mon State and the disease is spread by the bite of infected mosquito in the rainy season. Generally, more DHF cases predominate during the raining season and highest number of cases was recorded in July (Maung Maung Mya *et al.*, 2016).

Currently, there is no effective antiviral treatment or preventive vaccine for dengue (Xu *et al.*, 2016). *Aedes* larval control is the most effective method for controlling dengue disease (Singh and Taylor-Robinson, 2017). For the prevention of dengue fever, the topographical factors that influence the key breeding sites of *Aedes aegypti* and *Aedes albopictus* must be more closely investigated.

There are several factors influencing *Aedes* mosquitoes, including water container types, seasons and socio-

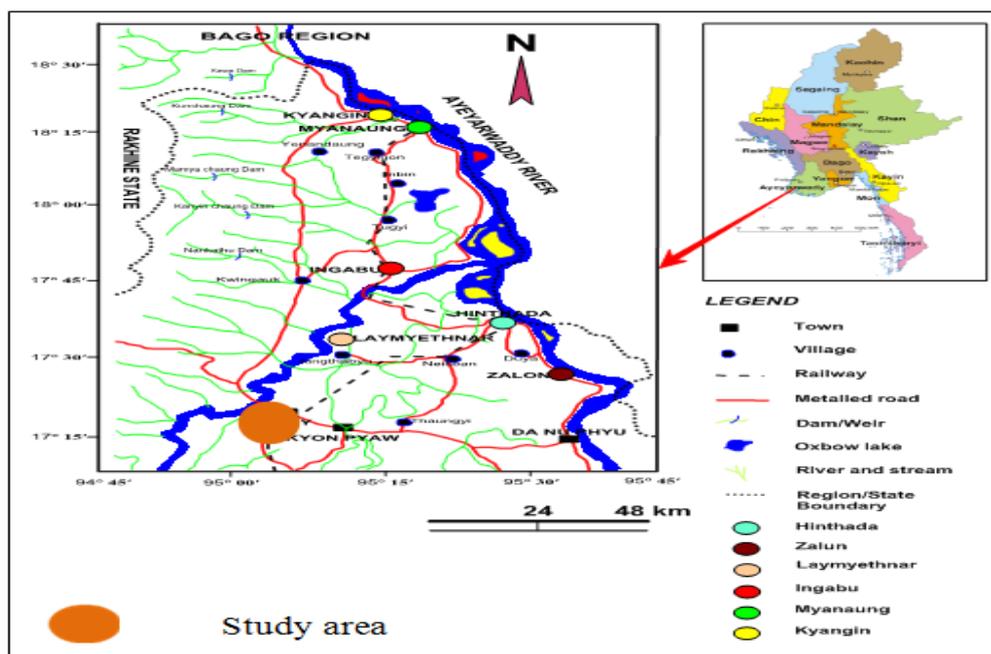
culture practices, topographic, climatic and vectorial factors (Chumsri *et al.*, 2018). Container type is probably the most important factor determining the key breeding sites of mosquito species. *Aedes aegypti* preferred to breed in indoor containers and *Aedes albopictus* preferred to breed in outdoor containers, but both species preferred to breed in artificial, dark-colored, or without lid containers than in natural, light-colored, or with lid containers, respectively (Wongkoon *et al.*, 2007, Rajesh *et al.*, 2013).

Water storage practices in household for multipurpose use in city and rural areas provide year-round breeding opportunities for the vector. In States and Regions, *Aedes* breeding sites become established only in raining season when the local people storage rain water for the domestic usages. In drawing up strategies for *Aedes* control, it is essential that distribution and density of the mosquitoes should be studied and clearly understood. Therefore, the present study was investigated in Laymyethnar Township because of its high infestation level for *Aedes* larvae and pupae in Hinthada District and to determine the seasonal distribution of *Aedes* larvae in various water storage containers during the study period.

MATERIALS AND METHODS

Study area

Lal Tan Ngal village, Laymyethnar (between 18° 30' N and 96° 15' E) with high infestation level for *Aedes* larvae among the six areas in Hinthada District, Ayeyawady Region was chosen as study area. In Lal Tan Ngal village, a total of 186 population live in 50 houses, out of this population 30 were children. Container survey was done to investigate the seasonal distribution of *Aedes* larvae in the study period. The inspected sites were chosen randomly in this area.



Map of the study site

Study period

The study period was from October 2017 to September 2018.

Study design

The study was conducted using non-intervention descriptive field investigation method. All potential breeding sites were examined in order to carry out the systematic study. Larval positive containers of different container categories and types were recorded and compared with each other. The breeding sources were divided into major, minor and miscellaneous sources. Concrete tank, concrete jar, concrete barrel, over-head tank, metal drum, plastic drum, glazed or unglazed earthen jar and glazed earthen bowl were considered as major sources (above as size of 30 liters). Other sources such as flower vases, small glazed earthen jars, small glazed earthen bowl, earthen pot, plastic bowl, metal bucket, plastic bucket, rubber bucket and flower bowl were considered as minor sources (below as size of 30 liters). Miscellaneous container categories contain discarded utensils, hollow bamboo poles, bowls, old cans, discarded tyres, discarded bottles, broken earthen pots and bowls and coconut shells.

Collection of larvae

During the study period, larvae were collected in different positive containers by using the sweeper and counted with the dropper. The collected larvae were kept in 70% formalin for species identification in laboratory.

Larval indices

Larval survey was carried out in the selected sites. Larval examination method of Sheppard *et al.*, (1969) was used to confirm the presence of larvae in the containers.

Larval indices were calculated as follows:

(a) Container Index (CI) =

$$\frac{\text{No. of positive containers with } Aedes \text{ larvae} \times 100}{\text{Total number of containers examined}}$$

(b) House Index (HI) =

$$\frac{\text{No. of positive houses for } Aedes \text{ larvae} \times 100}{\text{Total number of houses examined}}$$

(c) Breteau Index (BI) = No. of positive containers per 100 houses

(d) Key container = 500 and above larvae positive per container

(e) Key premises = 3 and above positive containers with *Aedes* larvae per house

(f) Pupae/house = No. of pupae per house

(g) Pupae/container = No. of pupae per container

(h) Pupae/person = No. of pupae per person

(i) Pupae/child = No. of pupae per child under 12 years

Data collection method

Standard sheet for data collection was developed and noted down for the particulars including total water holding containers with water and percentages of positive containers.

Statistical analysis

Field data were recorded in appropriate forms and statistical analysis was conducted by using Microsoft Excel. Larval indices, Pupal indices, Key containers, Key premises, Chi-square and P value (SPSS version 23) were calculated. Percentage positivity was used for comparison in selected study area.

RESULTS

Different containers harboring *Aedes aegypti* larvae in Laymyethnar Township

The present study was carried out in Lal Tan Ngal village, Laymyethnar Township, Hinthada District to determine the seasonal distribution of *Aedes aegypti* larvae in different water storage containers. A total of 50 households were studied to examine the larval positivity rate of various water storage containers in the study period.

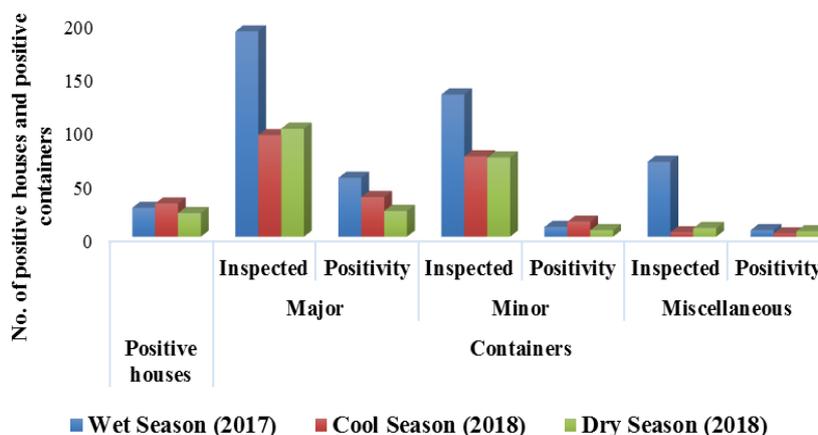


Fig. 1: Number of different water storage containers harboring *Aedes aegypti* larvae during the study period.

Fig. 1 shows that in wet season (2017), a total of 332 water storage containers (129 major, 133 minor and 70 miscellaneous) were inspected and found 55 (42.64%) major containers were positive for *Aedes* larvae. Highest number of larvae for positivity was examined in concrete jar (36.36%), followed by bago jar (30.91%), concrete tank (16.36%), glazed earthen bowl (10.91%), concrete barrel (3.64%) and lowest in plastic drum (1.82%). In Key containers (500 and above larvae positive container), 13 containers in concrete jar, 5 in concrete tank, 3 in bago jar, 1 in plastic drum, concrete barrel and glazed earthen bowl were found as Key containers. Out of 133 minor containers, 9 (6.77%) containers were positive for larvae during the study period. In positivity, 55.56% in small bago jar, 22.22% in earthen pot and plastic bowl (with each) were recorded respectively. Three containers in small bago jar and one in plastic bowl were found for Key containers. In 70 miscellaneous containers, 66.67% in broken bago jar, 16.66% in broken plastic bowl and broken earthen pot (with each) were studied to be positive for *Aedes* larvae. As the Key container, there was only one in broken bago jar.

In cool season (2018), a total of 174 containers were inspected and 37 (21.26%) of major containers were recorded as positive containers for *Aedes* larvae. Concrete jar was occurred to be the highest positivity (48.65%), followed by bago jar (32.43%) and the lowest in concrete tank (18.92%). For Key container, there were 6 in concrete jar, 3 in concrete tank and bago jar (with each). In minor, the highest number of positivity was study in small bago jar (35.71%), followed by earthen pot and plastic bowl (21.43% each) and the lowest in plastic bucket, rubber bucket and small glazed earthen bowl (7.14% each). There were not Key containers in minor ones. In positivity of miscellaneous containers, 66.67% in broken bago jar and 33.33% in broken earthen pot were examined as positive containers. Only one Key container was found in broken bago jar in the study period.

In dry season (2018), a total of 101 major water storage containers were inspected and found that 24 (32.76%) of 101 containers were positive with *Aedes* larvae. Out of

these, concrete jar was found with the highest positivity (54.17%), followed by bago jar (29.17%). Lowest positivity rate was found in concrete tank (16.66%). Three Key containers were found only in concrete jar and found over 500 *Aedes* larvae. Of 74 minor containers, 6 (8.11%) containers were positive with larvae. The positivity of minor containers was occurred in plastic bucket (50%), earthen pot (33.33%) and small bago jar (16.67%) respectively. In miscellaneous, out of 183 containers, 8 containers were inspected and found 5 (62.5%) water storage containers were positivity with larvae. The larval positivity was studied to be in broken earthen pot (60%), followed by broken plastic bowl and discarded tyre (20% each). The Key containers of minor and miscellaneous containers were not recorded in dry season (2018).

In *Aedes* larval positivity, 54% of households, 42.64% of major, 6.77% of minor and 8.57% of miscellaneous containers were found to be positive for *Aedes* larvae in wet season (2017). In cool season (2018), 62% of the houses, 38.93% of major, 18.67% of minor and 75% of miscellaneous containers were found to be positive for *Aedes* larvae. The study showed that 44% of households, 23.76% of major, 8.11% of minor and 62.5% of miscellaneous containers were investigated to be positive for breeding sources for larvae in dry season (2018). Highest positivity rate of containers was studied in miscellaneous containers i.e. 75% in cool season (2018) during the study period. Concrete jar provided highest positivity and was predominated one in all container types throughout the study period.

In positive major containers, positivity was highly significant ($P < 0.05$) from 55 (42.64%) to 24 (23.76%), and in minor and miscellaneous, there were not significant ($P > 0.05$) from 9 (6.77%) to 6 (8.11%) and from 6 (8.57%) to 5 (62.5%) respectively in the survey. In larval positivity of different containers, the positivity rates were highly significant in all seasons throughout the study period. The present study showed that there were significant differences according to seasonal positivity in various containers in the study area.

Seasonal positivity of Key containers and Key premises in study area

Table 1: Seasonal positivity of Key containers and Key premises in Laymyethnar Township.

Indices	Survey			Significant Difference P value
	Wet	Cool	Dry	
Key container	29 (41.43%)	13 (24.07%)	3 (8.57%)	$P < 0.05$ (Highly Significant)
Key premises	9 (18%)	8 (16%)	1 (2%)	$P < 0.05$ (Highly Significant)

Table 1. shows that in wet season (2017), 9 households (18%) were found as Key premises and 29 containers (41.43%) were found as Key containers. In cool season (2018), 8 households (16%) were recorded as Key premises and 13 containers were recorded as Key containers. Only one household (2%) was examined as Key premise and 3 Key containers (8.57%) were

examined in dry season (2018). Highest percentage of Key premises and Key containers were found to be positive for *Aedes* larvae in wet season (2017). Percentage of Key containers and Key premises were highly significant ($p < 0.05$) from wet to dry. Key containers and Key premises of wet (2017) were found to be higher positivity than cool and dry (2018) (Table 1).

Seasonal occurrence of larval indices in study area**Table 2: Seasonal occurrence of larval indices in Laymyethnar Township.**

Indices	Survey		
	Wet	Cool	Dry
HI	54	62	44
CI	21.08	31.03	19.13
BI	140	108	70

HI = House Index, CI = Container Index, BI = Breteau Index

Table 2 shows that a total of 50 households from Lal Tan Ngal village in Laymyethnar Township were randomly selected and seasonal occurrence of larval positivity were found 27 (54%) in wet (2017), 31 (62%) in cool (2018) and 22 (44%) of the households in dry season (2018) were positive with *Aedes* larvae in various water storage containers. Larval indices of wet (2017) such as House Index (HI) 54, Container Index (CI) 21.08 and Breteau Index (BI) 140; HI (62), CI (31.03) and BI (108) in cool (2018); and dry (2018) with HI (44), CI (19.13) and BI (70) were investigated respectively. In wet season (2017), HI, CI and BI were found to be higher positivity when compared with HI, CI and BI of dry (2018), although HI and CI of cool (2018) were higher positivity than wet and dry seasons in study area.

Seasonal occurrence of Pupal indices in study area**Table 3: Seasonal occurrence of Pupal indices in Laymyethnar Township.**

Indices	Survey		
	Wet	Cool	Dry
Pupae/House	28.6	2.8	1.8
Pupae/Container	4.31	0.8	0.49
Pupae/Person	7.69	0.75	0.48
Pupae/Child	47.67	4.67	3

Table 3. shows that pupal indices were gradually decreased from wet season (2017) to dry season (2018). In wet season (2017), pupae/house, pupae/container, pupae/person and pupae/child were declined from 28.6, 4.31, 7.69 and 47.67 to 1.8, 0.49, 0.48 and 3 in dry season (2018) in the study period respectively. Wet season was found to be more predominated one for pupal indices than other seasons throughout the study period.

DISCUSSION

Mosquito-borne diseases are a major public health threat in Myanmar. *Aedes aegypti* is a vector of dengue in urban areas but now the species are distributed in rural areas in Myanmar. The rapid growth of population and industrial zones in urban and semi-urban areas has resulted in an alarming increase in mosquito's density. Dengue is the most important mosquito-borne virus disease in world. Untreated water in containers originated from pipe supply, tube wells, surface wells, ponds and rain water also encourage mosquito density.

The larvae of *Aedes aegypti* were highly present in concrete jar of the major container in study area, followed by big bago jar and concrete tank, but in wet season (2017), plastic drum and big glazed earthen bowl were also positive with larvae. Because which containers were mostly placed under the gutters to keep rain water in raining season. Most households used concrete jars, big bago jars, concrete tanks and plastic drums to store water for household use. Metal drums and bago jars were highly positive for *Aedes* larvae and metal drums are regarded as Key containers in Tha Kay Ta, Shan Chaung and Dagon North Township, Yangon Region (Ni War Lwin, 2013) and Mingalar Ywar Thit village and Taung Nar village, Hpa-an Township (Maung Maung Mya *et al.*, 2016). In the study area of Laymyethnar Township, the concrete jars are usually and widely used to store water for multipurpose throughout the study period. Thus, it was highly positive container with *Aedes* larvae among the water storage containers.

Aedes larval distribution study in Tha Kay Ta Township (Ni War Lwin, 2013) revealed that the high number of *Aedes* larvae positivity were in minor containers such as spirit bowls and small bago jars. And then, *Aedes* larval occurrence in Hpa-an Township, the highest positivity rate of minor containers was in flower pots in household (Maung Maung Mya *et al.*, 2016). The present study investigated that the high number of *Aedes* larval positivity were in small bago jars and earthen pots throughout the study period. And, these containers were widely used for domestic usages by villagers in the study area.

A total of 70 miscellaneous containers in wet (2017), 4 in cool (2018) and 8 in dry (2018) were inspected for *Aedes* larvae. In study area, broken bago jars were high number of positive containers for larvae, followed by broken earthen pots and broken plastic bowls in the study period. Present study found that one broken bago jar each were Key containers in wet (2017) and cool (2018) for *Aedes* larvae. Bamboo trees are abundantly grown in study area and cutting bamboo stumps are always full of rain water which provided good breeding places for *Aedes* mosquitoes. Maung Maung Mya *et al.*, (2015) found car tyres, bamboo stems and tree holes were Key containers in Hpa-an area. In present study, man-made bamboo bowls which were also used for chicken food containers in the village but the high number of larvae was not present in these containers. In positive major containers, positivity was highly significant ($p < 0.05$) from wet to dry and in minor and miscellaneous, there were not significant ($p > 0.05$) from wet to dry in the container survey. In larval positivity of different containers, the positivity rates were highly significant in all seasons throughout the study period. And then, there were significant differences according to seasonal positivity in various containers in the study area.

Aedes aegypti and *Aedes albopictus* larvae were observed as co-breeders in concrete tank, concrete jar,

earthen pot and small bago jar. Same observation of *Aedes aegypti* and *Aedes albopictus* were observed in a spirit bowl in Hmawbi Township, Yangon Region (Maung Maung Mya *et al.*, 2005).

Most of the major containers were found as Key containers, such as concrete jars and bago jars throughout the container survey. The results of the present studies agreed with the results of other researchers that they had found high number of Key containers and Key premises in bago jars in Tha Kay Ta, North Dagon, Insein, Shwe Pyi Thar and Kamayut in raining season (Myint Myint Chit, 2009 and Ni War Lwin, 2013). Tin Mar Yi Tun (2007) mentioned that in pre-monsoon survey, 62.19% of major containers were positive for *Aedes aegypti* in North Dagon Township, followed by 50% of Pazundaung area. Percentage of positive miscellaneous containers of Latha Township was higher than that of other Townships and the highest percentage of positive premises (70.59%) was found in North Dagon in post monsoon period Yangon Region. In present study, 9 (18%) Key premises in wet (2017) was higher than 1 (2%) Key premises in dry (2018). During the study period, 1 (2%) of Key premises and larval positivity in water storage containers in dry (2018) were lower than other seasons. Maung Maung Mya *et al.*, 2016 stated that the distribution of *Aedes* larvae in wet season was higher than in cool and dry seasons. The present study examined that the wet season was predominated one in all seasons. In Key containers, 29 (41.43%) in wet (2017) was also higher than 13 (24.07%) in cool (2018) and 3 (8.57%) in dry (2018). The number of Key containers and Key premises were also significantly reduced ($p < 0.05$) from wet (2017) to dry (2018).

Lal Tan Ngal village in Laymyethnar Township is high risk of DHF due to high larval indices and high number of positive containers in Hinthada District. In the present study, larval positivity in water storage containers and Key premises were high in wet season (2017), because most of the water storage containers were not covered and full of rain water. The environment of study area had highest humidity (75-80%) and moderate temperature (25-32°C). These conditions are favorable for the breeding of *Aedes* larvae. Plenty of oviposition sites is available for *Aedes* mosquitoes and life span of the larvae was shortened during raining season. This also supports the increase of *Aedes aegypti* density in monsoon period. However, Pe Than Htun *et al.*, (2010) revealed that sometime, mosquito density increased and DHF outbreak occurred in hot season because water shortage caused lack of cleaning in Dala Township, Yangon Region. The present study agreed that the highest number of larvae and high larval positive containers were mainly and usually found in untreated or uncleaned water storage containers frequently in study area.

The larval indices of HI, CI, BI, Key containers and Key premises were significantly reduced from pre-intervention to post-intervention period was observed by Myint Myint Chit (2009) and Ni War Lwin (2013) in Yangon areas. Similarly, larval indices of HI, CI, BI, Key containers and Key premises were slightly reduced from wet (2017) to dry (2018) in study area. However, HI and CI in cool (2018) were found to be higher positivity than other seasons.

Ni War Lwin, (2013) reported that pupal indices (Pupae/house, Pupae/container, Pupae/person and Pupae/child) were also significantly reduced from pre-intervention to post-intervention period. The finding of this study was also agreed that the pupal indices were also significantly reduced from wet (2017) to dry (2018) in selected study area. The present field study demonstrated that *Aedes aegypti* larvae were removed effectively in water storage containers because of cleaning or treating various containers frequently in this area.

During the study period, larval positive water storage containers in Key premises and Key containers were placed mostly under the roof gutters and full of rain water which are favorable for gravid female *Aedes aegypti* to lay their eggs in these containers. Similarly, Maung Maung Mya *et al.*, 2016 revealed that the major containers, major breeding sources, which are usually placed under the roof gutters just outside the house are usually replenished by rainfall. Large water containers such as concrete tanks, concrete jars, bago jars, drums and earthen pots are found to be the main sources for breeding of *Aedes aegypti* due to these containers are never completely emptied.

Aedes larval control is the most effective method for controlling of dengue disease. The sweeping method in larval control is a very effective control method; it can remove 95% of larvae within 10 minutes by alternate top and bottom sweeping procedures using cotton net sweeper. In the view of the above, the spread of dengue and distribution or prevalence of disease vectors to this area should be a matter of great concern to public health authorities and socio-culture practices, and there is urgent need to create awareness of populations in village. The larval control should be done in and around the village and especially every household has to be aware of vector-borne diseases.

According to the survey, if the water storage containers are frequently cleaned and covered completely, there will be the reduction of number of positive containers, controlling for diseases and preventing for breeding sources of *Aedes* mosquitoes. The result of present work indicates that the monthly or weekly survey will be to promote socio-culture practices and provide awareness of vector-borne diseases for villagers and may be useful for elimination of breeding sites and reduction of eggs and larvae of *Aedes* mosquitoes in this study area.

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