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DENGUE MOSQUITO IMMATURE PRODUCTION IN RESIDENTIAL AND NON-RESIDENTIAL HOUSES OF LALITPUR DISTRICT, NEPAL

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Master of Science in Tropical Ecology

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ABSTRACT

In Nepal, entomological surveys on *Aedes aegypti* and *Aedes albopictus* were focused more on residential houses ignoring non-residential houses. The result of this project suggests that non-residential premises may have the chances to produce a large proportion of the dengue mosquito immature production. However, the total number of dengue vectors were found higher in residential houses compared to non-residential houses. It is because fewer non-residential houses were found in the study area and sampled (n = 32) than that of residential houses (n = 68). The Repeated sampling was conducted within 100 houses, 8 times in four-month period at two seasons, pre-monsoon (May, June) and post-monsoon (August, September). The *Stegomyia* indices: House Index, Container Index and Breteau Index were found higher in non-residential houses than that of residential houses. The statistical analysis using negative binomial regression model in between two houses type for immature stage of dengue mosquitoes (*Aedes aegypti* and *Aedes albopictus*) shows that there are no significant differences between house type, that is $p>0.05$. The analysis shows strong significant differences with very low p-value ($p<0.05$), when compared two seasons. Seven different types of container classified by shape, use, and material for both species (*Aedes aegypti* and *Aedes albopictus*) contributed about 72-74% and other miscellaneous 26%. So vector control should be focus targeting all productive containers. Mosquitoes abundance in the study area was influence by climatic variables, mosquito density which was highest in fifth survey after monsoon when there was relatively a low rainfall (5.9 mm), humidity (95.4%) and high temperature (23.28°C).

Keywords: Dengue, Immature, *Aedes aegypti*, *Aedes albopictus*, Residential, Non-residential, Container

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1. Introduction

Dengue fever is a mosquito borne viral disease, which is spreading rapidly in new areas in recent years. The disease has increased 30 fold, extending its range in new countries, from urban to the rural areas, in the past 50 years (WHO 2009). Dengue is considered one of the most important vector borne diseases globally (Gubler 1998). The disease has a long history and considered as one of the oldest diseases with its first record in Chinese medical encyclopaedia in 992 (Gubler 2006). The first epidemic of dengue fever occurred in 1779-1780 AD in Asia, Africa and America (Gubler & Clark 1995).

1.1 Epidemiology of dengue fever

Dengue is divided into three types, classical dengue fever (DF), and the more severe forms dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS) (Hadinegoro 2012). Nearly 2.5 billion people are living in dengue endemic countries in the World WHO (2008). Dengue transmission is common for the countries which lies on tropical and sub-tropical regions, the endemic of dengue is found in more than 125 countries in the World (Murray et al. 2013). According to Bhatt et al. (2013), 96 million dengue infections were recorded globally in 2010, of which 70% were from Asia, specially in densely populated areas, among this 34% of the total dengue infections were recorded from India alone, America contributes 14% and 16% of the total global dengue infections were from Africa.

1.2 Disease control

Dengvaxia, a vaccine for dengue, was introduced in several countries in 2016, but does not give complete protection for the disease (Pasteur 2016). Mosquito vector control remains the main way to control the disease (Jacobs 2000, Koenraadt et al. 2007). The most common way to control dengue vectors is well-organized larvae control methods, (Ponlawat et al. 2005). Pupal survey methods with total pupae count is being more effective methods for identifying the most adult productive containers in the dengue infection area (Nathan et al. 2006). The larvae of dengue vectors can be control by reducing mosquitoes breeding household containers, removing discarded containers from residential and non residential houses, also by spraying larvicides and predatory fish in water holding containers. Adult mosquitoes can be controlled by spraying insecticides as well as continuous efforts by the community in the dengue affected area (Baak-Baak et al. 2014, Dos et al. 2010, Kroeger et al. 2006).

1.3 Dengue virus and vector

The virus that causes dengue fever is the dengue virus (DEN), which is a small single stranded RNA virus of genus *Flavivirus* and family Flaviviridae, which consist of four dengue serotypes, that is, DENV 1, DENV 2, DENV 3, AND DENV 4 (WHO 2009). The dengue vector that transmit dengue virus from infected person to person is *Aedes* mosquitoes that is, *Aedes aegypti* and *Aedes albopictus* (Gubler 2002, Gratz 2004). Both of these species are members of the subgenus *Stegomyia*, which are also called as *Stegomyia* mosquitoes (Sota & Mogi 1992). *Aedes aegypti* is considered as the main vector and *Ae. albopictus* the secondary vector, commonly called the Asian tiger mosquito (Christophers 1960, Gratz 2004). It is known that *Aedes albopictus* alone is also responsible for dengue transmission and outbreak (WHO 2009). Both of the mosquito species are important vector for the dengue virus transmission (Ponlawat & Harrington 2005, Rodhain & Rosen 1997, Bonizzoni et al. 2013).

1.4 Origin and distribution of dengue vectors

The study by Braks et al. (2004) on the mosquito *Aedes aegypti* shows that, it was first originated in the tropical region of Africa and then entered into the South America in 16th century. During 18th and 19th centuries when the global shipping industry were expanding in South America, the port cities grew and became more urbanized which produced favourable breeding places for *Aedes aegypti* (Gubler 2002). *Ae. aegypti* was first introduced into the Asian continent in the end of 19th century, whereas, *Ae. albopictus* is a native species of Asia (Tabachnick 1991). In recent years *Ae. albopictus* is widely distributed in many countries of the Asia, Africa, Americas and Europe (WHO 2009). Likewise, *Ae. aegypti* have become widely distributed in tropical region of the Asia, South America and Africa (Braks et al. 2003).

1.5 Dengue mosquito characteristics

Common breeding habitats for *Aedes aegypti* is in artificial water storage containers which contain clean water closely associated with human dwellings (Christophers 1960). *Ae. albopictus* prefer to breed more in natural water holding containers like tree holes, crevices, leaf axils and crack holes (Bonizzoni et al. 2013). The eggs of these *Stegomyia* species can be viable for longer period of time and can survive in adverse climatic conditions, such as long droughts and winter. *Ae. aegypti* is more desiccation-resistant than the eggs of *Ae. albopictus* (Sota and Mogi 1992). The larval stages of both species feeds on microorganism, organic detritus and other food particles present inside the water holding containers (Braks et al. 2004).

The adult stages of both species are day-biting mosquitoes, which prefer to feed in early in the morning and late afternoon (Ho et al. 1973). The females of both species feeds on human blood but *Ae. albopictus* prefer to feeds more on blood of other animals than human, *Ae. aegypti* rarely feed on other animals (Lambrechts et al. 2010). The adult *Ae. aegypti* is considered as highly anthropophilic in nature and well adapted to urban areas where there is high human population with low vegetation (Braks et al. 2003). Whereas *Ae. albopictus* is zoophilic in nature and more common in rural areas in outdoor natural forest where there is high vegetation (Ho et al. 1973). The female *Ae. aegypti* disperse for food, oviposition and searching for a mate (Muir & Kay 1998, Trips & Hausermann 1986). According to Honório et al. (2003) dispersal for oviposition of this mosquito is pertinent for the disease propagation.

Invasion by one species, *Ae. aegypti* can affect the abundance and distribution of the other species, *Ae. albopictus* (Lounibos 2002). The density of *Ae. aegypti* is high in human settlements with high populations and low socioeconomic status, when there is deficiency in water supply households tend to store water in containers, which is a risk factor for *Ae. aegypti* proliferation, (Tauil 2001).

1.6 Dengue vector in residential and non residential sites

A study on dengue vector in Brazil shows that non-residential premises were key sites for vector surveillance because these sites contain large number of potential breeding sites compared to residential sites for immature *Ae. aegypti*, such as discarded tires, garbage recycling centres, bus stations, school, metal workshops, cemeteries, hospitals, shopping centres and transportation companies (Dos et al. 2010). Abundance of immature dengue mosquitoes were found higher in non-residential sites which are present near to residential houses than that of residential houses (Baak-Baak et al. 2014). According to another study (Vezzani et al. 2001) in Argentina, the density of *Ae. aegypti* is high in cemeteries and is considered more important in non-residential areas than others. The pupal demographic survey conducted in non-residential areas of Peruvian city of Iquitos shows that such areas can be highly productive for *Ae. aegypti* breeding production compared to residential sites, as highly productive large unused containers filled with water were present in these non-residential areas (Morrison et al. 2013).

1.7 Seasonal influence on dengue vectors

Different season can influence vector development and dynamics. The density of mosquitoes is usually being higher in the post monsoon season, after the monsoon season, containers are filled up with fresh and clean water, which become favourable place to breed for dengue mosquitoes. (Oo et al. 2011, Gautam et al. 2015). When there is heavy rainfall during the monsoon season, mosquito abundance is low because floods can sweep away the immature mosquitoes from the containers and the breeding habitat may be disrupted (Vijayakumar et al. 2014). Climatic determinants such as temperature, rainfall and humidity play important roles for the abundance of vector populations (Sharma et al. 2005). Like wise many vector borne diseases, including dengue fever, are also influenced by climatic condition, specially temperature fluctuation can affect the dengue virus transmission by *Aedes* mosquitoes (Lambrechts et al. 2011).

1.8 Dengue fever in Nepal

Nepal is a land-locked country, which is surrounded by India on three sides (East, West, and South) and China on the north. The altitude range of Nepal is between 90 meter to 8848 meters above sea level (Malla et al. 2008). Dengue fever (DF) is a relatively new disease in Nepal. It was first recorded in 2004 from Chitwan district low land region of Nepal and has emerged in new areas of the country in recent years (Pandey et al. 2004). The first dengue outbreak from Nepal was in 2006 from nine districts of low land region of Nepal. The nine districts were Kathmandu, Banke, Parsa, Dhading, Jhapa, Rupandehi, Dang, and Kapilbastu (Pandey et al. 2008, Malla et al. 2008). Dengue fever occurs in tropical and sub-tropical region of Nepal (Dhimal et al. 2015). The first cases of dengue fever from Kathmandu was detected in October 2006 and there were more dengue cases from Kathmandu valley during dengue outbreak in 2010 (Pandey et al. 2013). The disease has spread widely in new geographical areas from tropical region of Nepal to sub-tropical regions, from lower elevation to higher Himalayas region (Dhimal et al. 2014). The data on dengue up to the year 2014 shows that, all together 2442 people in Nepal have been confirmed with dengue fever and five people died due to dengue fever from 32 district Ministry of Health and Population (2015). Chitwan and Jhapa districts have higher dengue incidence than other districts from Nepal upto the year 2014 (Acharya et al. 2016).

The Asian tiger mosquito, *Ae. albopictus*, is indigenous species for Nepal, which was first reported in 1950 (Peters & Dewar 1956). Another dengue vector *Ae. aegypti* was first recorded in 2006 during the first dengue outbreak in the nine districts (Malla et al. 2008). According to Gautam et al. (2009), Pandey et al. (2013) *Ae. aegypti* for the first time was reported from the Kathmandu and Lalitpur district in 2009. *Ae. albopictus*, however was previously known from the Lalitpur district from 1990 (Darsi & Pradhan 1990). Both *Ae. aegypti* and *Ae. albopictus* have expanded their geographical range and were commonly found in middle mountain regions of central Nepal including Kathmandu Valley (Kathmandu and Lalitpur district) (Dhimal et al. 2015). Previous studies found that adults as well as immature stage of dengue mosquitoes has commonly occur in Lalitpur district of Nepal (Gautam et al. 2009, Pandey et al. 2013, Gautam et al. 2015, Dhimal et al. 2015).

1.8.2 Dengue virus in Nepal

All four dengue virus serotypes have been detected in Nepal since 2006, during the first outbreak in the country Malla et al. (2008). During the outbreak dengue patients from Dang district had no travel history and *Aedes* mosquitoes were also reported from the district, which suggest that there is endemic cycle of dengue fever in Nepal (Malla et al. 2008). In recent years, all four serotypes are expanding into the new geographical areas of the country, Nepal, which will further increase the risk of dengue outbreaks in new areas (Pun 2011). There are four seasons in Nepal, the pre monsoon, monsoon, post monsoon, and winter. The density of dengue vectors and its fluctuation in Nepal depends on this seasonal variation. The abundance was high in post-monsoon comparing to other seasons, therefore post monsoon season is also called the dengue season in Nepal (Dhimal et al. 2014). From Lalitpur district also the density of *Ae. albopictus* and *Ae. aegypti* is higher in post monsoon season compared to pre-monsoon season (Gautam et al. 2015).

In the context of Nepal, the study on dengue fever, dengue virus and dengue vector surveillance has been conducted previously from many parts of the country, from lower elevations to higher elevation. However, there is a lack of studies on breeding site characteristics and immature production in residential and non-residential houses. Most of dengue vector surveillance was only focused on residential houses often neglecting non-residential premises, which might be potential breeding sites in larger volume for *Ae. aegypti* and *Ae. albopictus*.

2. OBJECTIVES

The objectives of this study are given below:

1. Compare immature production of *Aedes aegypti* and *Aedes albopictus* in residential and non-residential houses in Lalitpur district of Nepal.

2. Determine the most productive containers for *Ae. aegypti* and *Ae. albopictus* in the study area.

2.3 Compare immature production of *Ae. aegypti* and *Ae. albopictus* in the pre-monsoon and post-monsoon season in the study area.

3. METHODS

3.1 Study area

The study was done in two wards (9 and 22) of Patan city, Lalitpur district, Nepal. Patan city is also called Lalitpur Sub-Metropolitian city. There are a total 30 wards present in the Lalitpur Sub-Metropolitian city. I choose these two wards because the area is located inside the ring road area of Kathmandu Valley; many mosquito data have been collected previously from outside the ring road area of Patan city, but not from inside the ring road. Lalitpur Sub-Metropolitan city is located between N 27 °39 ″ and E 27° 41″. The city is five km southeast of Kathmandu district. The city covers an area of 15.5 km^2. The city is one of three major cities of the Kathmandu valley. It is one of the oldest cities of Nepal consisting of old housing structures. It has many historical places such as temples, pagodas, stupas, sacred buildings, and monasteries. According to the population census data of 2011-2012, there were a total of 254,308 people (123,752 females and 130,556 males) and 62,893 households present in the city (Government of Nepal 2016a, Government of Nepal 2016b).

The Lalitpur district with Patan as its district headquarter expands its range up to 385 km^2 and the elevation of the district ranges between 457 m to 2831 m above sea level (Government of Nepal 2016b)). Figure 1 shows the map of study area, two wards inside the Patan city of Lalitpur district, Nepal. Figure 2 shows how residential and non-residential houses were located in the study sites using geographical coordination, Arc GIS and google earth. Non-residential houses types were shown separately in figure 3. Likewise, figure 4 shows location of total residential house types searched in the study area.

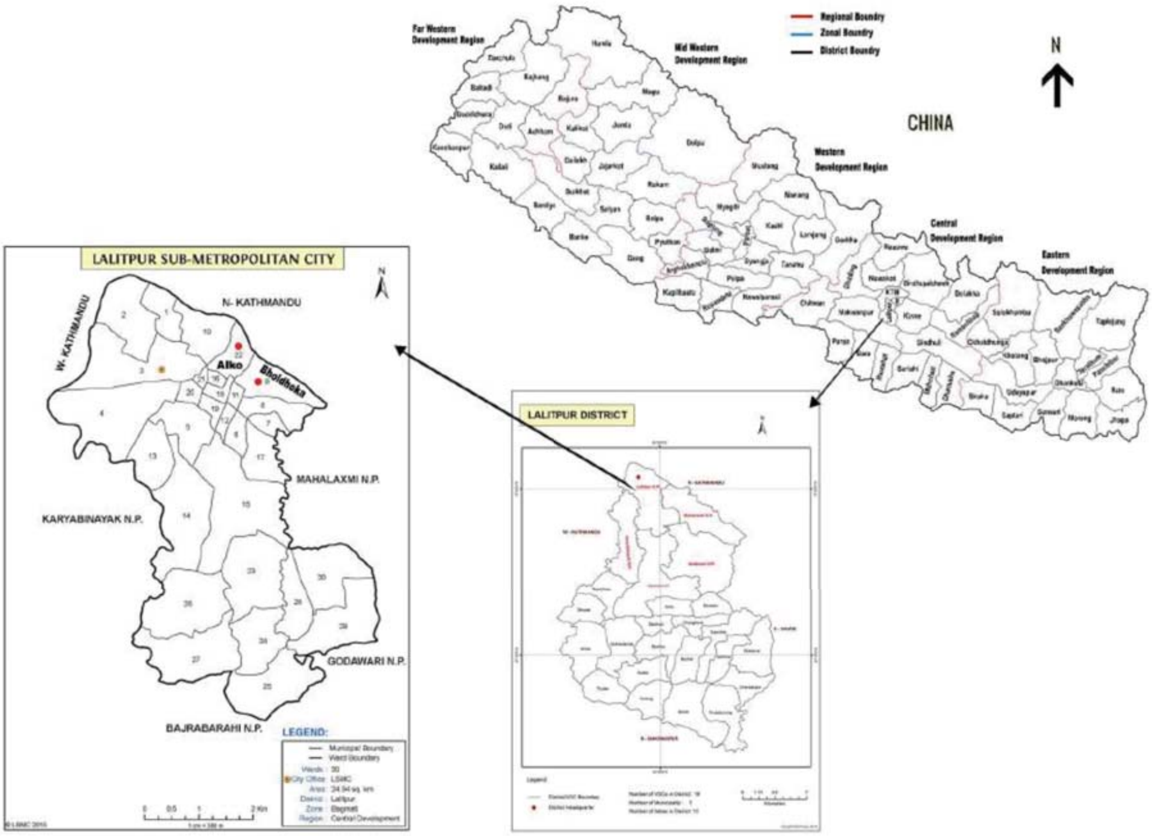

Figure 1. Map of study area

Figure 2. Map showing residential and non-residential houses in two wards. Green square indicate residential and red square indicate non-residential houses.

Figure 3. Map showing non-residential houses in study area.

Figure 4. Map showing residential houses in the study sites.

3.2 Study design

A longitudinal entomological survey was conducted in residential and non-residential house types of Lalitpur district. One hundred houses were sampled in two selected wards.

The non-residential houses type includes, cement block factories = 2, mud statue factories = 3, metal workshops = 2, tyre repair shop = 1, temple = 2, furniture factories = 2, government and private office = 6, rice mill = 1, electronic shop = 1, restaurants = 6, garbage recycling centre = 1, Kindergarten and school = 2, groceries shop = 3. Total 32 non-residential houses were searched and 68 residential houses were taken from nearby non-residential houses. Repeated sampling was done eight times during a four-month period (two surveys in each month) in exactly the same houses. The non-residential houses types can be distinguished from residential by its characters such as people did not stay or sleep during night in such houses. The survey was conducted in two different seasons, that is pre monsoon (May, June) and post monsoon (August, September) seasons.

3.3 Entomological survey

Two teams, each consisting of two data collectors carried out the mosquito collections. All water-holding containers in each selected house were searched for the presence and absence of immature mosquitoes. Larvae and pupae were collected using large-sized steel spoons and dippers. All immature mosquitoes present inside containers were transferred to plastic bags and labelled giving house code, container code, date and locality using permanent markers. Based on the shape, use, and materials (SUM) method (Koenraadt et al. 2007), all wet containers present inside and outside of each house were recorded. Data were collected on other associated variables, that is, presence of cover (yes or no), location (indoor or outdoor), size (length, height and opening), water depth, shade (yes, no or partially), under roof (yes no or partially), water source (rain fed or manually), insecticide used (yes or no) and container washed (yes or no). Geographical coordinates of all hundred houses as well as elevation were recorded using a global positioning system (GPS) device (Garmin eTrex 10).

3.4 Laboratory work

All the immature mosquitoes collected from the field were brought to the laboratory at the Natural History Museum, Kathmandu, Nepal for rearing and identification. All immature stages of mosquitoes were transferred to petri dishes from the plastic bag. With the help of a dropper larvae and pupae were transferred to plastic cups and covered with thin muslin cloth and rubber band. Plastic cups were kept in the laboratory under normal temperature conditions for rearing and labelled with the same code as in the plastic bag using permanent markers. The labelled plastic cups were checked once a day for adult emergence. Adult mosquitoes were transferred to test tubes by using aspirator and killed with chloroform. Larvae that did not emerge to adults and died were preserved in 70 % alcohol in vials and then prepared as permanent slide.

Adult mosquitoes were identified using taxonomic key and a dissecting microscope, hand lenses (10X triplet hand lens) and pointed forceps. *Aedes aegypti* and *Aedes albopictus* mosquitoes were identified to species level where as others were identified to genus by using taxonomic key, Rueda (2004), Fenemore (2006). The slides containing larvae were observed under compound microscope and identified using the keys and descriptions of Rueda (2004) and the book of Fenemore (2006).

3.5 Weather data

Data on temperature, rainfall and humidity were collected from a nearby meteorological station of the Meteorological and Hydrological Forecasting Division (Government of Nepal 2016c).

3.6 Data analysis

All data collected were then entered into Microsoft Excel. Container characteristics were summarized using Excel pivot tables. Immature mosquito infestation and percentages in residential and non-residential houses were calculated. Container productivity of *Aedes aegypi* and *Aedes albopictus* positive containers were classified by shape, use and material and ranked from highest to lowest. *Stegomyia* indices such as house index (percentage of houses positive for immature dengue mosquitoes), container index (percentage of containers positive for immature dengue mosquitoes), and Breteau index (number of immature dengue mosquito positive containers per 100 houses) were calculated for residential and non-residential houses for pre-monsoon and post-monsoon season.

Statistical analysis was done using negative binomial regression model to test the significance difference between house type in two different season (pre-monsoon and post-monsoon) at 95% confidence level for both species (*Aedes aegypti* and *Aedes albopictus*).

4. RESULTS

4.1 Container characteristics

Data collected from 100 houses of which 68 were residential and 32 non-residential revealed a total of 1779 wet containers of which 1259 (71%) were from the residential houses and 520 (29%) were from non-residential houses (Table 1) and maximum, minimum and average size of the containers and water depth in residential and non-residential houses are shown in table 2. There was no active vector control in the area, with none of the containers being treated with chlorine, temephos, or fish.

Table 1. Container characteristics in residential and non-residential houses of Lalitpur district, Nepal

	Residential houses (n=68)	%	Non-residential houses (n=32)	%	Total (n)	Total (%)
No. of wet containers	1259	71%	520	29%	1779	100%
Location						
Outdoor	694	68%	332	32%	1026	58%
Indoor	565	75%	188	25%	753	42%
Cover (lid)						
Yes	615	76%	190	24%	805	45%
No	644	66%	330	34%	974	55%
Filling method						
Rain	439	65%	232	35%	671	38%
Manual	820	74%	288	26%	1108	62%
In shade						
Yes	385	73%	142	27%	527	29%
Partially	97	63%	57	37%	154	9%
No	777	71%	321	29%	1098	62%
Under roof						
Yes	735	74%	260	26%	995	56%
Partially	22	56%	17	44%	39	2%
No	502	67%	243	33%	745	42%

Wash before refill						
Yes	715	76%	229	24%	944	53%
No	544	65%	291	35%	835	47%

Table 2. Size of the container and water depth

	Residential house	Non-residential house
Maximum		
Length (cm)	200	200
Width (cm)	100	100
Height (cm)	250	250
Opening (cm)	200	200
Minimum		
Length (cm)	5	5
Width (cm)	2.5	2.5
Height (cm)	5	8
Opening (cm)	4	5
Average		
Length (cm)	41.9	46
Width (cm)	20.5	22.37
Height (cm)	56.9	57.3
Opening (cm)	40	44.7
Water depth (cm)		
Maximum	197	206
Minimum	1	1
Average	38.3	37.7

4.2 Shape, use and material

The main container types were drums (793), buckets (504), pots (270), tanks (94), gallons (55), tires (42) and jars (21). Among these, 72% of the drums were in residential houses and 28% in non-residential houses. Of the buckets, 76% were in residential houses and 24% in non-residential houses. The corresponding residential and non-residential figures for pots were 68% and 32%, for tanks 56% and 44%, for gallons 76% and 24%, and for jars 67% and 33%, respectively.

Pots were made from either metal, plastic or clay, and drums were either plastic or metal. Most of the plastic drums were black, yellow and blue in colour, whereas metal drums were blue or brown. Buckets were made from plastic or metal, tires from rubber, and tanks from cement. Most of the plastic pots were used for washing such as hand and face washing, brushing, and cleaning. Metal pots in non-residential houses were garbage, but in residential houses metal pots were used for irrigation and drinking water for pets, some were thrown away near to the home here and there. Clay pots were used for decoration such as flower pots and other small pots for irrigation and drinking water for pets. Drums were used for dishwashing, clothes washing, and bathing. Buckets were used to store water for washing hand, face and feet and for toilet use as well as cooking. Large cement tanks were used for all types of washing and drinking in residential houses and for making statue, cement blocks and rings in non-residential houses. Jars and gallons were used to store drinking water. Tires were not in used and were discarded lying near non-residential houses (workshops, repairing shop and recycling centre).

4.3 Mosquito immature infestation

4.3.1 General

A total of 136 containers (136/1779 = 7.6%) were infested with *Aedes aegypti* larvae and pupae. These were pots (n = 58), drums (n = 42), buckets (n = 24), tires (n = 10), and tanks (n = 2). For *Aedes albopictus* all together 152 containers (152/1779=8.5%) were found positive for larvae and pupae. These were pots (n = 62), drums (n = 43), buckets (n = 37), tires (n = 9) and a tank (n = 1). A total of 122 containers (6.9%) were infested with *Culex* species; these were drums (n = 44), pots (n = 32), buckets (n = 23), tanks (n = 18), and tires (n = 5). Thirty-four containers (2%) were positive for other *Aedes* mosquito pots (n = 14), drums (n = 10), buckets (n = 7), tires (n = 2) and tank (n = 1).

4.3.2 Mosquitoes in residential and non-residential houses

All together 2107 larvae and pupae were recorded from the whole survey. A total of 484 immature *Aedes aegypti* were collected, 304 from the residential houses and 180 from the non- residential houses. A total of 776 immatures of *Ae. albopictus* were collected, 479 from residential houses and 297 from non-residential houses. The detail is given in Table 3. The number of *Aedes albopictus* (n = 776) is highest compared to other species, second abundant mosquito was *Culex* species (n = 713), followed by *Ae. aegypti* (n = 484), other *Aedes* species (n = 96), *Anopheles* species (n = 24) and other unidentified species were 14.

Table 3. Number and proportion of mosquito immatures collected in residential and non-residential houses in Lalitpur district, Nepal in May, June, August and September 2016

Species	Residential		Non-residential		Total	
	Number	%	Number	%	Number	%
Ae. aegypti	304	63	180	37	484	100
Larvae	209	63	121	37	330	100
Pupae	95	62	59	38	154	100
Ae. albopictus	479	62	297	38	776	100
Larvae	264	61	169	39	433	100
Pupae	215	63	128	37	343	100
Anopheles spp.	12	50	12	50	24	100
Culex spp.	448	63	265	37	713	100
Aedes spp.	64	67	32	33	96	100
Unidentified	6	43	8	57	14	100
Sum	1313		794		2107	

4.3.3 *Stegomyia* indices by house type

The House Index, Container Index and Breteau Index for immature dengue mosquitoes was higher in non-residential houses than in residential houses (Table 4).

Table 4. Stegomyia indices of immature dengue mosquitoes (Ae. aegypti and Ae. albopictus) in residential and non-residential houses in Lalitpur district, Nepal, 2016

	House type		
	Residential	Non-residential	Total
Total no. of wet containers encountered	1259	520	1779
Average no. of wet containers per house	2.3	2.1	2.2
No. of positive houses	102	63	165
No. of positive containers	110	65	175
Container Index (CI), %	8.7	12.5	9.8

House Index (HI), %	18.8	24.6	20.6
Breteau Index (BI)	20.2	25.4	22
No. of pupae positive containers	71	39	110
Total no. of pupae	310	187	497
Pupae per House Index (PHI)	57	73	62

CI = Percentage of water holding containers infested with immature dengue mosquitoes.

HI = Percentage of houses infested with immature dengue mosquitoes.

BI = Number of dengue mosquito positive containers per 100 houses.

PHI = Number of pupae per house.

4.4 Seasonal distribution of immature mosquitoes

In the pre-monsoon season the highest number of mosquitoes recorded was of *Culex* species, the second highest was *Aedes albopictus*, followed by *Aedes aegypti* and then other *Aedes* species (Figure 5). For the post-monsoon season the highest number of immature mosquitoes collected were *Aedes albopictus*, and then *Aedes aegypti* followed by *Culex* species (Figure 5).

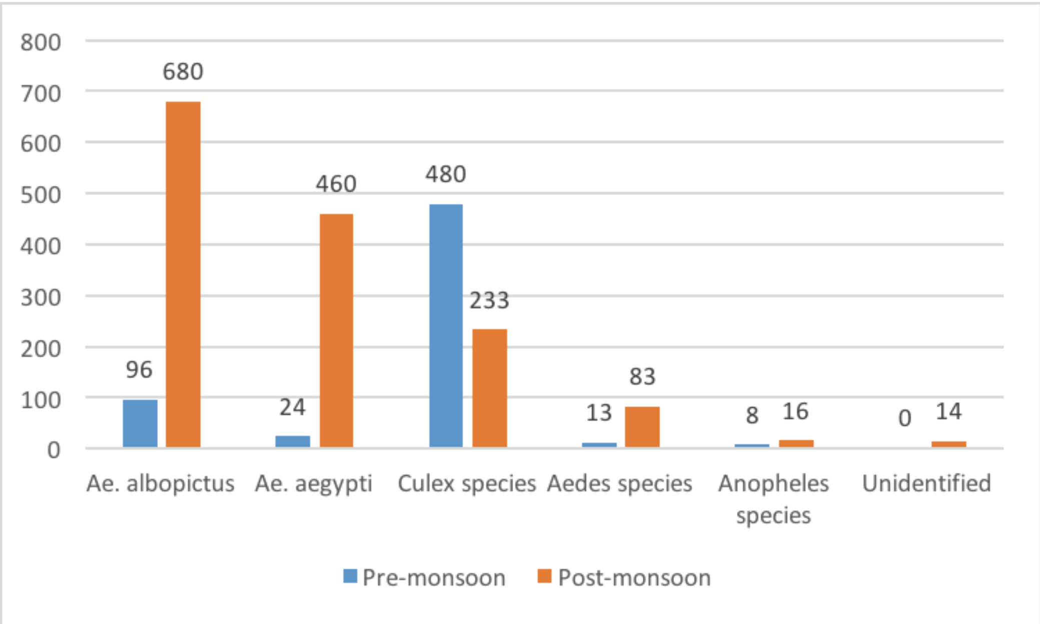

Figure 5. Total immature mosquitoes collected in of Lalitpur district, Nepal during pre-monsoon (May-June) and post-monsoon (August-September) seasons in 2016.

4.4.1 *Stegomyia* indices by season

The larval and pupal indices were higher in the post-monsoon season compared to the pre-monsoon season (Table 5).

Table 5. Stegomyia indices of immature dengue mosquitoes (Ae. aegypti and Ae. albopictus) in pre-monsoon (May-June) and post-monsoon (August-September) seasons in 2016, Lalitpur district, Nepal

	Season		
	Pre-monsoon	Post-monsoon	Total
Total no. of wet containers encountered	946	833	1779
Average no. of wet containers per houses	2.3	2.1	2.2
No. of positive houses	25	140	165
No. of positive container	30	145	175
Container Index (CI), %	3.1	17.4	9.8
House Index (HI), %	6.3	35.0	20.6
Breteau Index (BI)	7.5	36.2	22
No. of pupae positive containers	6	104	110
Total no. of pupae	25	472	497
Pupae per House Index (PHI)	0.06	1.18	62

4.5 Container productivity

4.5.1 For *Aedes aegypti*

The most productive containers for *Aedes aegypti* immatures classified by shape, use and material were plastic drums used for washing, although they did not produce more than 16% of all immatures collected (Table 6). Containers were ranked from most to least productive as shown in Table 6. As many as seven different container classes (various shape, use and material combinations) only produced 72% of all *Ae. aegypti*.The various categories consisted of many different shape, use and material combinations as follows: cement and plastic tanks used for washing; mud and metal pots used for washing; plastic and metal pots used for irrigation; glass and metal pots without use; metal and plastic pots used for pets; mud pots use for flowers; mud drums use for washing; plastic drums used for irrigation; plastic drums use for drinking, mud and metal buckets use for washing, plastic buckets used for irrigation, wood and plastic buckets without use, and plastic buckets use for drinking.

Table 6. Most productive Aedes aegypti containers as classified by shape, use and material.

	Container class						
Rank	Shape	Use	Material	No. positive containers	*Ae. aegypti* larvae + pupae	Container productivity	Cumulative productivity
1	Drum	Washing	Plastic	21	78	16.1 %	16.1 %
2	Pot	Garbage	Plastic	25	74	15.3 %	31.4%
3	Bucket	Washing	Plastic	14	48	9.9%	41.3 %
4	Tire	Garbage	Rubber	10	50	10.3 %	51.6 %
5	Pot	Washing	Plastic	11	38	7.9 %	59.5 %
6	Drum	Washing	Metal	6	31	6.4 %	65.9 %
7	Drum	Dishwashing	Plastic	11	30	6.2 %	72.1 %
8	Various	Various	Various	37	135	27.9 %	100 %
Total				136	484	100 %	

Container productivity = Percentage of total pupae produced by each container class.

4.5.2 For *Aedes albopictus*

For *Ae. albopictus* the containers were ranked in the same way as for *Ae. aegypti* (Table 7). The most productive containers for *Ae. albopictus* immatures were discarded plastic pots and produced 18.4% of all immatures collected. Seven different container classes produced 73.4% of all *Ae. albopictus*. The various category consisted of many different shape, use and material combinations as follows: plastic tanks used for washing; metal pots used for washing; plastic and metal pots used for irrigation; metal and glass pots with no use; plastic pots use for pets; mud and plastic flower pots; metal and mud drums use for washing; plastic drums used for drinking; cement drums used for dish washing; metal and mud buckets use for washing; plastic and mud buckets use for irrigation, wood and plastic bucket with no use; plastic buckets used for drinking; and plastic buckets used for dish washing.

Table 7. Most productive Aedes albopictus containers as classified by shape, use and material.

Rank	Shape	Use	Material	No. positive containers	Ae. albopictus larvae + pupae	Container productivity	Cumulative productivity
1	Pot	Garbage	Plastic	26	143	18.4 %	18.4 %
2	Bucket	Washing	Plastic	18	103	13.3 %	31.7 %
3	Drum	Washing	Plastic	22	95	12.2 %	43.9 %
4	Pot	Washing	Plastic	13	67	8.6 %	52.5 %
5	Drum	Dishwashing	Plastic	13	64	8.2 %	60.7 %
6	Pot	Garbage	Mud	8	53	6.8 %	67.5 %
7	Tire	Garbage	Rubber	9	46	5.9 %	73.4 %
8	Various	Various	Various	43	205	26.4 %	100 %
Total				152	776	100 %	

Container productivity = Percentage of total pupae produced by each container class.

4.5.3 Residential and non-residential houses

a) *Aedes aegypti*

The *Ae. aegypti* immature mosquito production per container was highest in non-residential houses compared to residential houses (Figure 6).

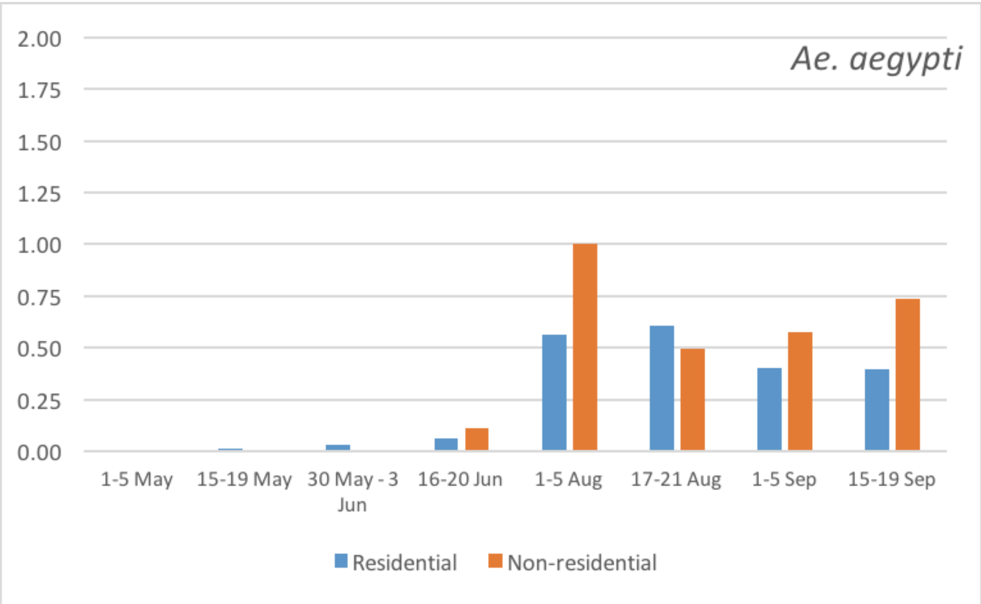

Figure 6. Mosquito production of Ae. aegypti in residential and non-residential houses

b) *Aedes albopictus*

For *Ae. albopictus*, the highest productivity was found in non-residential houses than that of residential houses. Detail is given below in Figure 7.

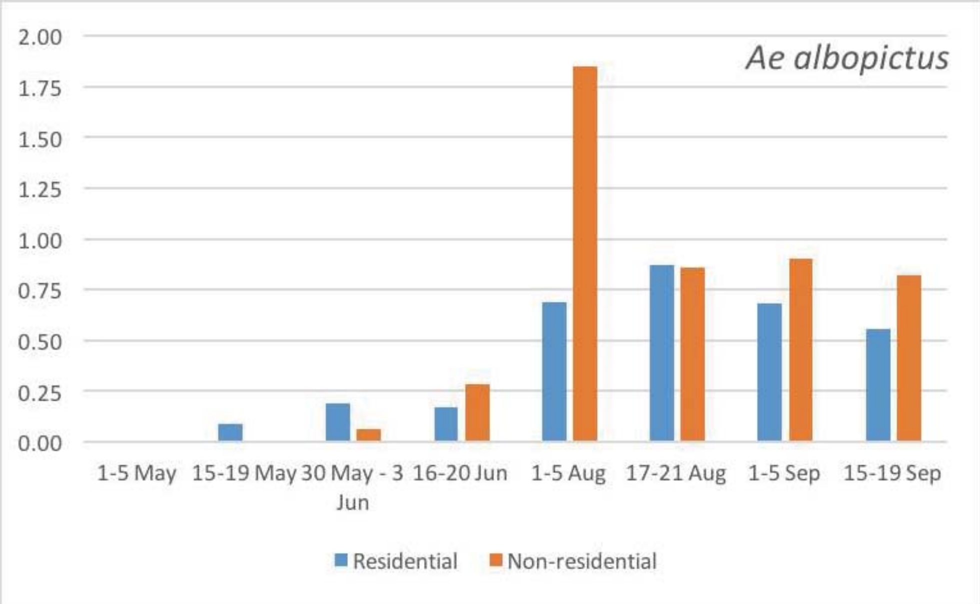

Figure 7. Mosquito production of Ae. albopictus in residential and non-residential houses

4.6 Abundance of immature dengue mosquitoes in overall field survey

The overall abundance of immature dengue mosquitoes was highest in the 5th survey for both *Ae. aegypti* and *Ae. albopictus* (Figure 8). This survey was the first done in the post-monsoon.

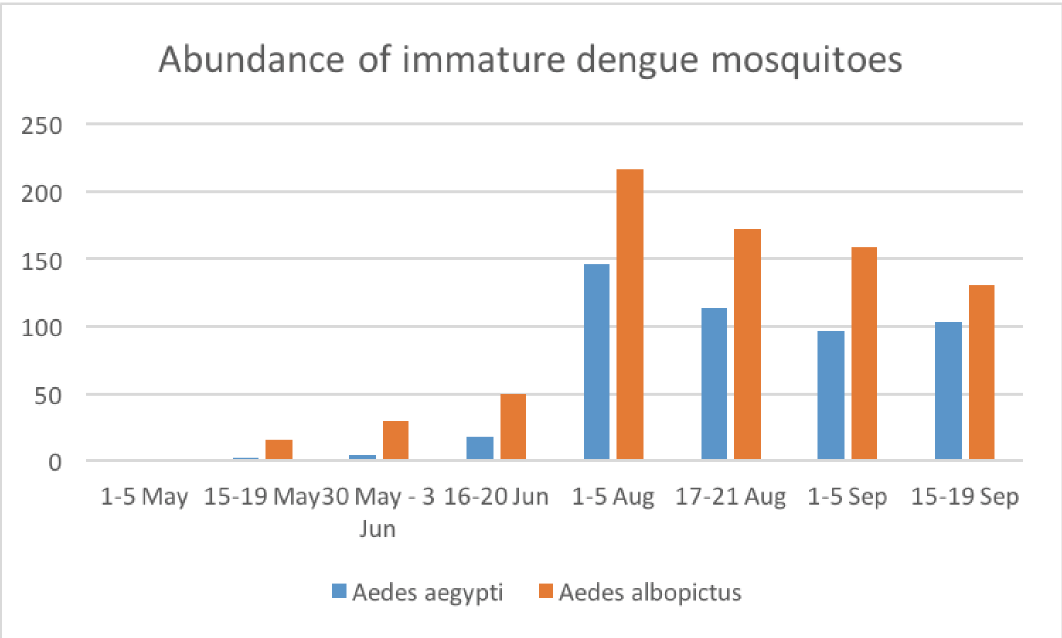

Figure 8. Abundance of immature dengue mosquitoes in Lalitpur district, Nepal.

4.7 Mosquito density comparisons between house type and season

The statistical analysis on comparing immature *Ae. aegypti* (larvae + pupae), *Ae. albopictus* (larvae + pupae) and *Culex* species between houses types in each season and between seasons were done by using negative binomial regression model. Table 8 shows the result from the analysis.

Table 8. Incidence rate ratios (IRR) ([95% confidence intervals]) of immature mosquitoes per container in relation to house type (for each season) and season (across house types) in Lalitput, Nepal, 2016.

Variable	**Level**	**Ae. aegypti**		**Ae. albopictus**		**Culex spp.**
		Larvae	**Pupae[1)]**	**Larvae**	**Pupae**	
Pre-monsoon (n=946)						
House type	Non-residential	1	-	1	1	1
	Residential	0.81 [0.16-4.13) p=0.805	-	1.12 [0.26-4.72] p=0.882	1.29 [0.10-16.73] p=0.845	0.90 [0.38-2.14] p=0.807
Post-monsoon (n=833)						
House type	Non-residential	1	1	1	1	1
	Residential	0.71 [0.39-1.30] p=0.274	0.67 [0.35-1.30] p=0.242	0.59 [0.30-1.18] p=0.134	0.67 [0.36-1.27] p=0.220	0.43 [0.16-1.13] p=0.087
Across house types (n=1779)						
Season	Pre-monsoon	1	1	1	1	1
	Post-monsoon	14.48 [8.27-25.34] p<0.0001	1.16×10^8 [--] P=0.98	5.79 [3.34-10.05] p<0.0001	14.45 [8.03-25.98] p<0.0001	0.55 [0.30-1.00] p=0.051

1) No *Ae. aegypti* pupae collected in the pre-monsoon season.

4.7. Weather information

The average rainfall and relative humidity for the time period in 2015 corresponding to the survey times (1-8) in 2016 are compared in Figure 9 (rainfall) and Figure 10 (relative humidity). The average minimum temperature was 16.96°C which was in first survey and the average maximum temperature during field survey was 23.28°C which was in fifth survey.

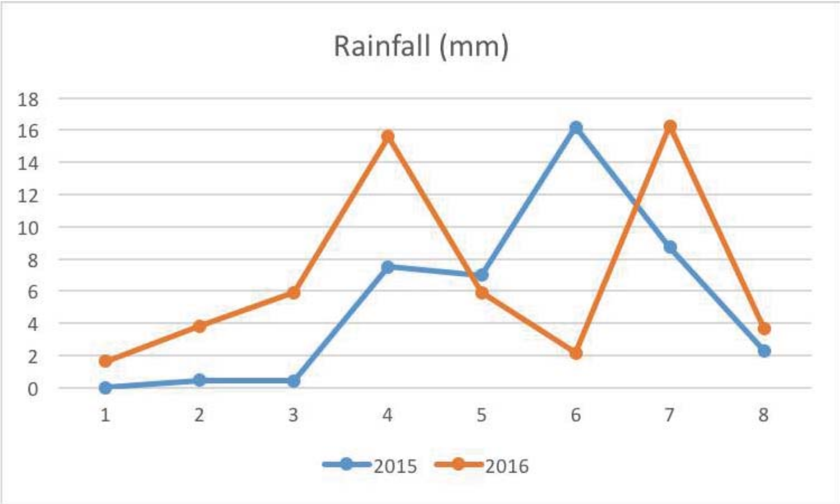

Figure 9. Average rainfall of the year 2015 and 2016 during field survey.

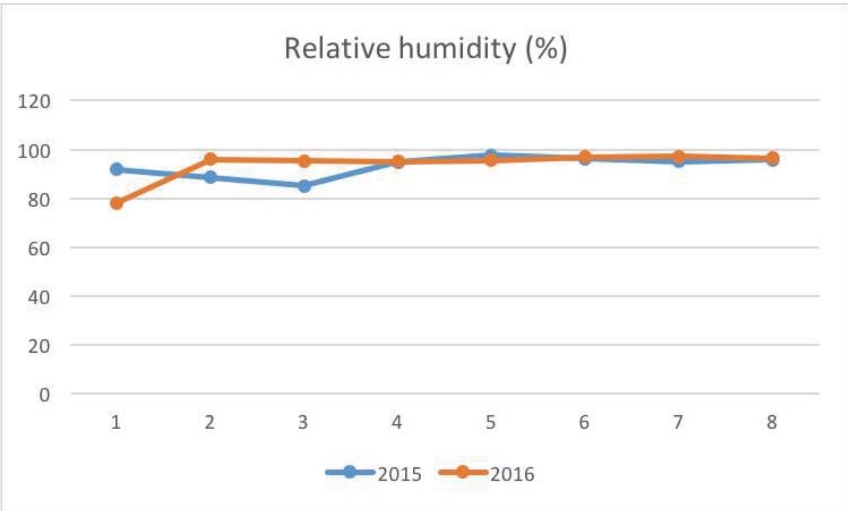

Figure 10. Average relative humidity of the year 2015-2016 during field survey.

5. DISCUSSION

No data have previously available on the immature dengue mosquito production separating in two different house types (residential and non-residential) from Lalitpur district of Nepal with altitude ranges from 1110–1325 meters from the sea level. That is why, this is the first study on the topic, which suggests that more immature dengue mosquito production was in non-residential houses than that of residential houses type though there is not any significant differences between two house types.

There were more number of immature dengue vectors in the residential houses than in the non-residential houses. But the *Stegomyia* indices, Container index, House index, and Breteau index were higher in non-residential houses compared to residential houses. The number of mosquitoes per containers was higher in non-residential (0.60) houses than in the residential houses (0.40). However, when testing the differences there were no significant differences between house types for all species (*Ae. aegypti*, *Ae. albopictus*, *Culex* species). It means that P value is always greater than 0.05 in both season (pre-monsoon and post-monsoon) for *Ae. aegypti* (larvae and pupae), *Ae. albopictus* (larvae and pupae) and *Culex* species. Non-residential houses in my study includes garbage recycling centres (1), metal workshops (2), tyre repair shop (1), cement block factories (2), Offices (6), School (2), groceries shop (3), temple (2), restaurants (6), electronic shop (1), furniture factories (2), rice mill (1), and mud statue factories (3).

This may be due to few containers were searched in non-residential houses compared to residential houses. The reason for higher production of mosquitoes in non-residential houses would be presence of the houses near to highly infested residential houses with high vegetation in outdoor area. And most favourable breeding containers such as discarded plastic pots, and rubber tires fill up with fresh rain water were found in non-residential houses such as in repairing shop and recycling centres.

The study by Dos et al. (2010) on *Aedes aegypti* infestation in residential and non-residential premises in Rio de Janeiro, Brazil shows that the mosquito infestation is higher in residential houses compared to non-residential premises but some of the non-residential houses (recycling centres) are highly infested with *Ae. aegypti* mosquito than residential houses specially those houses which are present near to highly infested residential houses. This shows that highly productive potential breeding containers were present in non-residential premises.

In my study, the density (mosquitoes/containers) were lower in residential houses compared to non-residential houses, but overall there were no significant differences in mosquito productivity between house types. According to (Morrison et al. 2013) when compared *Aedes aegypti* pupae production in non-residential sites in the Amazonian city of Iquitos, Peru with the residential sites that had been surveyed a few years earlier, the non-residential sites have few number pupae/hector compared to residential sites. But the higher percentage of pupae has been recorded from the non-residential sites.

According to (Baak-Baak et al. 2014) the study conducted in residential premises and non-residential sites in Merida city of Mexico shows that, more number of immature *Aedes aegypti* production was recorded from non-residential urban environment specially in vacant lots where there were abundant vegetation and often being located near residential premises and contain large or small size discarded water filled containers which become favourable place to breed adult mosquito and suitable place for the immature development compared to residential houses. According to (Lagrotta et al. 2008) non-residential premises such as tyre repair shops, metal workshops are infested highly with mosquito *Aedes aegypti* than residential premises.

This finding shows that the most productive containers for *Aedes aegypti* classified by shape, use and materials were plastic drums used for washing purpose, and discarded plastic pots. They were responsible for 31.3% of larvae/pupae production. Like wise discarded plastic pots and plastic buckets used for washing contributes higher container productivity (31.7%) for *Aedes albopictus*. As many as seven different container classes (various shape, use and material combinations) only produced 72-74% of all immatures *Ae. aegypti* and *Ae. albopictus*. Most of the middle size plastic drums and buckets used for washing in residential houses were kept outside with lid open and were in dark coloured (black, blue and yellow) and that of small size discarded plastic pots which were found in outdoors location of non-residential houses with vegetation around can accumulates any kind of water become favourable breeding place for adult dengue mosquitoes. No mosquitoes were found in light and transparent coloured plastic gallons and jars and very few mosquitoes were collected from large sized plastic and cement tank. This is because most of the tank we searched were few in number and dry (no water), those which have water were always covered with lids. Gallons and Jars were also few in number and were in light coloured (transparent and sky blue) which were always covered with lid.

The study by (Koenraadt et al. 2007) in Kamphaeng Phet, Thailand shows that the most productive containers classified by shape, use, and material for *Aedes aegypti* pupae were earthen jars and cement tank used for washing purpose, which were responsible for 59% pupae production. The large sized containers with dark coloured and organic materials harbour more immature dengue mosquitoes than that of light coloured containers (Baak-Baak et al. 2014). Discarded tires, metal drums, plastic drums, mud pots were considered most productive container for *Aedes aegypti* and *Aedes albopictus* from Lalitpur and Kathmandu district of Nepal (Gautam et al. 2015). From my study the most productive containers from Lalitpur district for *Aedes aegypti* and *Aedes albopictus* are plastic pots, drums and buckets. This may be due to fewer discarded tires were found around the study area, but the discarded tires searched were all found positive for dengue mosquitoes.

The number of larvae/pupae of *Ae. aegypti* and *Ae. albopictus* were higher in the post-monsoon (August and September) season compared to the pre-monsoon (May and June) season. The larval/pupal indices, Container index, House index, and Breteau index were also higher in post-monsoon survey compared to pre-monsoon survey. The statistical analysis negative binomial regression model at 95% confidence interval showed that there were highly significant differences between the pre-monsoon and the post monsoon season indicating that more mosquitoes were found in post-monsoon than in pre-monsoon for both mosquitoes *Ae. aegypti* and *Ae. albopictus*. That is P-value is very low than 0.05 which indicates strong significance differences. And for the *Culex* species more mosquitoes were found in pre-monsoon than post-monsoon (P = 0.51).

As pre-monsoon season is dry season, it means that most of the containers were dry in that time and wet containers also did not contain fresh water because of shortage of water, but after monsoon when there is heavy rain in monsoon many containers present in outdoors were fill up with fresh water. Which become favourable breeding place for adult dengue mosquitoes. According to (Gautam et al. 2015 and Dhimal et al. 2015) abundance of dengue mosquitoes follow seasonal patterns in Nepal. The larva/pupae abundance of *Ae. aegypti* and *Ae. albopictus* in Lalitpur and Kathmandu district of Nepal, were significantly higher in monsoon and post- monsoon season compared to pre-monsoon and winter season when the containers were fill up with fresh water. In my study also immature mosquito (*Ae. aegypti* and *Ae. albopictus*) abundance in Lalitpur district, Nepal was significantly higher in the post-monsoon season compared to the pre-monsoon season.

Overall 2107 immature mosquitoes were collected during field survey, which includes *Aedes albopictus*, *Aedes aegypti*, *Culex* species, *Aedes* species, *Anopheles* species and other unidentified species. The number of *Aedes albopictus* from residential houses and in post monsoon season is found more than other species. Mosquito abundance is high in first week of August (5th field) for both *Aedes aegypti* and *Aedes albopictus*. This is because fifth survey is the first survey after monsoon, it means that at that time most of the containers contain fresh water. Which become suitable place for immature development and oviposition for adult *Ae. aegypti* and *Ae. albopictus*.

More containers were found outdoors than indoors, most of the containers were not covered with lids. The water sources for many containers were manual water (well and tap) most of the outdoor containers contain rain water. The containers in residential houses were washed frequently than the container present in non-residential houses. None of the containers in the study area were treated with insecticides for mosquitoes control. This is because there were not any dengue cases recorded till now and very few people know about the risk of disease transfer. So to prevent from the mosquito bite during night people used mosquito coil and bed nets.

This study found that the most abundance species is *Aedes albopictus*, followed by *Culex* species. This is due to presence of abundant vegetation in and around the study area. As *Ae. albopictus* prefer high vegetation and is considered as endemic species for Nepal. The study by (Baak-Baak et al. 2014) in Maxico also found that the most abundance species was *Aedes aegypti* followed by *Culex quenquifaciatus* because of abundant vegetation in study area. Previous study by (Dhimal et al. 2015) on *Aedes aegypti* and *Aedes albopictus* on Lalitpur district shows that abundance species was *Aedes albopictus* than *Aedes aegypti* this study agree with that study that *Aedes albopictus* was found most abundance species from the Lalitpur district of Nepal.

From my study I found that more number of immatures of *Aedes aegypti* as well as *Aedes albopictus* has been recorded from outdoor containers than that of indoor containers this finding is similar to the study conducted in central Nepal and India (Dhimal et al. 2015, Vijayakumar et al. 2014).

When comparing average rainfall and relative humidity of each field survey of this year to previous year, there is heavy rainfall in 4th (end of June) survey and 7th survey (first week of September) in this year (2016) but in the previous year (2015) heavy rainfall is in 6th field (end of August). In my study when there is high rainfall mosquito density is relatively low, but after heavy rainfall in fourth survey and after monsoon season mosquito abundance increased in post monsoon from fifth survey. The average minimum air temperature is 16.96 °C which is in first field and the maximum temperature is 23.28 °C which is in fifth field survey. More mosquito abundance for *Ae. aegypti* and *Ae. albopictus* is in fifth survey when there is slightly low rainfall (5.9 mm), high humidity (95.4%) and higher temperature (23.28°C).

Rainfall can also influence the vector population, that is increased in rainy days may increase larval habitat of the dengue vectors in new habitat and thus increase adult survival (Wongkoon et al. 2013). However, when there is continuous rainfall can have negative effects on larval development (Gubler et al. 2001). In Nepal dengue outbreak starts to occur from the end of August (Sharma 2010). This study is similar with the study by (Honorio et al. 2009) that presence of *Aedes aegypti* is high when there is high temperature, in our study also immature dengue mosquito abundance is high in fifth survey when there is high temperature. Ho

6. CONCLUSION

This finding summarized that more potential mosquito breeding containers are found in non-residential houses types compared to residential houses types. However, mosquito abundance is low compared to residential houses. And no significance difference were found between house types. This may be because there were fewer containers and few number of non-residential houses found in the study area than residential houses.

Seven different container classes (various shape, use and material combinations) only produced 72 – 74% of immature dengue mosquitoes, so almost all of the containers searched were found productive from the study area. The containers in non-residential houses near to residential houses contain more immature *Ae. albopictus* and *Ae. aegypti*. In non-residential houses and surrounding outdoors of the study sites contain more unused disposable plastic, metal and mud pots and discarded tires with vegetation. In such containers rain water stored during monsoon and become favourable places to breed for dengue mosquitoes.

Dengue fever is an emerging disease for Nepal, migrating from the lowlands upwards to higher altitudes. My study area, Patan city has an elevation range between 1100-1300 meter. It means that there is a higher chance of risk of dengue transmission in future. Vector surveillance and with larval/pupal control methods in Nepal were only focused on residential houses ignoring non-residential houses. This study suggests that further vector control programme in Lalitpur district should be focused on almost all kind of productive wet containers in residential and non-residential houses. More studies should be carried out in future so as to quantify the immature dengue mosquito production in residential houses verses non-residential houses. So further studies focusing on non-residential sites will be carried out in the Patan city of Lalitpur district, Nepal.

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APPENDIX

This section contains pictures of the non-residential houses, residential houses and different containers types during the field visit.

Garbage recycling centre

Cement block factory

Furniture factory

Metal repairing centre

Workshop

Restaurant

Temple

Statue factory

Government office

Residential house type

Residential house type

Metal and plastic drums

Residential houses

Plastic and mud pot

Wet containers in residential house

Plastic pots and buckets in residential house (Outdoor)

Plastic drums and bucket in residential houses (Indoor)

Plastic pots in residential house (Outdoor)

Mud and plastic pots in non-residential house

Underground cement tank

Plastic bucket and pots use for washing

Plastic tanks

Discarded tire in non-residential house

Immature mosquitoes rearing in laboratory

Adult mosquitoes in test tube

Adult *Aedes albopictus*

Adult *Aedes aegypti*

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