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A Study on the influence of climatic factors on the preferential breeding places of *Aedes*, the Dengue vector, in Delhi, India

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Abstract
In India, outbreaks of dengue fever/dengue hemorrhagic fever (DF/DHF) have been recorded in almost all parts of the country, including the National Capital Territory of Delhi. A severe outbreak was reported from Delhi in 1996 and again in 2006 and 2010. Reports are available for the prevalence of *Ae.aegypti* in Delhi since 1964 *Ae.aegypti* is most prevalent mosquitoes preferred to breed in artificial containers in and around the houses. Therefore the present study is to find out season wise key containers for larvae and pupae production of *Aedes* in a highly endemic area for dengue in the capital city of India Delhi. Besides larval survey, pupal survey was also conducted during 2016 as pupal production is a better proxy for adult mosquito reproduction than traditional breeding indices and is more appropriate for directing dengue control programs. Monthly surveys were carried out randomly in 8140 houses and 18799 containers were checked. Three key containers such as plastic containers, coolers, and cement tank harboured 80 % of *Aedes* larvae. Among them plastic storage and coolers are recorded as perennial source of *Aedes* infestation that contributed 70 % of the immature breeding. We observed how different types of water holding containers, contribute to the breeding of *Aedes* in Delhi. Plastic storage used to store potable water in and around houses which ensured year round availability of water, acted as mother foci. The overall larval indices house index (HI), container index (CI), Breteau index (BI) and pupal index (PI) were calculated as 2.52, 1.22, 2.83 and 1.90 respectively. There was a significant relationship observed among the season. Therefore, public health attention is required to control breeding in such containers. Present study indicates there is a seasonal variation of breeding preference of *Aedes aegypti* and *Aedes albopictus* in different containers in Delhi, so this is important for implementation of appropriate season wise control measures for the prevention of dengue outbreaks in the future.

Keywords: Dengue, *Aedes aegypti*, *Aedes albopictus*, key containers, pupal survey, breeding, Delhi, India

1. Introduction
Dengue fever is one of the most important rapidly rising mosquito transmitted infection in the world. Every year newer areas of the world are invaded by this dreadful dengue infection. Dengue fever (DF) and dengue hemorrhagic fever (DHF) are vector borne diseases of global public health importance in tropical, subtropical, and temperate regions of the world.

In the past five decades, the dengue incidence was reported to have increased 30-fold upsurge worldwide between 1960 and 2010, due to increased population growth rate, global warming, unplanned urbanization, inefficient mosquito control, frequent air travel and lack of health care facilities [1, 2, 3, 4]. Two and half billion people are at risk of tropical, subtropical and temperate areas of the world [4, 5, 6, 7]. Dengue is endemic in south-east Asia and is emerging as a major public health problem in India. Dengue outbreaks are occurring with increasing frequency and intensity in India. Dengue infection affects more than 100 countries, including Europe and the United State (USA) [8].

The first reported case of dengue like illness in India was in Madras in 1780, the first virologically proved epidemic of DF in India occurred in Calcutta and Eastern Coast of India in 1963-1964 [9]. The first epidemic of dengue fever was recorded in 1967 [5] after that many outbreaks were recorded from Delhi [10, 11, 12, 13, 14].

Now it is important to study seasonal breeding preference of *Ae.aegypti* as Zika virus and dengue virus share the same mosquito vector, i.e. *Ae.aegypti*. *Ae.aegypti* most likely originated in Africa; since then, the mosquito has been transported globally throughout the tropical, subtropical, and parts of the temperate world, through global trade and shipping activities [15].

Ae.aegypti mosquitoes have a high vectorial capacity for DENV, CHIKV, *ZIKV, and* yellow fever which has a 400-year history, at present, occurs only in tropical areas of Africa and the Americas and is transmitted by *Aedes* mosquitoes. Population growth and increased individuals movement, urbanization, and the limited financial and human resources are attributed to the emergence and reemergence of the disease [11, 16, 5]. *Ae.aegypti* is the most efficient vector for arboviruses because it is highly anthropophilic, frequently bites, and thrives in close proximity to human [17]. Infected *Ae.aegypti* [14] and *Aedes albopictus* [18] females may transmit the virus to their next generation transovarially.

Therefore, present study was undertaken to identify most productive breeding sites for larvae as well as for pupae of *Aedes* in Delhi and furfure to identify the key containers for pupal production. It is important to know the seasonal variation for breeding preference of *Aedes* in different types of containers so that season wise planning can be done. In view of this seasonal preference of breeding of *Aedes* mosquito in all the seasons of Delhi were studied and presented in the paper. This is useful for season wise planning of vector control measures focused in the key containers.

2. Materials and Methods

NCT of Delhi is the largest metropolis by area and the second-largest metropolis by population in India. It is situated at 77º.15′ E and 26 º.15′ N. It occupies 1,485 km^{2}area of which 900 km^2 is classified as urban. Delhi has a population of 16.7 million (2011 Census). The city has three local bodies- New Delhi Municipal Council (NDMC), Delhi Cantonment Board and Municipal Corporation of Delhi (MCD). MCD covers nearly 97% of the total area of the city. The NCT of Delhi receives 611 mm of rainfall on an average annually mainly from July to September. The highest monthly average high temperature is 41°C in May and the lowest monthly average low temperature is 7°C in January. The average annual relative humidity is 49.2% and average monthly relative humidity ranges from 25% in April - May to 73% in August [2]. The study has been done on the basis of four seasons-Winter season (January and February), pre-monsoon (March, April & May), monsoon (June, July, August and September) and post-monsoon (October, November and December).

2.1 Entomological Surveys

Entomological Surveys for dengue vectors were carried out in different localities of Delhi in 2016. Repeated surveys were carried out on monthly basis in sentinel localities, were randomly selected for survey. Localities are colonies or urban settlement.

In each survey, about 50 houses in each locality were searched for breeding of *Aedes* using single larval technique [19, 20]. Only water holding containers were checked. The data on larval collections were recorded in the pre-designed and pre-tested survey forms. House with the presence of immatures (larvae & pupae) was marked as positive and *Aedes* mosquito immature collected by using WHO standard methods [21]. A container containing any amount of water was considered as wet container and the wet container containing any number of immature (larvae, pupae or both) was considered as positive container. All kind of indoor and outdoor breeding habitats were examined to collect the *Aedes* immatures by following the dipper method [22].

Some specific categories of containers were: Plastic water storage containers which comprised of plastic drums, gallons and cans to store water for domestic purpose; desert coolers are used to cool inside of houses, which have a water tank and a fan, planted pots usually kept indoors or around the houses and solid waste comprises of unused cups, glasses, ceramic pipes, helmets and polythene etc. Most widely used water tanks in Delhi are syntex tanks either kept on ground or on the roof having lids but hardly closed and dried for reuse.

The immature were counted and reared in laboratory for adult emergence and species identification. HI, CI and BI were calculated. Pupal counts were used to calculate the pupal productivity indices: PI and pupae per container (PPC) according to standard methods [23, 4].

Month wise data has been pooled together as per four seasons to know the variation of seasonal dynamics of vectors in view of control strategy. As per standard WHO method HI, CI, and BI were calculated [24]. HI was defined as the percentage of houses infested with larvae and/or pupae and CI defined as the percentage of water holding containers infested with larvae or pupae. BI was calculated as the number of positive containers per 100 houses inspected. Pupal counts were used to calculate the pupal productivity Indices; PI and pupae per Container (PPC) according to standard method [19, 24].

3. Results

3.1 Breeding preference habitats

During 2016, total of 18799 different types of containers were checked (Table 1), immature of *Aedes* infestation were found maximum in plastic water storage container (35%) and coolers (35%) followed by cement tanks(10%), other solid waste(8%), syntax tanks(5%), plastic unused(3%), earthen and planted pot (2%) (Fig1).

It seems from the result that number of containers with immatures was significantly more as compare to number of containers with pupae. Immatures of *Aedes* were found in 230 containers (Table 1), whereas, pupal production was recorded only in 33 containers.

In Delhi, plastic storage and coolers were the most favorable containers for larvae and pupae growth of *Aedes* (Fig 1 & Fig 2). However, in some abundant container like earthen, planted pots, other solid wastes where number of immature were found more but number of pupae were less, it seems most of the immatures did not reach up to pupal stages. Thus unused containers with temporary water collection had low pupal production.

Total of 579 *Aedes* adult mosquitoes merged out from immature and pupae collected during survey out of which 314 were male 265 were female. The emerged adult mosquitoes identified up to species level were *Ae.aegypti* (570), *Aedes albopictus* (4) and Ae. *vittatus* (5). Table 2 shows the season wise prevalence details. During the study period, *Ae.aegypti* was most predominant *Aedes* larvae that were found throughout the year but could not be reared to adult in winter season due to slow development at extreme low temperature. *Ae.aegypti* breeding was found maximum during monsoon. However, *Ae.albopictus* and *Ae.vittatus* were found in monsoon season only.

3.2 Seasonal variation of *Aedes* breeding habitats

Season-wise variation in breeding indices and preference of particular container are given below:

3.2.1 Winter Season

During winter months, low breeding indices were recorded and PPC was also low. During 2016, 1638 houses and 3311 containers were searched in winter season, house index was 0.18 and container index was 0.09. However, BI and PI was 0.18 and 0.31 respectively (Table 1).

Table 1 shows that during winter, *Aedes* breeding was limited to three types of permanent containers which are Plastic storage, cement & cooler (Fig 1a). Maximum immature collection of *Aedes* was found in plastic storage followed by cooler and cement tanks (Fig 3). However, pupae could be collected from only one container i.e. cooler (Fig 4).

During winter, due to extreme low temperature, slow development takes place. Breeding is limited to few permanent principal key containers such as plastic storage, cement tank & coolers. Plastic storage used to store potable water in and around houses which ensured year round availability of water, acted as mother foci during winter. Larvae and pupae stay there for long period of time due to slow development at extreme low temperature [25]. Therefore, vector control may be required to focus in these containers during winter season.

Aedes eggs can withstand at edges and sides of the containers for long period of desiccation, and may hatch when the temperature becomes suitable. *Aedes* mosquitoes infected with dengue virus can pass the virus from generation to generation through their eggs [26]. Therefore, vector control may be focused in these permanent containers even during winter season, so as to prevent their proliferation. Edges and sides of all water containers and tanks should be scrubbed and cleaned with household detergent to remove possible deposited *Aedes* eggs of previous season to eliminate mosquito eggs so as to prevent their proliferation. Fig 1a shows that during winter *Aedes* breeding was limited to three types of containers.

3.2.2 Pre-monsoon

Table 1 shows that during pre-monsoon, 0.89% of containers were infested with immature, whereas, pupal production recorded only in 5 out of 50 containers. Pupae per container were 0.61%. During the survey the number of containers with pupae was found less number as compared to containers with immature, hence development till pupae stages could be reached in less number of containers since *Aedes* mosquito is a climate sensitive vector. During the study period, *Ae.aegypti* preferred to breed significantly more in Plastic storage 38% followed by cooler 22 % and cement tank 20 %, syntex 14 % and plastic unused 6 % (Fig 1b & Fig 3). Pupal density was preferred in cement tank (40%), followed by plastic storage (20%), cooler (20%) and syntax (20%) (Fig 2a & Fig 4). During the season the field collected larvae reared were 100 % *Ae.aegypti* (Table 2).

3.2.3 Monsoon

During monsoon 3628 houses were checked, of which 3.94 % were found positive whereas total of 9119 containers were searched and 1.83% containers were found positive with *Aedes* breeding. Average pupal index was recorded as 3.03% in 2016 (Table 1). Maximum numbers of pupae were collected during this season as compared to other season. Maximum breeding was recorded in coolers (40 %) followed by 32 % plastic (Fig 3). About 11% breeding was recorded in solid waste (Fig 1c). Total 167 containers infested with immature stages of *Aedes*, pupae could be collected only from 23 positive containers**.** Maximum number of pupae were collected by perennial breeding source that is cooler (57%) followed by cement tank, plastic storage, solid waste, plastic unused and planted pots (Fig 2b & Fig 4). Larvae collected during survey and reared in laboratory till adult comprised of all the three different species of *Aedes i.e. Ae. aegypti*, *Ae. albopictus* and *Ae. vittatus* (Table 2).

3.2.4 Post-monsoon

During this season, there was a declining trend of average breeding indices as compared to monsoon (Table 1). Pupal Index was recorded as 1.38 while PPC was 0.77. Larval and pupal production was found maximum in plastic, cooler and syntax tank (Fig 1d, Fig 3 & Fig 4). However, out of 10 positive containers with immatures pupae were present only in 1 container. Pupae production was higher in plastic storage than cooler (Fig 4). Number of containers with immature and pupae were significantly more during monsoon and pre-monsoon. However, percent proportion of containers with pupae productivity was found high during monsoon and post-monsoon (Table 1).

Table 1: Season-wise *Aedes* breeding indices in Delhi during 2016.

Month	HS	+ve	HI	CS	+ve	CI	BI	Pupa No.	PI	PPC
Winter	1638	3	0.18	3311	3	0.09	0.18	5	0.31	0.15
Pre-monsoon	2440	49	2.01	5591	50	0.89	2.05	34	1.39	0.61
Monsoon	3628	143	3.94	9119	167	1.83	4.60	110	3.03	1.21
Post-Monsoon	434	10	2.30	778	10	1.29	2.30	6	1.38	0.77
Total	8140	205	2.52	18799	230	1.22	2.83	155	1.90	0.82

Table 2: Season and sex-wise distribution of *Ae. aegypti*, *Ae. albopictus*, and *Ae. vittatus* in Delhi in 2016.

Season	Species	No. of Male	No. of Female	Total Mosquitoes	%
Winter	*Ae.aegypti*	0	0	0	0
	Ae.albopictus	0	0	0	0
	Ae. vittatus	0	0	0	0
	Total	0	0	0	0
Pre-monsoon	*Ae.aegypti*	80	73	153	100
	Ae.albopictus	0	0	0	0
	Ae. vittatus	0	0	0	0
	Total	80	73	153	100
Monsoon	*Ae.aegypti*	211	181	392	97.75

	Ae.albopictus	2	2	4	0.99
	Ae. vittatus	4	1	5	1.24
	Total	217	184	401	100
Post-monsoon	*Ae.aegypti*	17	8	25	100
	Ae.albopictus	0	0	0	0
	Ae. vittatus	0	0	0	0
	Total	17	8	25	100

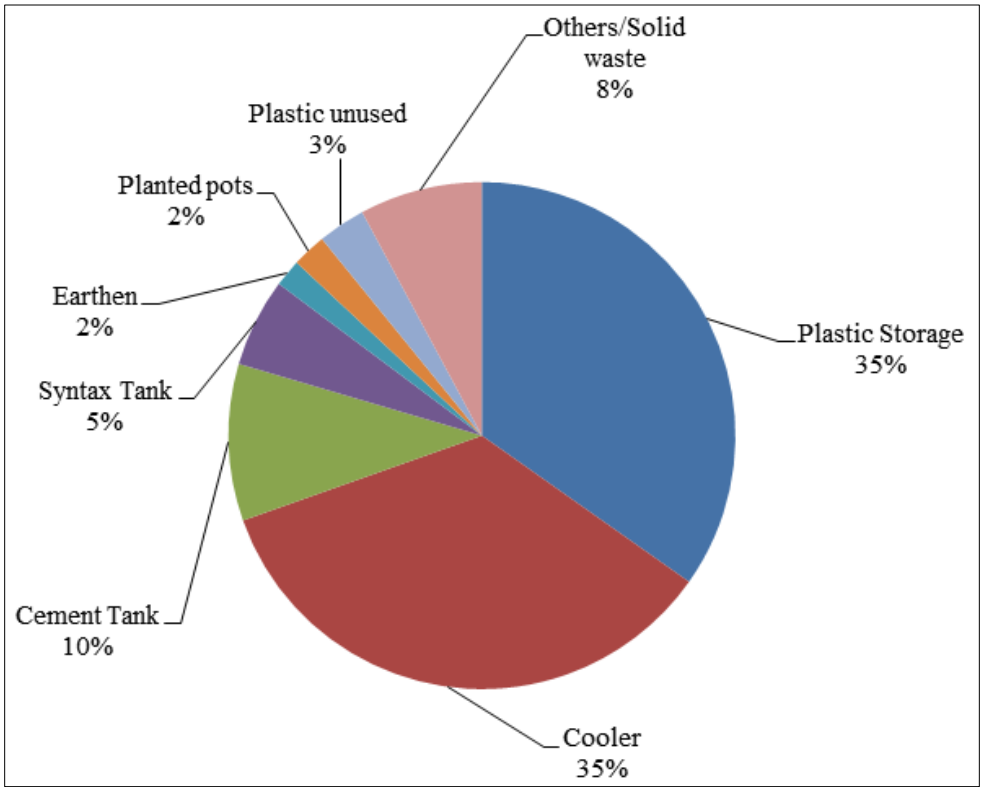

Fig 1: *Aedes* immature container preference in Delhi during-2016.

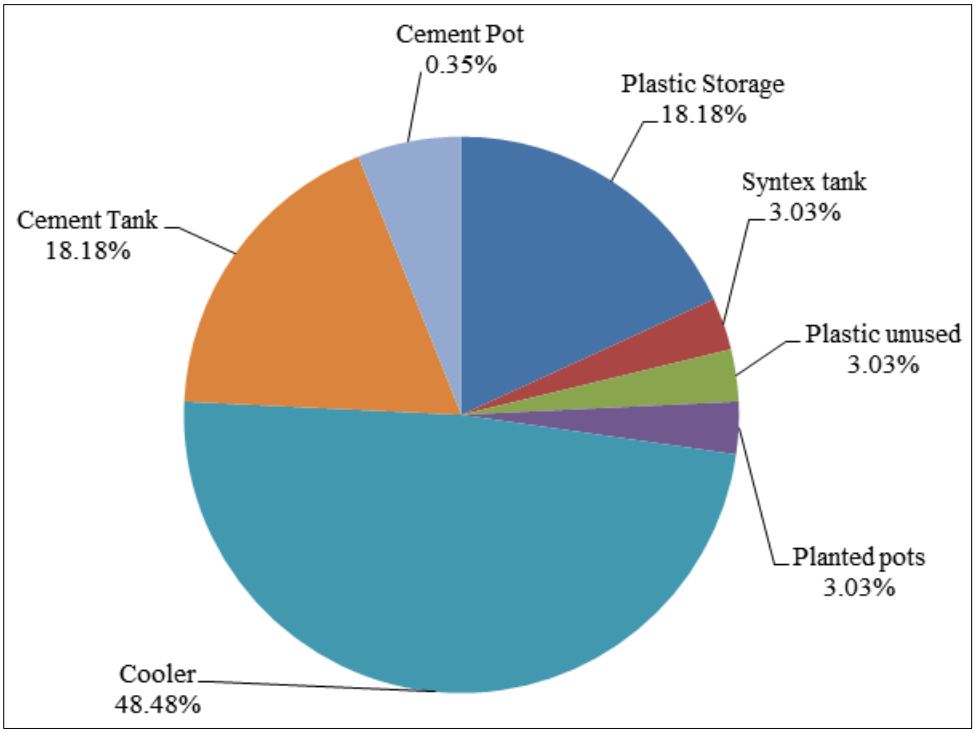

Fig 2: *Aedes* pupal container preference in Delhi during 2016.

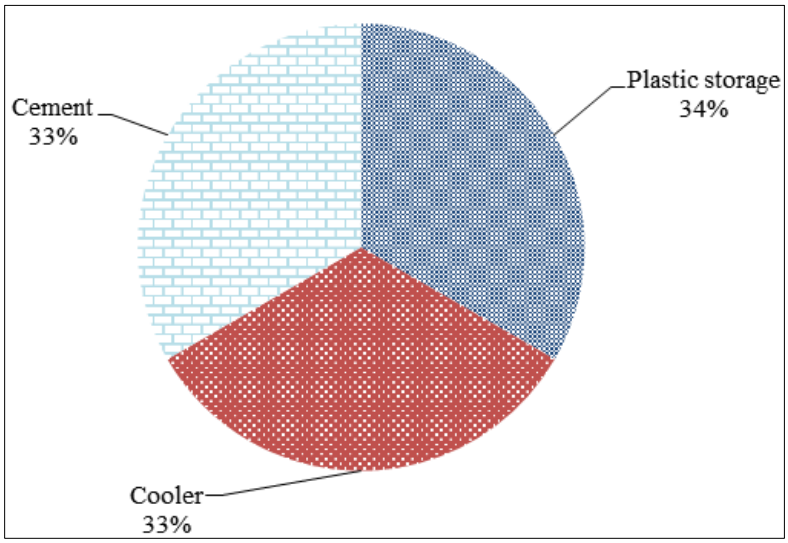

Fig 1a: *Aedes* immature container preference in Delhi in winter season during-2016

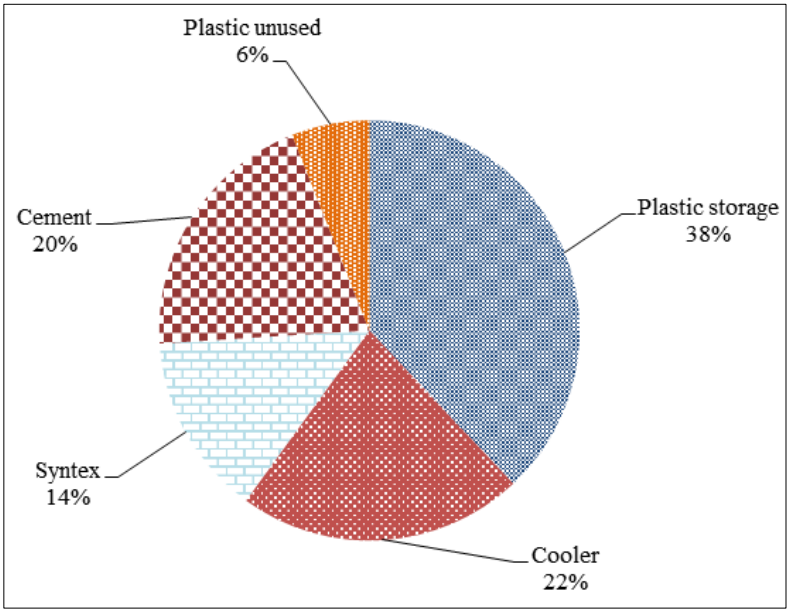

Fig 1b: *Aedes* immature container preference in Delhi in pre-monsoon season during-2016

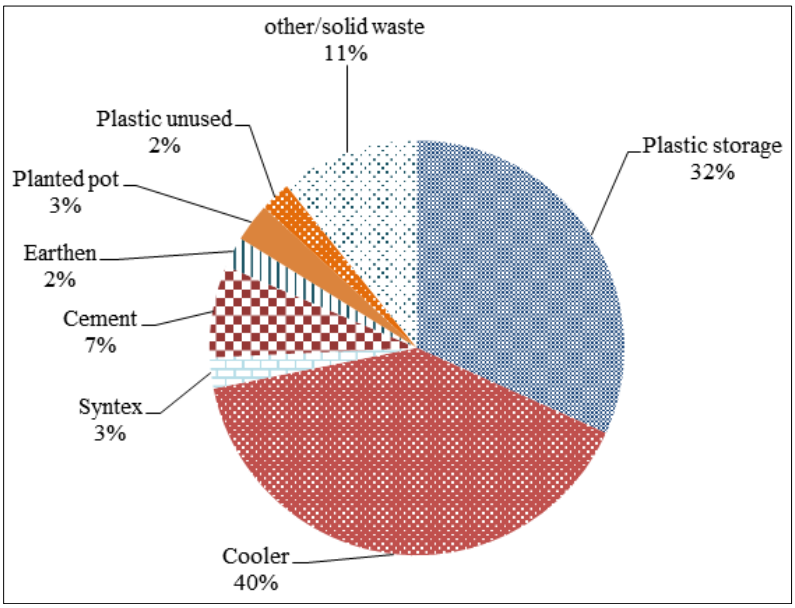

Fig 1c: *Aedes* immature container preference in Delhi in monsoon season during-2016

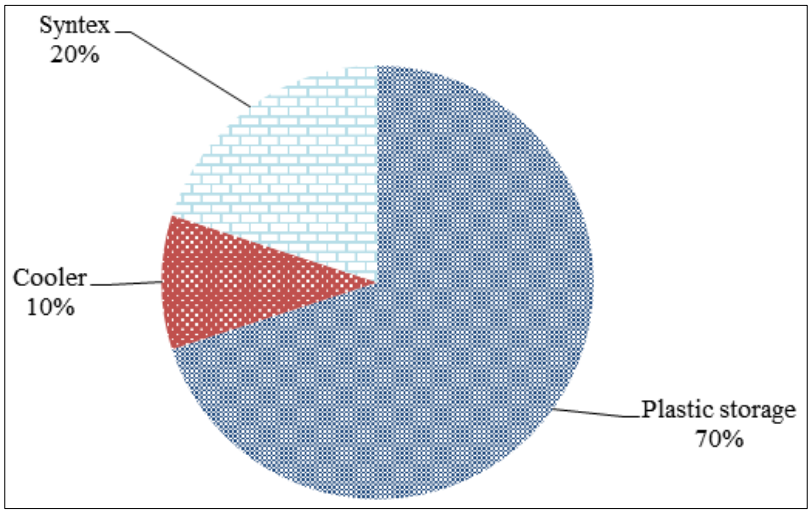

Fig 1d: *Aedes* immature container preference in Delhi in post-monsoon season during-2016

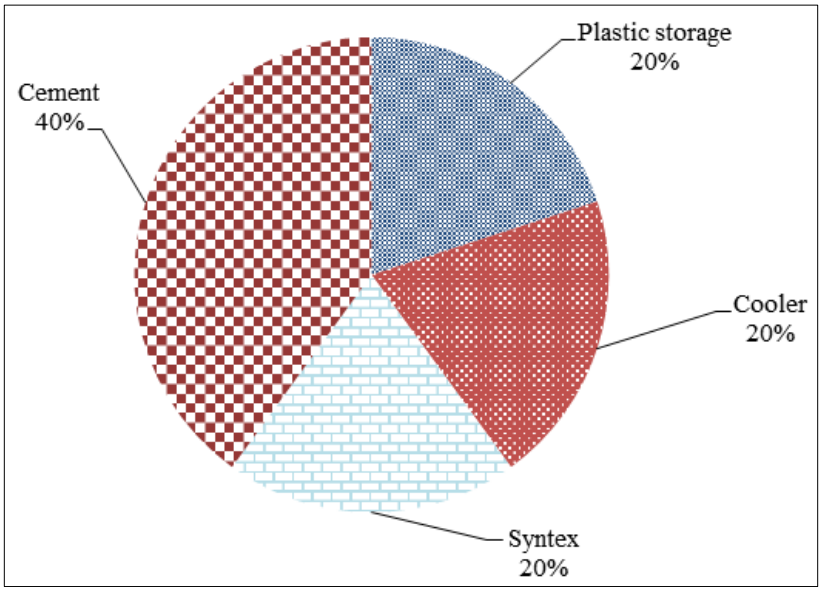

Fig 2a: *Aedes* pupal container preference in Delhi in pre-monsoon season during-2016

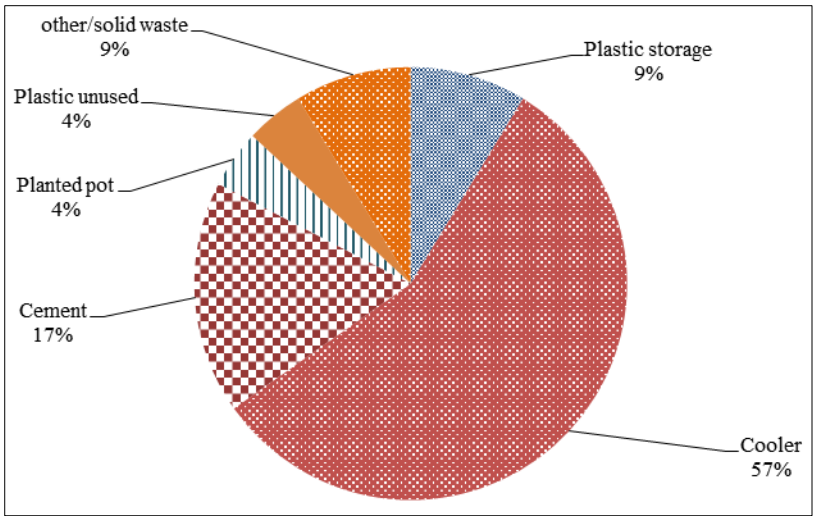

Fig 2b: *Aedes* pupal container preference in Delhi in monsoon season during-2016

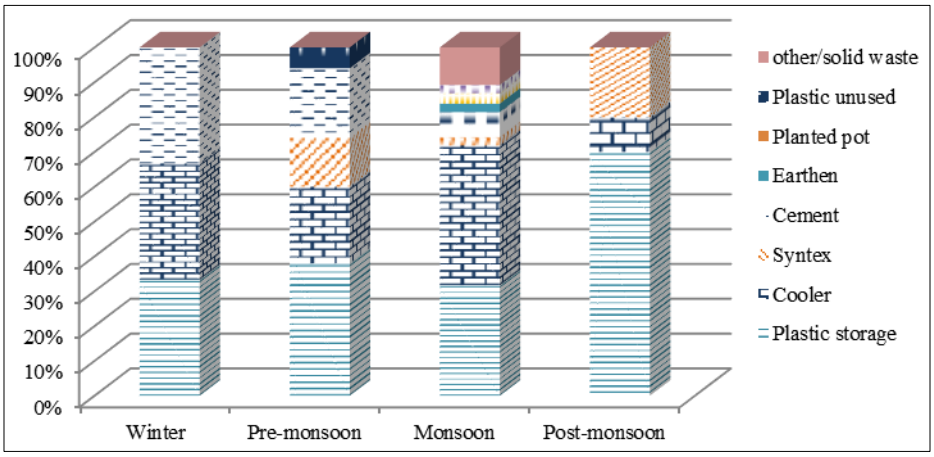

Fig 3: Season-wise *Aedes* immature container preference in Delhi during-2016

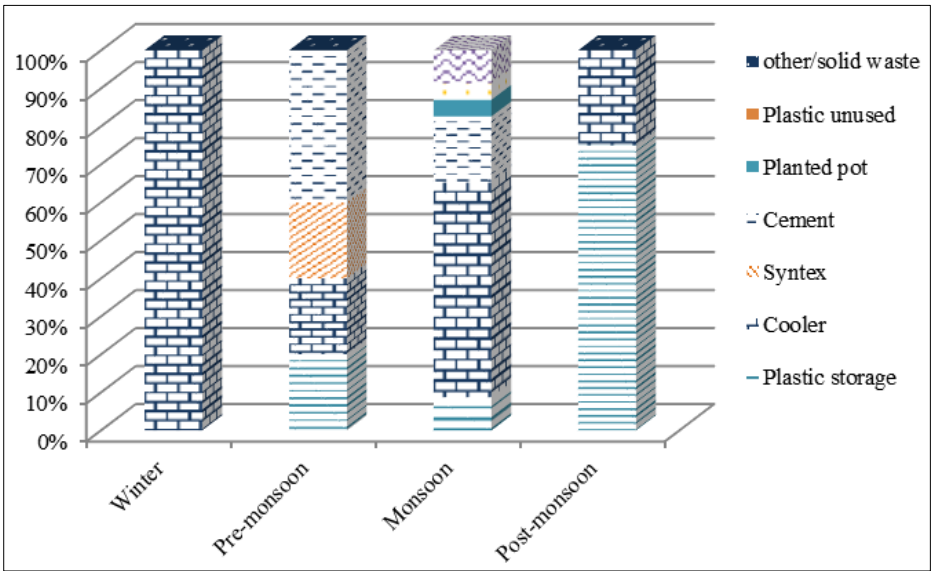

Fig 4: Season-wise *Aedes* pupal container preference in Delhi during-2016

4. Discussion

Worldwide studies have proposed that ecological and climate factors influence the seasonal prevalence of both the vector *Aedes aegypti* and *Aedes albopictus* [27, 28]. The result of this study raises a number of public health concerns that need to be addressed. *Aedes* mosquito is a climate sensitive vector. Three species of *Aedes* viz. *Aedes aegypti*, *Aedes albopictus*, and *Aedes vittatus* were found in study area during the entomological survey of which only *Aedes aegypti* was the dominant species prevalent in the domestic and peri-domestic container habitats, as reported earlier also[29, 8]. *Aedes albopictus* and *Aedes aegypti* were also found to co-breed in same type of breeding habitat in several localities of Delhi. Mixed breeding of *Aedes aegypti*, *Aedes albopictus* and *Aedes vittatus* has been also recorded in some manmade habitats in the few localities of Delhi [30]. All three species of *Aedes* mosquito were found in different localities of Delhi, but *Aedes aegypti*, was the most prevalent and widely distributed as reported earlier [29, 8]. Analysis of the seasonal percentage proportions of different *Aedes* species showed that *Aedes aegypti* was the predominant species in pre-monsoon (100 %), monsoon (97.75%), post monsoon (100 %), but *Aedes albopictus* (0.99 %) and *Aedes vittatus* (1.24 %) was reported only during monsoon season. Monsoon is the time of peak transmission of Dengue virus. Breeding of all three species was also detected in man-made containers during the monsoon season.

The Pre-monsoon season is normally characterized with acute water shortage when most residents usually resort into mass water storage in different containers. The common breeding habitats observed in the study area were, plastic storage, coolers, syntex, cement, Earthen, Planted Pot, Plastic unused and solid waste. The majorities of residents in Delhi store tap water in containers for domestic use. These containers, if not properly covered, could serve as breeding sites for disease vectors. On the other hand, the prolific breeding of the mosquitoes outdoors signals the danger associated with indiscriminate disposal of unwanted containers, the act that is common in many areas of the city [1]. Containers that retained water for long periods of time make good or suitable breeding habitats for mosquitoes such as the artificial containers [31, 32].

Our study shows that Plastic storage containers were common breeding site on every localities of Delhi which are perhaps mother foci/key containers for *Aedes* mosquitoes. *Aedes* breeding in plastic storage has been significantly increased from 27 % (2008-2009) [30] to 35 % in our study. Thus percent preference of breeding has been enhanced in plastic storage in comparison to earlier studies; breeding was more in cement tank. In the mean-time our studies shows same percentage of breeding i.e. 35 % also reported in cooler. Plastic storage,

cooler, cement tank, contributed 80 % of total *Aedes* breeding of immature and these containers also contributed 85 % of total pupal production. These containers were found to be perennial and key containers for production of larvae as well as pupal. Identification of key containers breeding of *Aedes* is important for control intervention in those containers. The containers were abundantly located close to human habitation and were potentially more durable than natural containers [33]. Plastic containers and discarded water bottles that can hold water for a long time may act as preferred breeding sites for *Aedes*. Such probable breeding sites provide a means of storing water in communities/residents where the supply of pipe water is absent or scarce [34]. The type of containers, water quality and conditions of water containers are also important for mosquito breeding [35].

Our studies show that pupal development take place only in few key containers. Three key containers (coolers, plastic storage, and cemented tank) harboured 85% of *Aedes* pupae. However, present study indicated the pupae development in specific containers in different season, which is useful for planning of targeted control measures. Since the pupae of dengue vectors emerge to become adults. Controlling the key breeding sites that produce the most pupae could have the great impact on the adult population [25]. Therefore, it needs to target pupal habitats for prevention and control of Dengue.

It was recorded that number of pupae production was also found maximum during monsoon followed by pre-monsoon, post monsoon & winter. Average pupal Index ranged from 0.31 to 3.03 in all four season of Delhi. Pupal Index is important to know the intensity of transmission and was considered the better and alternate indicator for adult mosquito abundance than traditional larvae surveillance [36].

In Delhi, there is variation in pupal production as in different containers in all four seasons and it is found varied in different containers accordingly to the dry and wet season in Kerala [26, 37].

In terms of dengue transmission, Dhar-Chowdhury *et al*. (2017) [38] associated the household dengue risk factors with the use of different types of containers for storing water by the households. In this regard, we have found that the containers located with stored tap water or water in the discarded household containers which remains uncovered for substantial period of time are positive breeding grounds for immature *Aedes*.

This identification of key indoor containers can facilitate the crafting of mosquito control massages specific to each type of containers. Previous studies have regarded this strategy as best practice in prevention and control of dengue in America [39] and Australia [40].

The study highlights the need for pre-emptive exclusion of breeding in positive localities to prevent the occurrence of potential outbreaks [41].

There is, therefore, a need for public health education campaigns that focus on the dangers inherent in the indiscriminate disposal of containers and storage of water inside the house as this serve as a potential breeding sites for the mosquito vectors, In general, larval predation of mosquitoes is less prevalent in temporary habitats than it is in large, permanent habitats [42, 43].

For the control of container breeding mosquitoes it is possible to use different methods in integration and these include covering water holding containers [44, 45]. Targeting specific type of water holding containers would enable a more focused approach to vector control than attempting to eliminate all water-holding containers [46], using appropriate biological control agents [44], public health education [47, 31, 48] creating knowledge and awareness of the residents on mosquito borne diseases [48], eliminating water-filled unused containers [47, 31] draining of containers once a week [45] and proper waste management system for all housing areas [31].

5. Conclusion

Dengue, a viral illness, has no specific treatment or vaccine available till date. Thus, reduction of infection and control at source remains the best strategy for prevention of local transmission. Entomological surveillance is an appropriate tool to identify key containers in which mosquito breed. Our study observed how different types of water-holding containers in different season contribute to the breeding of *Aedes* which may act as a vector for dengue transmission. Disease control measures need to be implemented vigorously during the pre-monsoon season to prevent outbreaks in the transmission season. Pupal index is the key predictor of an outbreak of dengue. In regard to seasonality, we found that monsoon season is the most suitable period for *Aedes* proliferation. An effective vector control programme should be established in Delhi by using the knowledge gained from this study to prevent outbreak of dengue in future. Hence, targeting the mother-foci during pre-monsoon season is needed to control dengue cases. This can be augmented by community participation involving all stakeholders, public sector offices, schools and other departments, and introducing "search-and-destroy" campaigns for better control of dengue.

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