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Surveillance of relative prevalence of dengue vectors in Agra city

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Abstract
Present paper highlights the annual and month wise prevalence of *Aedes aegypti* and *Aedes albopictus* for the years 2009 to 2012 in Agra region. Relative prevalence of *Ae. aegypti* was much higher than that of *Ae. Albopictus*, however, there was a significant increase in the population of *Ae. albopictus* from 2009 to 2012 suggesting the spread of this invasive species in the city. Moreover, the study showed that coolers were the main breeding sites of *Ae. aegypti* in the urban area whereas tire dumps and cement tanks were the preferred larval habitats for the *Ae. albopictus*. The significant interaction between positive larval habitats and species suggest that coolers, unused tires and garden pots and pits were the major contributors for the maintenance of *Aedes* population in the city. These sites should be given primary attention for controlling mosquito vectors and thereby preventing the spread of dengue and chikungunya.

Keywords: Artificial containers, Dengue, Chikungunya, Survey, Relative prevalence

1. Introduction

In recent years, the outbreaks of dengue and dengue haemorrhagic fever have become a serious public health concern worldwide. According to WHO estimates dengue is emerging at the rate of 50 million new infections per year in almost hundred countries of the world including India. The first outbreak of dengue hemorrhagic fever was recorded in 1963 in Kolkata and later spread all over India. The serious outbreak to hit the capital city Delhi was noticed in the last decade with 10,252 cases and 423 deaths [1]. There have been reports of large-scale outbreaks of this virus in southern India. At least 80,000 people in Karnataka state and Andhra Pradesh are known to have been affected since December 2005 [2]. However, recent reports of large-scale outbreaks of fever caused by chikungunya virus infection in several parts of southern India have confirmed the recent emergence of this virus [3, 4]. The above scenario represents major outbreaks of dengue reported in the last decade from various regions of India including the capital Delhi, Rajasthan, Kerala, north eastern part and adjoining areas [5, 6, 7, 8, 9]. Uttar Pradesh being the largest state of India has also experienced dengue outbreaks and only a few reports are available on the epidemiological surveys of this disease [10].

Control of disease transmission in tropical and subtropical regions has become more challenging as container breeding mosquito habitat increased with exponentially increasing human population and uncontrolled urbanization [11]. *Ae. aegypti* and *Ae. albopictus* are container breeder mosquitoes and recognized as vectors for transmission of dengue and chikungunya worldwide. Artificial containers are considered as major sources for the breeding of these species which include coolers tires, cement tanks, plastics, bottles, pots, flower vases etc. Agra, the city of Taj is one of the dengue endemic cities of Uttar Pradesh, India and cases of dengue are reported every year. However, an outbreak of chikungunya occurred in 2009 in Dayalbagh area and also in other parts of Agra. Only trace studies are performed on entomological surveys of dengue vectors. Knowledge on breeding patterns of these vector populations is necessary for the implementation of effective control measures. Therefore, an effort was made to assess the relative prevalence of *Ae. aegypti* and *Ae. albopictus* in Agra and the contribution of various larval habitats in maintaining *Aedes* population in the city.

2. Materials and Methods

The present study was conducted in Agra city (27°10N, 78°05E) during January 2009 to December 2012 (Fig. 1). A survey was done to search favourable habitats for the breeding of dengue vectors. In the present study the term larval habitat (LH) would be used to depict a variety of containers preferred by the dengue vectors for breeding [12].

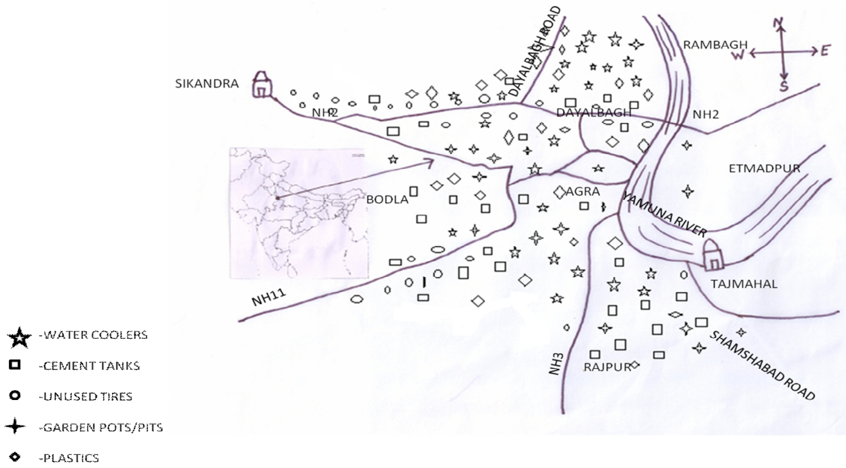


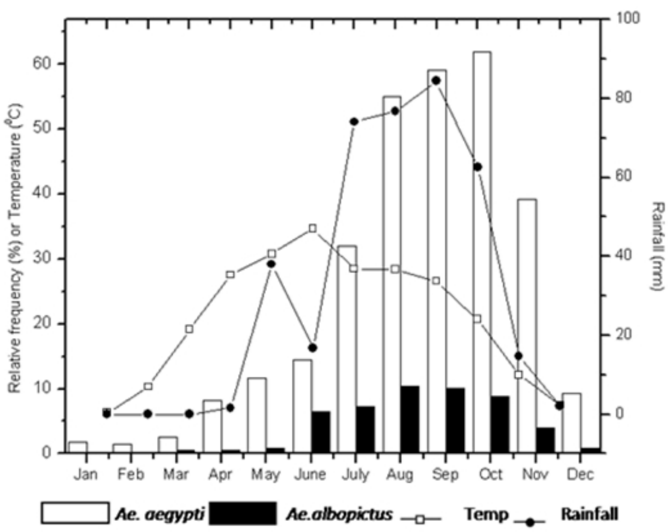
Fig: 1 Map of Agra city showing various collection sites

Five sites were selected for the study on the basis of predominant larval habitats in the area selected. Sampling was done from coolers (LH1) dominating area i.e. Dayalbagh whereas, highways were searched for collections from cement tanks (LH2) and unused tires (LH3). Rest of the samples were picked randomly from garden pots, pits and tree holes (LH4) and plastics (LH5) from the city. Monthly inspections were done from these areas to collect sufficient number of samples. Larvae and pupae were sampled from each positive larval habitat and collected in 100 ml sterilized plastic vials. The larvae were identified under light microscope (Olympus CH20 i) using appropriate taxonomic keys and confirmed by RAPD PCR using Operon primer OPA-02 (Operon Technologies, Alameda, CA) [13]. Meteorological data including mean monthly temperature and rainfall was obtained from the Indian Meteorological Department, Agra.

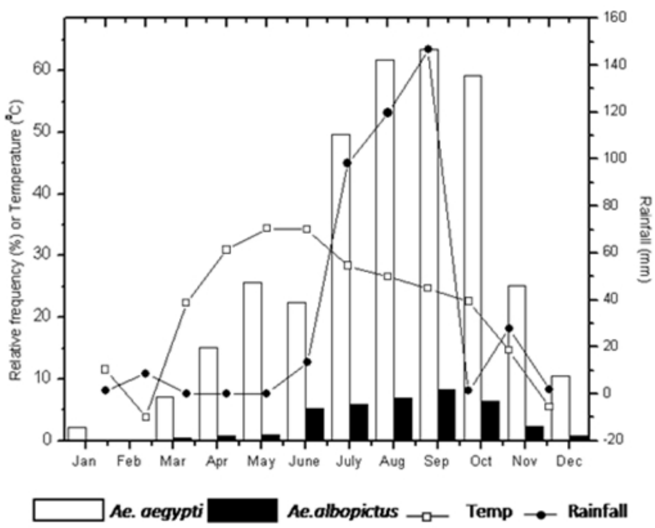
Statistical analysis: The relative frequency of immature stages of *Ae. aegypti* and *Ae. albopictus* was calculated by dividing the number of larval habitats positive for each of the species with total number of the habitats inspected per month. This was also calculated for each type of larval habitats separately. A two way ANOVA was applied on the number of positive larval habitats to justify the differences due to species and habitats. It was also done with frequency percentage considering species and months as variables. Cluster analysis was carried on the above data using WinStat in various types of artificial containers in terms of their importance as larval habitats. The statistical analysis was performed using Origin 6.0 and Graph Pad 4.0 softwares.

3. Results

A total of 10,430 larval habitats including a variety of artificial containers were searched for immature stages of dengue vectors annually. Relative prevalence of *Ae. aegypti* and *Ae. albopictus* were studied in terms of percent frequency and depicted as histograms in Fig. 2 and compared with the mean monthly temperature and rainfall. Both the species were predominated during July, August, September and October however *Ae. aegypti* was more prevalent species as compared to *Ae. albopictus.* Different habitats were preferred by each species as evident from Fig. 3. *Ae. aegypti* preferred to breed in coolers and garden pots and pits whereas *Ae. albopictus* predominated in unused tires and cement tanks.



2009



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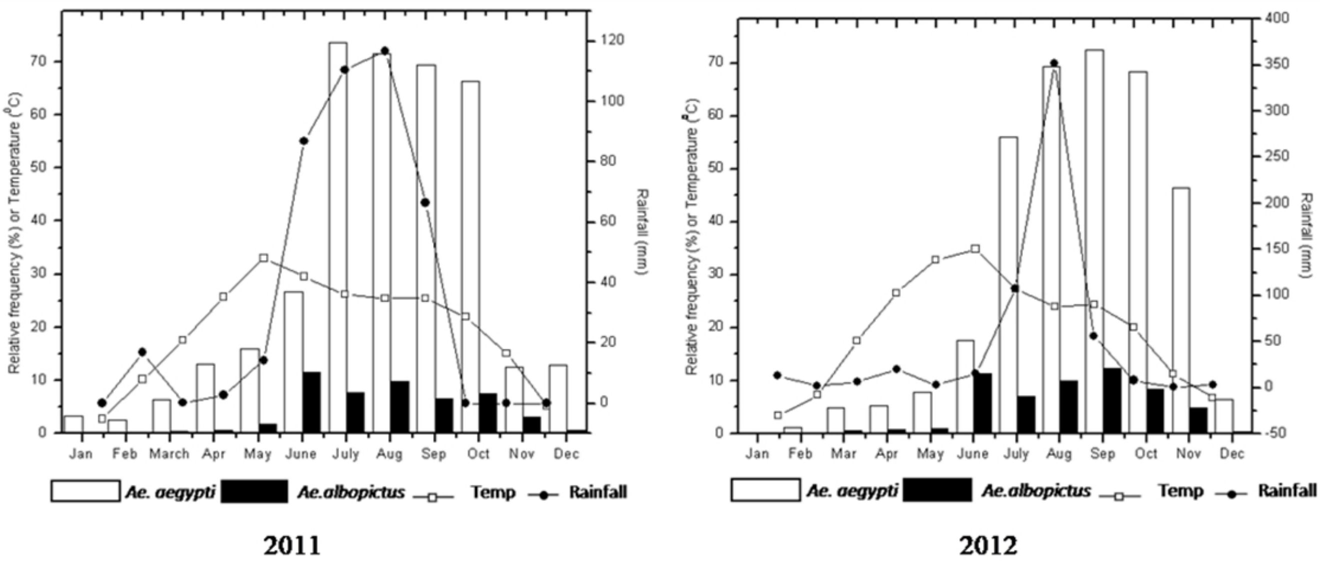


Fig: 2 Monthly prevalence of *Aedes* species and meteorological conditions (mean temperature and rainfall) in Agra during the period 2009-2012

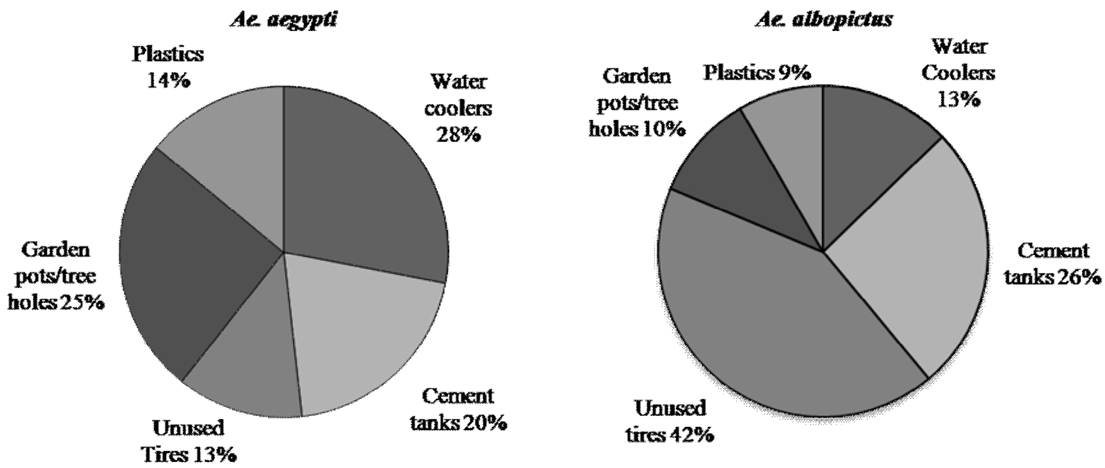


Fig 3: Average positive larval habitats for *Ae. aegypti* and *Ae. albopictus* during 2009-2012

The breeding pattern varied over a course of four years which is clearly represented in Fig. 4 depicting annual incidence of these dengue vectors. A gradual increase was observed in *Ae. aegypti* incidence from 2009 to 2011 whereas, it became static in 2012. However, a constant increase can be noticed in the annual incidence of *Ae. albopictus* throughout the study period clearly indicating its invasive potential in the city.

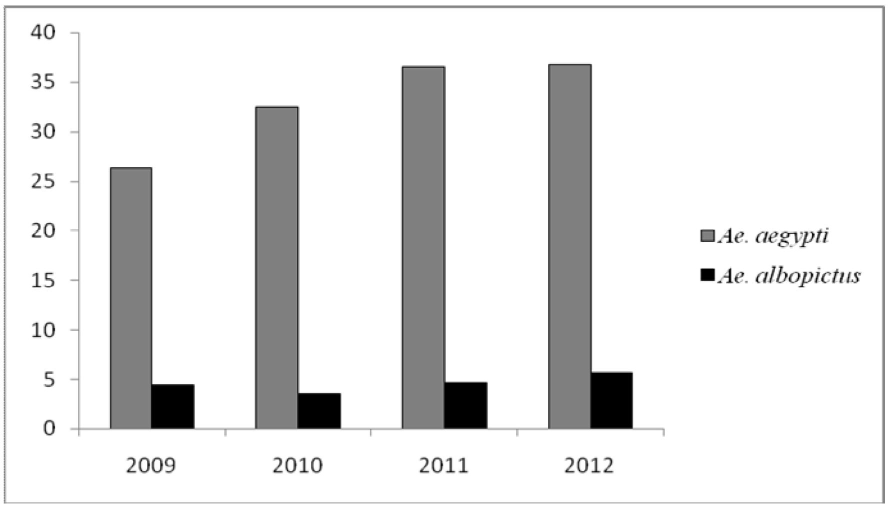


Fig 4: Annual incidence of *Ae. aegypti* and *Ae. albopictus* during the period 2009-2012

Significant variation was also noticed in the seasonal prevalence of these species (Table 1). Both the species predominated in monsoon followed by post monsoon, summer and winter. The results of two way ANOVA on positive larval habitats for these species exhibited significant relationship with species and non-significant relationship with larval habitats (Table 2). Bonferroni post-test indicated significant differences among *Ae. aegypti* and LH1 and LH 4 i.e. coolers and garden pots and pits whereas, no significant difference was found between *A. albopictus* and any of the larval habitat. Post-test between the species exhibited significant difference of species with LH1 and LH4 as observed in the first case.

Table 1: Seasonal pattern of percent positive larval habitats for *Ae. aegypti* (A) and *Ae. albopictus* (B)

Season	**Year**							
	2009		**2010**		**2011**		**2012**	
	A	**B**	**A**	**B**	**A**	**B**	**A**	**B**
Winter	1.93	0.20	3.83	0.23	4.02	0.26	2.19	0.23
Summer	11.32	2.56	20.95	2.30	17.9	4.31	10.48	4.61
Monsoon	49.15	9.32	58.25	6.95	71.48	8.14	65.70	9.81
Post Monsoon	39.50	4.96	35.11	3.56	33.55	4.14	45.21	5.21

Table 2: Results of two way ANOVA on the positive larval habitats of *Aedes* immatures

Source of Variation	**Df**	**Sum of squares**	**Mean square**	**F**
Species	2	18460000	9229000	30.67
Habitat	4	745200	186300	0.6191
Residual	8	2407000	300900	-
Bonferroni post test (Habitat vs. *A. aegypti*)				
Habitat	**Difference**	**t value**	**P value**	**Summary**
LH1	3515	4.531	P<0.01	**
LH2	2536	3.269	P>0.05	ns
LH3	1556	2.006	P>0.05	ns
LH4	3167	4.082	P<0.05	*
LH5	1764	2.274	P>0.05	ns
Bonferroni post test (Habitat vs. *A. albopictus*)				
Habitat	**Difference**	**t value**	**P value**	**Summary**
LH1	224	0.2887	P>0.05	ns
LH2	455	0.5865	P>0.05	ns
LH3	738	0.9513	P>0.05	ns
LH4	178	0.2294	P>0.05	ns
LH5	142	0.1830	P>0.05	ns
Bonferroni post test (*A. aegypti* vs. *A. albopictus*)				
Habitat	**Difference**	**t value**	**P value**	**Summary**
LH1	-3291	4.242	P<0.05	*
LH2	-2081	2.682	P>0.05	ns
LH3	-818	1.054	P>0.05	ns
LH4	-2989	3.853	P<0.05	*
LH5	-1622	2.091	P>0.05	ns

ns- non significant

Table 3: Results of two way ANOVA on percent frequency considering species and months as variables

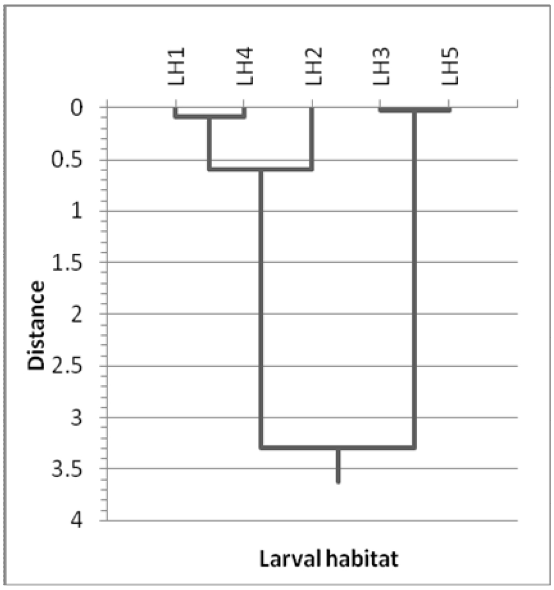
Source of Variation	**Df**	**Sum of squares**	**Mean square**	**F**
Interaction	11	11250	1023	31.56
Species	1	14340	14340	442.3
Month	11	19330	1757	54.21
Residual	72	2334	32.41	-
Bonferroni post test (*A. aegypti* vs. *A. albopictus*)				
Month	**Difference**	**t value**	**P value**	**Summary**
Jan	-1.740	0.4322	P>0.05	ns
Feb	-1.288	0.3198	P>0.05	ns
Mar	-4.705	1.169	P>0.05	ns
Apr	-9.648	2.396	P>0.05	ns
May	-14.08	3.498	P<0.01	**
June	-11.6	2.881	P>0.05	ns
July	-45.81	11.38	P<0.001	***
Aug	-55.10	13.69	P<0.001	***
Sep	-56.77	14.10	P<0.001	***
Oct	-56.21	13.96	P<0.001	***
Nov	-27.24	6.766	P<0.001	***
Dec	-9.105	2.262	P>0.05	ns

ns- non significant

A two way ANOVA on frequency percent of positive larval habitats was done month wise where, species and months exhibited significant values (Table 3). Bonferroni post test revealed significant differences between *Ae. aegypti* and *Ae. albopictus* in the month of July, August, September, October and November. A cluster analysis was performed which depicted clusters of larval habitats for both the species.

In *Ae. aegypti*, coolers were found to be closely related to garden pots and pits (LH4) and cement tanks (LH2) and distantly related to another cluster of unused tires (LH3) and plastics (LH5) (Fig. 5). For *Ae. albopictus* cooler and cement habitat formed one cluster and rest of the three larval habitats formed another cluster and both the clusters were found to have a significant difference in terms of linkage distance as indicated by Fig. 5.

Aedes aegypti



Aedes albopictus

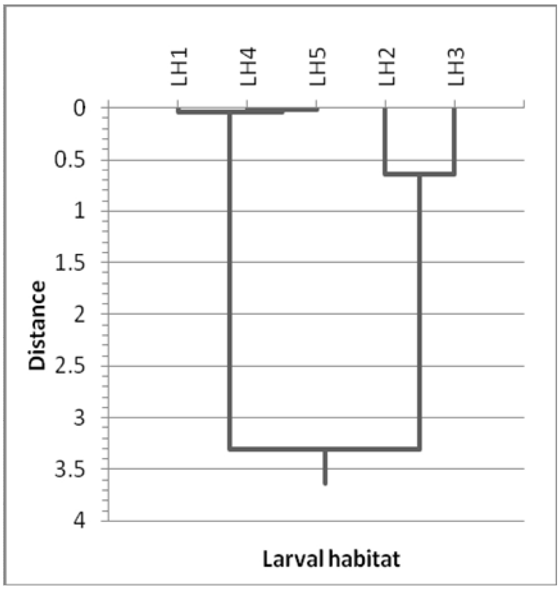


Fig: 5 Results of cluster analysis depicting similarities between larval habitats for *Aedes* immature sampled between Jan 2009-Dec 2012.

4. Discussion

In the present study extensive survey was carried out for the first time for four consecutive years in the city of Taj. It provides a clear picture on *Aedes* diversity, seasonal prevalence and various larval habitats in the Agra city. Both the dengue vectors were found to be most prevalent during monsoon followed by post monsoon. Similar trend of occurrence was observed by previous workers [14]. In contrast, reports are also available on maximum prevalence during post monsoon [5, 8]. The possible reason for the higher prevalence during July, August and September could be associated with high rainfall, mean monthly temperature and humidity during this period.

Only trace studies are available on the prevalence of *Ae. aegypti* from Agra region [15] however, this is first report on the occurrence of Asian tiger mosquito *Ae. albopictus* from this region. Although the prevalence of *Ae. aegypti* was considerably high, *Ae. albopictus* was found to be invading the city along the highways. They were encountered in maximum numbers form national highways where plenty of unused tires and cement tanks were observed as mosquito breeding sites form the repair shops. Moreover, they were found spreading in the city and collected from other habitats also. Further, the annual incidence pattern clearly depicted the invasion of this species in the city which may be alarming in the near future as their increase is related with a gradual fall in the abundance of *Ae. aegypti*.

Considering the larval habitats, coolers were found to host higher population of *Ae. aegypti* than *Ae. albopictus* which may be related to the size of container and its water holding capacity. On the other hand tires and cement tanks were available to *Ae. albopictus* for breeding along the high ways. Earthen and other plastic wastes were also accommodating these species as they contain ample amount of water needed for the breeding of these vectors. Especially, plastic pots and containers were the major larval habitats which hold water for longer duration than other habitats due to their non porous nature and thereby facilitating the breeding of dengue vectors [12]. Cluster analysis validated the significance of coolers and cement tanks in supporting the breeding of *Ae. albopictus* as they formed one cluster of habitat which was distantly related to other larval habitats as evident from the city map. The patterns of seasonal fluctuations in *Aedes* prevalence described in this study may help in determining the appropriate periods for the implementation of suitable control measures. Source reduction or eradication of larval habitat is to be considered essential for controlling mosquitoes and thereby reducing the outbreaks of dengue and dengue haemorrhagic fever**.**

5. Acknowledgements

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6. References

1. Kaul SM, Sharma RS, Sharma SN, Panigrahi N, Phukan PK, lal S *et al*. Preventing dengue and DHF. The role of entomological surveillance. J Com Dis 1998; 30:187-192.
2. Ravi V. Re-emergence of chikungunya virus in India. Ind J Med Microbiol 2006; 24:83-84.
3. World Health Organization. Chikungunya and dengue in the south-west Indian Ocean. Epidemic and pandemic alert and response (EPR). Disease outbreak news, 17 March 2006.

(http://www.who.int/csr/don/2006_03_17/en) Accessed Aug 2009.
4. Enserink M. Massive out break draws fresh attention to little known virus. Science 2006; 311:1085.
5. Sharma RS, Kaul SM, Sokhay J. Seasonal fluctuations of dengue fever vector, *Aedes aegypti* (Diptera: Culicidae) in Delhi, India. Southeast Asian J Trop Med Pub Health 2005; 36:186-190.
6. Sharma K, Angel B, Singh H, Purohit A, Joshi V. Entomological studies for surveillance and prevention of dengue in arid and semi-arid districts of Rajasthan, India. J Vec Borne Dis 2008; 45:124-132.
7. Rao BB. Larval habitats of *Aedes albopictus* (Skuse) in rural areas of Calicut, Kerala, India. J vec Borne Dis 2010; 47:175-177.
8. Barua S, Dutta P. Seasonal pattern of abundance of *Aedes albopictus* in urban and industrial areas of Dibrugarh District Assam. Asian J Exp Biol Sci 2012; 3:559-564.
9. Puwar T, Sheth JK, Kohli V, Yadav, R. Prevalence of chikungunya in the city of Ahmedabad, India during 2006 outbreak: A community based study. Deng Bull 2010; 34: 40-45.
10. Cecilia D. Current status of dengue and chikungunya in India. WHO South-East Asia J Public Health 2014; 3(1):22-27.
11. Preet S, Sneha A. Biochemical evidence of efficacy of potash alum for the control of dengue vector *Aedes aegypti* (Linnaeus). Parasitol Res 2011; 108:1533-1539.
12. Banerjee S, Aditya G, Saha GK. Household disposables as breeding habitats of dengue vectors: Linking wastes and public health. Waste Manag 2013; 33:233-239.
13. Gupta S, Preet S. Protocol optimization for genomic DNA extraction and RAPD-PCR in mosquito larvae (Diptera: Culicidae). Annals Biol Res 2012; 3:1553-1561.
14. Jomon KV, Sudharmini S, Valamparampil TT. *Aedes* mosquitoes in arboviral prone area of Kottayam district, Kerala, India. SB Aca Rev 2009; 16:171-178.
15. Bar A, Andrew J. Seasonal prevalence of *Aedes aegypti* larvae in Agra. Res Zool 2012; 2:14-17.