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Surveillance of *Aedes* mosquito species in villages of Jalna district, Maharashtra, India

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Abstract
Monthly ovitrap surveillance was conducted in ten villages of Jalna district, Maharashtra, India to study the presence and seasonal abundance of *Aedes* vector species. Ovitraps were installed indoors and outdoors in randomly selected locations, and the eggs retrieved from the positive ovitraps were hatched and reared for identification of the species. Total 4310 ovitraps were recovered with 1088 positive ovitraps, and the species found were *Ae. Aegypti*, *Ae. Albopictus*, and *Ae. Vittatus*. Ovitrap index of *Ae. Aegypti* was found to be significantly higher compared to *Ae. Albopictus* and *Ae. Vittatus*, and the monthly mean ovitrap index ranged between 7.4% to 53.0%, 0.3% to 12.7% and 0.3% to 1.0% respectively with no significant difference between indoor and outdoor ovitraps (P>0.05). Our observations revealed dengue vector *Ae. Aegypti* to be predominant species throughout the year in the study sites and its breeding association with human habitat. This study was undertaken, in concurrent to our phased evaluation studies on genetically engineered OX513A *Ae. Aegypti* strain, to generate baseline data on natural population of *Ae. Aegypti* for selection of test sites to demonstrate suppression of *Ae. Aegypti* population by sustained releases of OX513A strain male adults under open field conditions in future. In addition, our observations contribute to the knowledge on seasonal abundance of *Aedes* mosquito species to strategize for vector mosquito management in rural areas.

Keywords: *Aedes Aegypti*, *Aedes Albopictus*, *Aedes Vittatus*, ovitrap, dengue, chikungunya, zika

Introduction
Aedes mosquitoes are well known for transmission of arboviral diseases like dengue, chikungunya, zika and the yellow fever. *Aedes Aegypti* L. mosquito has historically been the primary vector responsible for transmission of dengue disease in India and worldwide, followed by *Aedes Albopictus* Skuse. Species considered as a secondary vector for transmitting the disease [1-2]. These two endemic vectors have different origins, *Ae. Aegypti* originating from African forests while *Ae. Albopictus* from South Asia forest and both have invaded worldwide. Although their short flight range is a limitation factor for migration, the rapid population growth, international trading and the ability of the *Aedes* eggs to undergo diapause and withstand desiccation have facilitated migration over long distances [3]. *Ae. Aegypti* is anthropophilic in nature seeking human blood from multiple individuals in short periods of time increasing their ability to spread the disease and potential transmission of disease [4]. Currently the global distribution of dengue disease is comparable to that of malaria, with transmission occurring in 128 countries and an estimated 4 billion people live at risk for epidemic transmission with 50-100 million cases reported every year [5-8]. In India, the number of dengue cases and severity of the disease has significantly increased since 2001. In addition to the increasing number of dengue cases, the geographical range of the disease has expanded from urban to rural regions [9].

Effective surveillance and monitoring of vector mosquito population is one of the crucial steps to understand the level of infestation in a particular locality for planning and managing the vector population well below the disease transmission threshold level [10]. Additionally, surveillance provides information for developing risk assessment, which in turn could be used to qualitatively or quantitatively predict the occurrence of vector-borne diseases or pest outbreaks [11-12]. Ovitrap is one among the cost-effective and sensitive tool recommended for surveillance, differentiating infestation levels, monitoring or prediction of disease out-break, and to assess impact of any control measures implemented [13-14]. Moreover, use of ovitrap for surveillance is considered to be more sensitive approach even during low levels of vector

Population than conventional methods like larval survey [15-16]. Spatial distribution and abundance of *Ae. Aegypti* are related with the impact of anthropogenic activities in the environment, and the abundance is associated with intra domiciliary water containers and or peridomiciliary premises [17]. Whilst, presence of *Ae. Albopictus* is more associated with the presence of vegetation in urban and rural areas, whereas its abundance is generally limited to spaces modified by human activity [18]. Abiotic factors like rainfall, temperature and relative humidity are key determining factors for the presence and frequency of these species. Relationship between these bio-ecological aspects and seasonal abundance can be well understood and analyzed by ovitrap surveillance method, which would serve for successful implementation of control measures to combat against disease outbreak [19].

This present study aimed to investigate the seasonal occurrence and abundance of *Aedes* mosquito species in villages of Jalna district, Maharashtra State, India. This study was undertaken in parallel to our on-going laboratory studies, a part of phased evaluation, on genetically-engineered OX513A *Ae. Aegypti* strain [20-23]. In this context, the surveillance also intended to generate data on distribution and seasonal abundance of *Ae. Aegypti* species, which could aid in selecting test sites in future for conducting open field trials on OX513A to demonstrate suppression of *Ae. Aegypti* population through sustained releases of OX513A male adult mosquitoes over a period of specified duration under Indian environmental conditions. Earlier studies on open field releases of OX513A male adults have shown high levels of suppression of *Ae. Aegypti* population demonstrating its potential use in the integrated vector management [24-28].

Materials and Methods

Study area

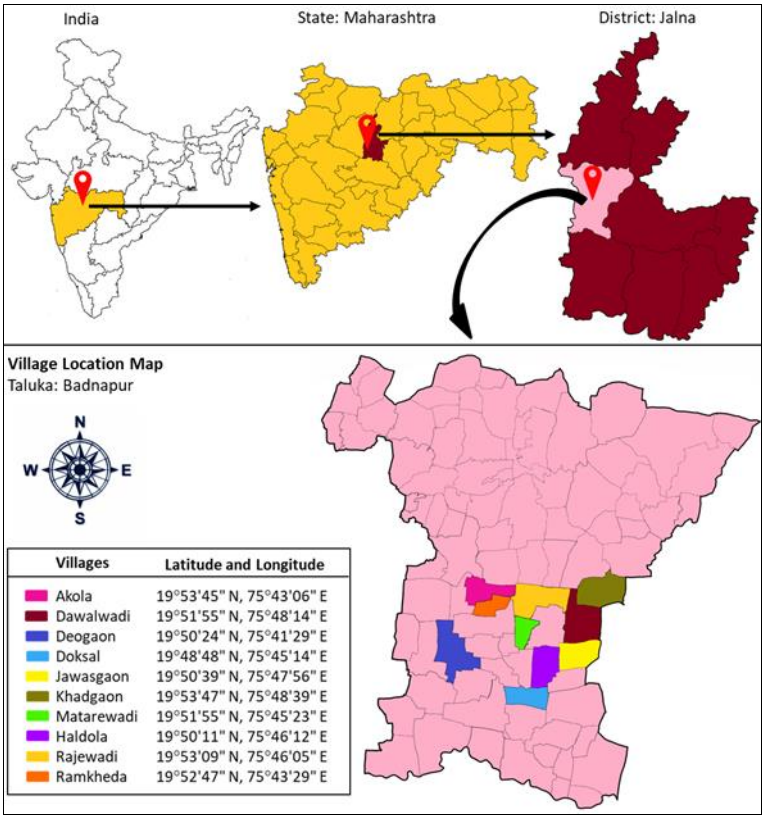


Fig 1: Map location of villages surveyed for *Aedes* mosquito population in Badnapur taluk of Jalna District, Maharashtra State, India.

Ovitrap surveillance study was conducted in ten rural villages of Badnapur taluk, Jalna district, Maharashtra state, India (Figure 1). The villages surveyed were Akola, Dawalwadi, Deogaon, Doksal, Haldola, Jawasgaon, Khadgaon, Matarewadi, Rajewadi and Ramkheda. Population in the villages ranged between 516 to 2500 and the area of the community living in villages ranged between 1.63 to 8.80 hectares (Table 1). Houses in the villages are constructed with burnt bricks, and or with mud, and have concrete roofs, and or cement or iron sheets and the size usually consist of 2 or 3 rooms. Drinking water is typically stored in earthen pots, metal or plastic containers, while water used for routine work is typically stored in barrels, cement tanks and in some houses, overhead tanks are used for storage of water.

Table 1: Population and area of community living in villages selected for surveillance of *Aedes Aegypti* L. mosquito

Villages	Population*	Area** (Hectares)
Akola	2150	8.80
Dawalwadi	1500	6.73
Doksal	1460	6.76
Haldola	900	8.00
Jawasgaon	1500	6.10
Deogaon	1000	7.85
Khadgaon	1370	4.25
Matarewadi	1500	2.13
Rajewadi	2500	8.44
Ramkheda	516	1.63

*Source - Gram Panchayat office; ** Source-Google maps

Ovitrap

Ovitrap surveillance was conducted during the period from October 2013 to November 2014 by adhering to the guidelines of World Health Organization (2009) [29]. Ovitrap consists of black plastic container of 300 ml volume (base diameter of 6.5 cm, opening diameter of 7.8 cm and 9.0 cm in height) filled with water, and a paper strip surrounding the inner side of the container and half immersed in the water. The traps were installed, indoors near dark potential resting sites and outdoors in shaded sites to avoid direct exposure to sunlight, in randomly selected houses / sites scattered over each study area. Indoors refers to inside premises of the house and outdoors refer to peridomiciliary of the houses but still within the immediate boundary of the house. Survey was conducted every month by installing 30-40 ovitraps both indoor and outdoor and after 6 days of ovitrap deployment, the paper strips from the ovitraps were recovered and transferred to laboratory for further analysis. Paper strips recovered were immersed in water for hatching and eggs hatched were reared till adult emergence for identification of species following the adult identification keys described by Barraud (1934) [30].

Rainfall and temperature data

Rainfall and temperature data for each month were taken from the records maintained using a rain gauge by research centre of "Maharashtra Hybrid Seeds Company Private Limited", located at Dawalwadi, Badnapur taluk, Jalna District, Maharashtra State, India. In the view, that all the villages selected for surveillance were approximately within the radius of 15 kilometers from Dawalwadi (one of the selected study area), the rainfall / temperature data by research center was considered for analysis.

Data analysis

The measures of infestation by populations of *Aedes* species eggs were expressed using the positive ovitrap index (OI) and mean number of adults per trap as follows [31].

Ovitrap Index (OI) = (Number of positive traps / Number of recovered traps) × 100

Mean number of adults emerged per trap = Total number of adults emerged / Number of recovered ovitraps

Data were subjected to statistical analysis by applying DMRT test (Duncan Multiple Range Test) and independent t-test at $p<0.05$ using the statistical software SPSS version 20. Correlation between ovitrap index and monthly rainfall was analyzed by pearson correlation coefficient.

Results

In the present study period of 14 months, total 4310 ovitraps were recovered with 1088 ovitraps positive for eggs of *Aedes* species in the study sites (Table 2). Recovery of indoor and outdoor ovitraps from the total ovitraps installation accounted to 2236 and 2074 ovitraps respectively. Identification of the species from the recovered positive ovitraps revealed presence of three *Aedes* species viz. *Ae. Aegypti*, *Ae. Albopictus* and *Ae. Vittatus*. Results indicated infestation of *Ae. Aegypti* and *Ae. Albopictus* species in all surveyed villages, while eggs of *Ae. Vittatus* was found only in ovitraps recovered from Haldola, Khadgaon and Rajewadi villages.

The most abundant species observed in the positive ovitraps collected was *Aedes Aegypti*. Among the total positive ovitraps (n=1088), 1020 (93.75%), 131 (12.04%) and 7 (0.64%) ovitraps were positive for *Ae. Aegypti*, *Ae. Albopictus* and *Ae. vitiates* population respectively. Ovitrap index (OI) of *Ae. Aegypti* for indoor and outdoor collection was found to be significantly higher ranging between 13.5% to 35.6% and 14.8% to 31.2% respectively than compared to OI of *Ae. Albopictus* ranging between 0.8% to 8.1% and 0.4% to 8.9% correspondingly. OI of *Ae. Vittatus* was negligible revealing maximum OI of 0.8% (indoor) and 0.9% (outdoor) in Haldola and Rajewadi village respectively. Infestation of *Ae. Aegypti* was high in three villages Akola, Khadgaon and Matrewadi with OI (indoor and outdoor) accounting to 57.5%, 65% and 57.3% respectively, compared to other villages during the surveillance period (Table 2). Interestingly *Ae. Aegypti* population was found throughout the survey period in all the villages with significantly lower ovitrap index during the dry spell from March to July 2014, while presence of *Ae. Albopictus* was not observed during the three months period between April to June 2014. During the surveillance period, few ovitraps were found positive for *Ae. Vittatus* species in the months of October 2013 and May, July and August 2014, with poor OI values accounting to less than 1%. OI of the three *Aedes* species observed ranged between 7.4% to 53.0%, 0.3% to 12.7% and 0.3% to 1.0% for *Ae. Aegypti*, *Ae. Albopictus* and *Ae. Vittatus* respectively (Table 3). Average number of emerged adults recovered from eggs per ovitrap was found to be in the range of 15.5 to 53.6, 0 to 25.0 and 0 to 11.6 for *Ae. Aegypti*, *Ae. Albopictus* and *Ae. Vittatus* respectively (Table 3).

The positive ovitraps were observed for existence of mixed breeding of the *Aedes* species. Mixed breeding of *Ae. Aegypti* and *Ae. Albopictus* populations was maximum with OI of 10.9% (30 positive ovitraps) during the post-monsoon season (Oct 2013) followed by OI of 4.3%, 2.8% and 1.3% in the months of August, October and November 2014 respectively (Table 4). And during other months mixed breeding of *Ae. Aegypti* and *Ae. Albopictus* was poor as indicated by OI ≤ 2%. While mixed breeding for population of *Ae. Aegypti* and *Ae. Vittatus*, and for population of *Ae. Albopictus* and *Ae. Vittatus* was negligible with only one ovitrap found positive during the survey period in the month of May and July correspondingly.

Our observations on the average number of emerged adults recovered from ovitraps during the 14 months surveillance period between the villages were statistically non-significant ($P>0.05$) for all the three *Aedes* populations (Table 5). Independent t-test between average OI of indoor and outdoor ovitraps observed in the villages during the 14 months survey period revealed non-significant difference ($P>0.05$) indicating equal preference for indoor and outdoor breeding (Table 6). Analysis of correlation between monthly rainfall data and average monthly ovitrap index of villages for the three species revealed positive correlation value ($r=0.574$) for *Ae. Albopictus* (Table 7 and Figure 2), and no correlation was observed for *Ae. Aegypti* and *Ae. Vittatus*. OI results of indoor and outdoor indicated *Ae. Aegypti* to be predominant species in the surveyed villages and its occurrence in all the seasons of the year.

Table 2: Average indoor and outdoor ovitrap index of *Ae. Aegypti*, *Ae. Albopictus* and *Ae. Vittatus* species in the villages surveyed from October 2013 to November 2014

Study village	No of recovered indoor ovitraps	Ovitrap Index *Mean±SE			comparison of OI between species [F value (*df*), *P* value]	No of recovered outdoor ovitraps	Ovitrap Index *Mean±SE			comparison of OI between species [F value (*df*), *P* value]
		Indoor					Outdoor			
		Ae. Aegypti	Ae. Albopictus	Ae. Vittatus			Ae. Aegypti	Ae. Albopictus	Ae. Vittatus	
Akola	214	28.0 ± 5.7^{abcA} (56)	0.9 ± 0.6^{bB} (2)	0^{aB}	F=22.8 (2), P<0.001	218	29.5 ± 6.0^{ab} (65)	0.7 ± 0.7^{c} (1)	0^{b}	F=23.8 (2), P<0.001
Dawalwadi	224	25.9 ± 5.7^{abcA} (60)	8.1 ± 4.9^{aB} (21)	0^{aB}	F=9.2 (2), P<0.01	207	22.9 ± 3.4^{ab} (47)	8.9 ± 3.2^{a} (19)	0^{b}	F=18.6 (2), P<0.001
Devgoan	225	22.9 ± 3.8^{abcA} (52)	2.0 ± 0.9^{b} (5)	0^{aB}	F=31.4 (2), P<0.001	209	20.8 ± 4.1^{ab} (41)	1.8 ± 1.4^{abc} (3)	0^{b}	F=21.6 (2), P<0.001
Doksal	214	13.4 ± 2.8^{cA} (30)	1.0 ± 0.7^{bB} (2)	0^{aB}	F=19.8 (2), P<0.001	208	14.8 ± 3.4^{b} (28)	1.4 ± 1.0^{bc} (2)	0^{b}	F=16.3 (2), P<0.001
Haldola	226	23.5 ± 4.2^{abcA} (56)	2.3 ± 1.3^{bB} (5)	0.8 ± 0.6^{aB} (2)	F=24.6 (2), P<0.001	210	22.7 ± 6.2^{ab} (47)	5.6 ± 3.6^{abc} (9)	0^{b}	F=8.2 (2), P<0.01
Jawasgoan	221	24.5 ± 6.1^{abcA} (56)	1.3 ± 0.7^{bB} (3)	0^{aB}	F=15.1 (2), P<0.001	219	21.7 ± 3.9^{ab} (47)	5.3 ± 2.4^{abc} (12)	0^{b}	F=18.6 (2), P<0.001
Khadgoan	217	33.8 ± 6.6^{abA} (72)	2.4 ± 1.1^{bB} (5)	0.5 ± 0.5^{aB} (1)	F=23.6 (2), P<0.001	214	31.2 ± 4.9^{a} (66)	3.6 ± 1.3^{abc} (8)	0.5 ± 0.5^{ab} (1)	F=33.2 (2), P<0.001
Matarewadi	234	35.6 ± 5.3^{aA} (83)	0.8 ± 0.5^{bB} (2)	0^{aB}	F=44.5 (2), P<0.001	192	21.6 ± 4.2^{ab} (39)	0.4 ± 0.4^{c} (1)	0^{b}	F=25.5 (2), P<0.001
Rajewadi	238	18.7 ± 3.0^{bcA} (46)	1.7 ± 0.9^{bB} (5)	0.5 ± 0.5^{aB} (1)	F=30.4 (2), P<0.001	199	21.5 ± 5.6^{ab} (45)	8.3 ± 3.9^{ab} (16)	0.9 ± 0.6^{a} (2)	F=7.1 (2), P<0.01
Ramkheda	223	20.2 ± 4.3^{abcA} (45)	1.9 ± 1.3^{bB} (5)	0^{aB}	F=18.6 (2), P<0.001	198	20.2 ± 4.6^{ab} (39)	2.4 ± 1.3^{abc} (5)	0^{b}	F=15.8 (2), P<0.001
F-value (df)		1.85 (9)	1.43 (9)	1.16 (9)			0.97 (9)	1.89 (9)	1.58 (9)	
P value		*P*=0.065	*P*=0.182	P=0.323			P=0.466	P=0.059	P=0.129	

*Figures represent mean of monthly OI for 14 months period; Figures in parentheses represent total number of positive ovitraps

Differences in the mean values indicated by the same small letters within the columns are non-significant at 0.05 level by One-way ANOVA using Duncan test.

Differences in the mean values indicated by the same capital letters within the rows for indoor and outdoor are non-significant at 0.05 level by One-way ANOVA using Duncan test

Table 3: Average monthly ovitrap index and number of adults recovered per ovitrap of *Aedes* species from the surveyed villages

Study period	Ovitrap index Mean ±SE			Number of adults recovered per ovitrap Mean ±SE			Rainfall (mm)	Temperature	
	Ae. Aegypti	*Ae. Albopictus*	*Ae. Vittatus*	*Ae. Aegypti*	*Ae. Albopictus*	*Ae. Vittatus*		Max	Min
Oct 2013	53.0 ± 4.4^{a}	12.7 ± 2.6^{a}	0.6 ± 0.4^{ab}	32.1 ± 7.5^{bc}	20.9 ± 3.5^{a}	0.6 ± 0.4^{b}	106	31	22
Nov 2013	35.5 ± 2.9^{b}	0.3 ± 0.3^{c}	0^{b}	40.3 ± 6.3^{ab}	0.9 ± 0.9^{b}	0^{b}	24	29.7	17
Dec 2013	19.3 ± 3.8^{def}	0.3 ± 0.3^{c}	0^{b}	25.5 ± 6.6^{bc}	0.6 ± 0.6^{b}	0^{b}	41	31.5	15.9
Jan 2014	22.6 ± 4.4^{cde}	1.4 ± 0.7^{c}	0^{b}	20.6 ± 3.2^{c}	1.4 ± 1.1^{b}	0^{b}	0	28.5	15.8
Feb 2014	26.8 ± 3.7^{bcd}	1.0 ± 0.7^{c}	0^{b}	32.3 ± 2.6^{bc}	0.5 ± 0.3^{b}	0^{b}	0	29.8	14.9
Mar 2014	19.4 ± 2.5^{def}	1.2 ± 0.6^{c}	0^{b}	23.1 ± 3.9^{c}	2.1 ± 1.6^{b}	0^{b}	86	31.6	18.7
April 2014	15.8 ± 2.8^{efg}	0^{c}	0^{b}	32.0 ± 3.6^{bc}	0^{b}	0^{b}	19.5	37.4	22
May 2014	8.2 ± 1.2^{g}	0^{c}	0.3 ± 0.3^{b}	19.3 ± 3.0^{c}	0^{b}	0.1 ± 0.1^{b}	1.5	38.9	25.5
June 2014	7.4 ± 1.2^{g}	0^{c}	0b	19.6 ± 4.0^{c}	0^{b}	0^{b}	0	38	26.9
July 2014	11.2 ± 1.7^{fg}	3.5 ± 1.1^{bc}	1.0 ± 0.5^{a}	53.6 ± 12.1^{a}	22.3 ± 7.5^{a}	11.6 ± 7.3^{a}	134	31.5	24.1
Aug 2014	30.4 ± 5.2^{bc}	7.8 ± 2.3^{ab}	0.3 ± 0.3^{b}	22.5 ± 2.4^{c}	25.0 ± 11.3^{a}	3.2 ± 3.2^{b}	310	30.3	23
Sept 2014	46.2 ± 3.7^{a}	9.2 ± 5.0^{a}	0^{b}	18.5 ± 3.0^{c}	23.4 ± 10.0^{a}	0^{b}	101	29.8	21.8
Oct 2014	19.8 ± 3.0^{def}	3.8 ± 2.0^{bc}	0^{b}	18.6 ± 2.8^{c}	11.1 ± 4.1^{ab}	0^{b}	11	31.6	20.6
Nov 2014	16.0 ± 0.8^{efg}	1.3 ± 0.4^{c}	0^{b}	15.5 ± 0.9^{c}	2.9 ± 1.3^{b}	0^{b}	28.5	30	17.7
F-value (df)	17.76 (13)	5.13 (13)	2.08 (13)	4.06 (13)	4.59 (13)	2.16 (13)	-	-	-
P value	P<0.001	P<0.01	P<0.05	P<0.001	P<0.001	P<0.05	-	-	-

Differences in the mean values of OI indicated by the same letters within the columns are non-significant at 0.05 level by One-way ANOVA using Duncan test.

Table 4: Ovitrap index of mixed breeding observed for *Ae. Aegypti*, *Ae. Albopictus* and *Ae. Vittatus* during the surveillance period in 10 villages

Study period	Ovitrap Index		
	Ae. Aegypti + *Ae. Albopictus*	*Ae. Aegypti* + *Ae. Vittatus*	*Ae. Albopictus* + *Ae. Vittatus*
Oct 2013	10.9 (30)	-	-
Nov 2013	0.7 (2)	-	-
Dec 2013	-	-	-

Jan 2014	0.3 (1)	-	-
Feb 2014	0.4 (1)	-	-
Mar 2014	0.8 (2)	-	-
April 2014	-	-	-
May 2014	-	0.4 (1)	-
June 2014	-	-	-
July 2014	-	-	0.3 (1)
Aug 2014	4.3 (14)	-	-
Sept 2014	0.6 (2)	-	-
Oct 2014	2.8 (11)	-	-
Nov 2014	1.3 (5)	-	-

Figures in the parentheses indicate number of positive ovitraps

Table 5: Number of adults recovered per positive ovitraps, ratio of mean adults recovered and ratio of mean OI of *Aedes* species in the villages during the survey period.

Village	Number of adults per recovered ovitrap Mean ±SE			Ratio of mean number of adults recovered	Ratio of mean of ovitrap index
	Ae. Aegypti	*Ae. Albopictus*	*Ae. Vittatus*	*Ae. Aegypti*: *Ae. Albopictus*: *Ae. Vittatus*	
Akola	25.5±3.0[a]	1.5±1.1[a]	0[a]	9.4: 0.6: 0	9.7: 0.3: 0
Dawalwadi	26.4±9.6[a]	7.9±2.8[a]	0[a]	7.7: 2.3: 0	7.4: 2.6: 0
Devgoan	27.4±4.1[a]	4.9±2.8[a]	0[a]	8.5: 1.5: 0	9.2: 0.8:
Doksal	27.3±6.4[a]	2.5±1.9[a]	0[a]	9.2: 0.8: 0	9.2: 0.8: 0
Haldola	25.2±4.4[a]	7.7±3.0[a]	0.9±0.8[a]	7.4: 2.3: 0.3	8.4: 1.4: 0.2
Jawasgoan	21.7±1.6[a]	4.7±1.9[a]	0[a]	8.2: 1.8: 0	8.8: 1.2: 0
Khadgoan	20.8±2.4[a]	5.1±2.4[a]	2.6±2.6[a]	7.3: 1.8: 0.9	9.0: 0.8: 0.2
Matarewadi	32.7±4.7[a]	0.4±0.3[a]	0[a]	9.9: 0.1: 0	9.8: 0.2: 0
Rajewadi	17.3±3.4[a]	6.2±2.9[a]	2.0±1.3[a]	6.8: 2.4: 0.8	7.8: 1.9: 0.3
Ramkheda	23.5±2.6[a]	4.1±2.3[a]	0[a]	8.5: 1.5: 0	9.0: 1.0: 0
F value (df)	0.782 (9)	1.186 (9)	1.068 (9)	-	-
P value	0.633	0.309	0.390	-	-

Differences in the mean values indicated by the same letters within the columns are non-significant at 0.05 level by One-way ANOVA using Duncan test.

Table 6: Comparative analysis ovitrap index and adults recovered per ovitrap during indoor and outdoor survey of *Ae. Aegypti*, *Ae. Albopictus* and *Ae. Vittatus* population in the villages during the survey period.

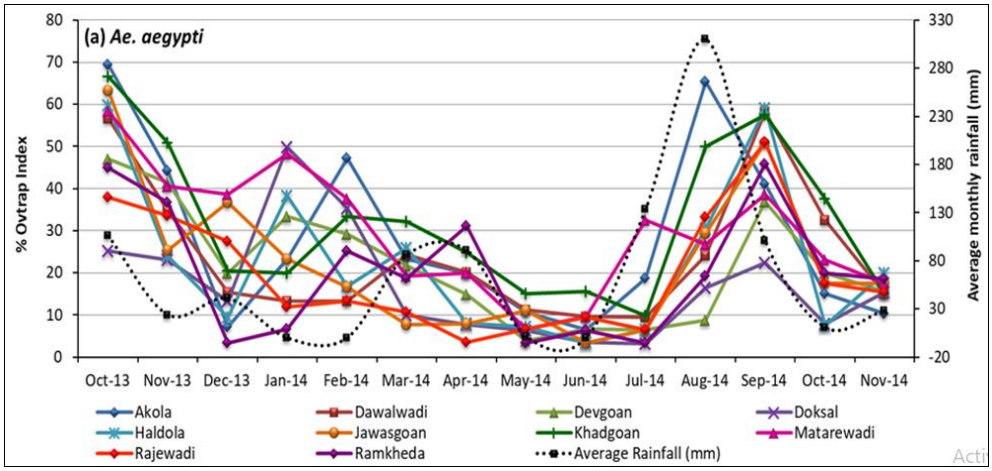
	Ae. Aegypti Mean ±SE		*Independent t-test [Sig. 2-tailed, P value; df]	*Ae. Albopictus* Mean ±SE		Independent t-test [Sig. 2-tailed, P value; df]	*Ae. Vittatus* Mean ±SE		Independent t-test [Sig. 2-tailed, P value; df]
	Indoor	Outdoor		Indoor	Outdoor		Indoor	Outdoor	
Ovitrap Index	24.6±1.6 (556)	22.7±1.5 (464)	P = 0.375; 278	2.2±0.6 (55)	3.8±0.7 (76)	P=0.086; 278	0.2±0.1 (4)	0.1±0.1 (3)	P=0.701; 278
Adults/recovered ovitrap	26.7±2.3	22.8±1.6	P = 0.169; 278	3.3±0.7	5.7±1.1	P=0.07; 278	0.2±0.2	0.9±0.6	P=0.252; 278

*Differences between mean values of indoor and outdoor ovitrap index were statistically analysed by independent t-test.
Figures in parentheses represent total number of positive ovitraps.

Table 7: Correlation coefficient (Pearson) for the relationship between monthly ovitrap index values and cumulative monthly rainfall (mm)

		Monthly Ovitrap Index		
		Ae. Aegypti	*Ae. Albopictus*	*Ae. Vittatus*
Monthly Rainfall (mm)	Pearson Correlation (r value)	0.304	0.574*	0.435
	Sig. (2-tailed)	0.290	0.032	0.120

*correlation is significant at the 0.05 level (2-tailed)



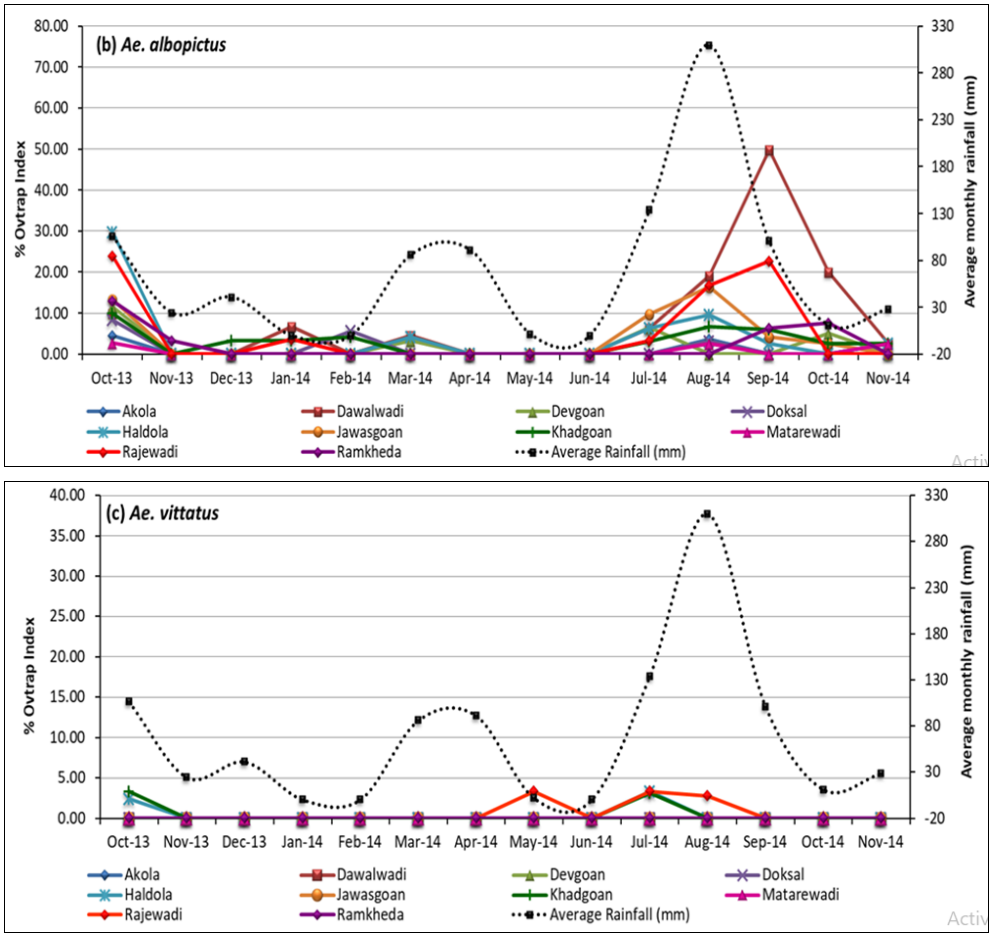


Fig 2: Average monthly rainfall and monthly ovitrap index of *Ae. Aegypti* (a), *Ae. Albopictus* (b) and *Ae. Vittatus* (c) in ten villages of Badnapur, Jalna District, Maharashtra State, India.

Discussion

Surveillance and monitoring is the basic function of any organized mosquito control program, as it provides crucial data on the presence of vector species, level of infestation and seasonal abundance, based on which control programs can be directed [32]. Indeed, without surveillance data, the implementation of mosquito control program practically would not only be difficult but would reduce the efficiency of any program [33]. In this study *Ae. Aegypti* population was found to be predominant among the three *Aedes* species observed in both indoor and outdoor surveillance. *Ae. Aegypti* and *Ae. Albopictus* are well known as potential vectors for transmission of arboviruses world-wide, while *Ae. Vittatus*, although capable of transmitting diseases, reports on its role in active disease transmission is limited [34]. In our surveillance, *Ae. Aegypti* population was found throughout the year representing slightly poor OI during the dry season from April to July. Interestingly, none of the ovitraps were found positive for *Ae. Albopictus* species in any of the study sites during the dry season from April to June. Notably, it is well known fact that presence and breeding habitat of *Ae. Albopictus* is more associated with vegetation area unlike *Ae. Aegypti*, which prefers to breed in and around the human periphery [35-36]. Our observations on the positive correlation between rainfall and ovitrap index for *Ae. Albopictus* may be due to their preferential breeding behaviour for mostly outdoor natural locations or habitats like tree holes and plants, including artificial containers with water. Earlier field surveillance studies on *Ae. Albopictus* have reported that average monthly container indices for outdoor collection were related to rainfall [37-39]. It was clear from the observations on the average number of adults recovered from the ovitraps that among the three species, the infestation rate of *Ae. Albopictus* and *Ae. Vittatus* population was very low in the study sites.

It was evident from the OI values, that *Ae. Aegypti* showed equal preference for both indoor and outdoor breeding. Similar studies on oviposition preferences of *Ae. Aegypti* in several suburban communities in Selangor have reported to possess equal preference for breeding in indoor and outdoor containers [40]. This equal preference for both indoor and outdoor breeding for *Ae. Aegypti* have an advantage to sustain breeding during all seasons and further indicates that there may be interspecies competition in outdoor for breeding in the natural environment [31, 40]. Although there was decrease in the OI during the summer season (i.e. April, May and June), *Ae. Aegypti* population was observed throughout the study period, this might be due to their breeding habit in manmade water storage containers, air coolers etc. Positive OI of *Ae. Aegypti* during all seasons indicates its ability to sustain population throughout the year and its non-dependence on rainy days, this is further supported by non-significant correlation between rainfall and OI. Studies suggest that *Ae. Aegypti* bites and rests indoors, while *Ae. Albopictus* bites both indoors and outdoors, and prefers to rest and breed outdoor [41]. This instinct behaviour of *Ae. Albopictus* to breed in outdoor natural breeding sites might have led to poor breeding in the ovitraps installed, leading to significantly lower OI compared to *Ae. Aegypti* population. Mixed breeding of *Ae. Aegypti* and *Ae. Albopictus* together was at a low frequency as indicated by OI in the surveyed villages.

It is important to emphasize that the *Ae. Aegypti* species was observed throughout the year in all the study villages, not

only revealing its potential to breed and proliferate around human, but the need for intervention, community awareness and efficient vector control program. Reports suggest that the entomological threshold level for ovitrap index as low as 10% for *Aedes* species in an area may indicate a possible risk for outbreak of dengue [33, 42]. Our findings reveal that OI for *Ae. Aegypti* greater than 10% except May and June 2014 during the pre-monsoon period with average OI of 8.2 and 7.4 respectively, indicating the risk of transmission and outbreak of disease in the surveyed villages. Studies on the use of visual larval surveillance system in comparison with ovitraping method to determine the presence of vectors have found ovitraping to be more sensitive than the visual inspection of larvae [19, 43-44]. Several reports suggest ovitraping method to be superior and convenient method compared to other methods of surveillance, to identify the vector population and infestation level [45]. In addition, to contributing information on the seasonal abundance of *Aedes* vector species, the present findings highlights the need for credible alternatives having potential to deliver and achieve effective vector control without posing risk to the environment for prevention of disease outbreak. Contemporary control approaches such as use of genetically-engineered OX513A *Ae. Aegypti* strain have potential to fill the gap of requisite alternative target-specific vector control tools [46].

Importantly, the present surveillance also intended to generate information on *Ae. Aegypti* population in sites under study, to explore suitable sites for evaluating OX513A strain following successful laboratory assessment of OX513A and regulatory approval for conducting open field trials in India [21]. OX513A strain is a self-limiting strain inserted with a dominant lethal gene construct, thus male adults of OX513A are intended to mate with female adults of *Ae. Aegypti* when released in the environment rendering mating events unsuccessful by passing a copy of lethal gene to all the offsprings leading to non-viable offsprings, with over 95% of the progeny dying before adulthood [46]. Our laboratory studies on life table characteristics, mating competition, insecticidal susceptibility tests and genetic variability studies based on COI gene in comparison with natural population of *Ae. Aegypti* have shown OX513A to be equally fit [21-23]. Earlier studies on open field releases of OX513A male adults have shown high levels of suppression of *Ae. Aegypti* population demonstrating its potential use in the integrated vector management [24-28]. Thus the present findings on abundance of *Ae. Aegypti* in the current study could possibly aid in selecting suitable sites for our future studies to demonstrate field performance of OX513A male adults and suppression of *Ae. Aegypti* population under open field conditions in India.

Conclusion

The study emphasizes that there is a greater invasion of *Ae. Aegypti* mosquito species in the human community due to its anthropophilic nature which calls for preventive measures to curtail disease transmission and epidemic outbreak. The information on the presence of vector species and its seasonal abundance in the rural areas could aid effective vector control at the right time. Further this study provides data pertinent for selection of sites for future studies to test genetically-engineered OX513A *Ae. Aegypti* strain under open field conditions within Indian environments with approval from regulatory bodies.

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