

Control of *Aedes aegypti* and *Ae. albopictus*, the vectors of dengue and chikungunya, by using pheromone C21 with an insect growth regulator: Results of multicentric trials from 2007–12 in India

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ABSTRACT

Background & objectives: *Aedes* mosquito control has gained much importance nowadays in view of rise in number of reported cases of dengue and chikungunya in India and other countries. In the present study, C21 attracticide (containing a pheromone and an insect growth regulator—IGR, developed by Defence Research and Development Establishment (DRDE), Gwalior, India) was tested for its feasibility for surveillance and control of *Aedes* mosquito in a multicentric mode from October 2007 to June 2012 in urban (Delhi, and Bengaluru district, Karnataka) and suburban (Alappuzha district, Kerala) settings of the country in three phases.

Methods: Across the randomly selected households in each study area, two to four containers treated with attracticide (experimental) and untreated (control) were placed and monitored by trained surveillance workers on weekly/fortnightly basis for determining the presence of eggs, larvae and pupae. Container positivity, percent larvae, egg and pupae collected were determined during different phases and analyzed statistically using SPSS 18.0.

Results: Container positivity was found statistically significant at Bengaluru and Alappuzha, Kerala while in Delhi, it was found non-significant. Eggs collected from experimental containers were significantly higher in comparison to control at all the locations except Delhi. Also larvae collected from control containers were significantly higher at all the locations except Bengaluru. Pupae collected from control containers remained significantly higher at all the locations as no pupal formation was recorded from experimental containers.

Interpretation & conclusion: The use of C21 attracticide hampered pupal formation, thus inhibiting adult population in the study areas. The study established that C21 attracticide was efficacious in the field conditions and has potential for use in surveillance and management of dengue and chikungunya mosquitoes.

Key words Attracticide; C21; chikungunya; dengue; surveillance

INTRODUCTION

Dengue is caused by dengue viruses of the family *Flaviviridae* and is transmitted by bites of *Aedes* (*Stegomyia*) mosquitoes¹⁻². Dengue presents a spectrum of severe clinical manifestations ranging from classic dengue fever (DF) to dengue haemorrhagic fever (DHF) to dengue shock syndrome (DSS). Annually, around one million confirmed dengue cases are being reported by the World Health Organization (WHO) worldwide³. In India, during 2001–06, dengue cases increased from 3306 to 12,317⁴.

Chikungunya is a mosquito-borne viral infection caus-

ing fever, rash and arthralgia⁵. Chikungunya is transmitted by single-stranded RNA alpha virus from the family *Togaviridae* and is almost exclusively transmitted by *Aedes* mosquitoes. As per WHO estimates, chikungunya has been identified in nearly 40 countries including India⁶. Different parts of the country witnessed series of focal outbreaks and minor epidemics of chikungunya fever during 1963–73⁷⁻¹⁰. After nearly three decades, outbreak of chikungunya occurred during 2006, reporting >1.39 million suspected cases affecting 210 districts in 13 states of India¹¹.

Ae. aegypti is the common epidemic vector for dengue and chikungunya virus. Other species such as *Ae.*

albopictus, *Ae. scutellaris* complex and *Ae. (Finlaya) niveus* have also been incriminated as secondary vectors for dengue and chikungunya which are generally considered less efficient epidemic vector than *Ae. aegypti*¹².

Surveillance and control of mosquito vector is very important for preventing transmission of dengue and chikungunya. Direct sampling of the adult population involves manual method of hand-catch in which a systematic search is made through homes and vacuum up all mosquitoes found using aspirators. The number of mosquitoes caught depends upon the experience and enthusiasm of the catchers and is therefore difficult to standardize. Further, due to the resting behaviour (rests under table/sofa/bed/cot *etc*), catching of *Aedes* mosquito becomes difficult. Additionally, larval sampling requires active search for breeding sites plus laboratory identification of immature larval stages is again very labour-intensive and poor indicator of the vector population density. Moreover, *Ae. aegypti* population rise during rains as breeding sites increase manifold due to water logging, thus resulting in increased level of incidence¹³⁻¹⁴.

Application of pheromones in the fields of agriculture, forestry, and urban pest management is well documented¹⁵. Major uses of pheromones in the integrated pest management (IPM) of the insects have been reported¹⁶. The most important use is in monitoring a population of insects to determine if they are present or absent in an area. Another major use of pheromones is to mass trap insects to remove large number of insects from breeding and feeding cycle in order to protect resources for human use. In this reference, aggregation pheromones can be of particular use which may lure many female insects to lay eggs at the same site resulting in mass egg production and reduction¹⁷.

The Defence Research and Development Establishment (DRDE), Gwalior isolated and identified an oviposition attractant pheromone, C21 hydrocarbon (strength—10 ppm with 7–10 days dissipation period) from the larval conditioned water of *Ae. aegypti*. Further, the attractant combined with IGR compound, produced a compounding effect of luring and killing. The attracticide was made keeping in view that the oviposition attractant should not influence the efficacy of IGR which inhibits the growth of larvae by hormonal imbalance and the IGR should not mask the oviposition attractant property of C21 compound. Laboratory evaluations of this attracticide have been reported to be effective in luring adults of *Ae. aegypti* to oviposit more number of eggs and inhibiting the larval growth into adult¹⁸.

Delhi, Karnataka and Kerala states are endemic for dengue and chikungunya. As per the data obtained from National Vector Borne Disease Control Programme (NVBDCP), in Delhi, dengue cases increased from 322

in 2001 to 3366 in 2006; in Karnataka from 220 in 2001 to 587 in 2005 and in Kerala from 41 in 2001 to 981 in 2006¹⁹. Chikungunya exhibited declining trend in all these places but may escalate in future whenever suitable ecological conditions prevail. Initially, small-scale field trials of attracticide carried out in Delhi and Kerala were found to be effective for surveillance and control of *Aedes* mosquitoes. Based on the encouraging results of the trials, multicentric trials were initiated in urban (Delhi and Bengaluru) and suburban (Alappuzha, Kerala) pockets of the country in three phases from 2007 to 2012 to test the feasibility of the attracticide compound for surveillance and control of *Aedes* mosquitoes.

MATERIAL & METHODS

Study site

The study sites in Bengaluru, Karnataka and Alappuzha, Kerala were covered by the National Institute of Malaria Research (NIMR) Field Units (FUs) while Delhi site was covered by NIMR Head Quarter (HQ). Randomized extensive entomological surveys in dengue and chikungunya affected areas of Delhi, Bengaluru and Alappuzha were conducted to collect baseline information, *e.g.* percent positivity/breteau index (BI). Collected baseline information helped further selection of localities. In Delhi, the highest percent positivity was recorded from Netaji Nagar (100%), Railway Colony in Tuglakabad (50%), Netaji Nagar–D Block and CPWD Colony (35.71%), Mayurkunj and Trilokpuri (33.33%), Valmiki Colony and Pachkuian Road (9.30%). In Bengaluru, the highest BI was recorded from Narayanpura and Sanjay Gandhinagar (23.74%) followed by Kanteerava Nagar (20.52%) and Ashok Nagar (19.32%), whereas in Alappuzha, the highest BI was recorded from Kadakkarapally (71.43%), Muhamma (59.26%), Ambalapuzha (52.6%) and Vettackal (46.67%) localities.

Based on the collected baseline data, the localities in the selected study sites were decided by a team of investigators from DRDE Gwalior, NIMR Delhi, NIMR (FUs) at Chennai and Bengaluru, Municipal Corporation of Delhi (MCD) and National Vector Borne Disease Control Programme (NVBDCP) and State Health Departments. The study was initiated in five localities of Delhi, namely Mayurkunj (Trilokpuri), Valmiki Colony (Panchkuian Road), Netaji Nagar, R.K. Puram (Sector 2 and 3) and Railway Colony (Tuglakabad) in October 2007; three localities of Bengaluru, namely Ashok Nagar, Kanteerava Nagar and Narayanpura and Sanjay Gandhi Nagar in January 2008 and three localities of Alappuzha

district of Kerala, namely Muhamma, Kadakkarapally and Vettackal in April 2008.

Duration

The study was conducted in three phases: Phase-1 (October 2007–March 2009), Phase-2 (October 2009–December 2010) and Phase-3 (April 2011–March 2012). During phase-1, eggs were counted while during phase-2 larvae were also counted. During phase-3, pupae were recorded along with eggs and larvae.

Target species

In Delhi and Bengaluru, experiments were conducted against *Ae. aegypti*, whereas in Alappuzha, Kerala, against *Ae. albopictus* (the most dominant species).

House selection

Single storey (suburban), 2–3 storey (urban) and multiple storey (urban) houses were considered.

Placement of containers

Containers were prepared using white bowls of 500 ml capacity. Oviposition pheromone named C21 (25.22 g) and IGR (0.32 g) compounds received from DRDE, Gwalior were mixed thoroughly in 1800 ml *n*-hexane and 200 ml acetone and kept as stock solution. Exactly, 396 ml of water was added to 4 ml of the above stock solution making a total volume of 400 ml, which was considered as experimental container. A separate stock solution of 1800 ml of *n*-hexane and 200 ml of acetone alone was also prepared. The control container consisted of 396 ml of water with 4 ml of this stock. The containers were placed in pair (one experimental and one control) within a distance of 1.5–2 ft indoor and outdoor in shaded condition.

Across randomly selected households in each study area, 2–4 containers were placed inside and outside the houses, *i.e.* bedrooms, living rooms, verandahs, bathrooms, motor rooms and in sheds adjacent to the houses. Householders were requested to help recording the eggs/immatures and also not to disturb the placed containers. After recording the data, containers were cleaned and re-

treated with attracticide as mentioned above at weekly and fortnightly intervals and data was pooled up on monthly basis.

Bengaluru: A total of 3043, 531 and 360 houses were visited per week during phase-1, phase-2 and phase-3 respectively. One experimental and one control container was placed in each house.

Delhi: A total of 3100, 249 and 244 houses were visited per week during phase-1, phase-2 and phase-3 respectively. During phase-1, 3312 containers were placed in experimental and control, whereas during phase-2 and phase-3, 249 and 244 containers were placed respectively.

Alappuzha, Kerala: A total of 1421, 221 and 222 houses were visited per week during phase-1, phase-2 and phase-3 respectively. During phase-1, 2993 containers were placed in experimental and control, whereas during phase-2 and phase-3, 221 and 222 containers were placed respectively.

Monitoring of containers and cross-checking

Containers (both experimental and control) were monitored by trained surveillance workers under the supervision of supervisors on weekly basis at Delhi and Bengaluru, and on fortnightly basis in Alappuzha as the houses were far away and sparsely located. Number of houses and containers placed in study area are described in Table 1.

Community participation

Meetings were organized at selected sites with local community and they were informed about the experiment to be carried out in their premises. Posters and pamphlets in English and Hindi were also distributed in the area to create awareness among people. Community consented and extended cooperation, and participated in the study with enthusiasm.

Data analysis

Surveillance workers recorded the observations as number of eggs laid, number of larvae and pupae present in experimental and control containers with weekly/fort-

Table 1. Number of houses and containers placed in study area

S.No.	Locality	Phase-1		Phase-2		Phase-3		Total	
		Houses	Containers	Houses	Containers	Houses	Containers	Houses	Containers
1.	Bengaluru	3043	6086	531	1062	360	720	3934	7868
2.	Delhi	3100	6624	249	498	244	488	3593	7610
3.	Alappuzha	1421	5986	221	442	222	444	1864	6872
	Total	7564	18696	1001	2002	826	1652	9391	22350

nightly re-treatment and replacement of containers. Positive containers were determined only on the basis of visual inspection of presence of eggs/larvae using 10x magnifier hand lens. Cross-checking of the results at various sites was done by a team from DRDE and NVBDCP along with NIMR members. Container positivity and percent positivity are described in Table 2 for Bengaluru, Delhi and Kerala. Number of eggs, larvae and pupae collected from experimental and control containers during different phases are described in Table 3. Student's *t*-test was attempted in SPSS 18.0 to compare this data. Month-wise data from experimental and control containers are presented in Figs. 1–3 for Bengaluru, Delhi and Kerala.

RESULTS

Bengaluru

A total of 3,76,045 containers were placed, out of which 8223 containers were found positive in which 5571 (68%) were experimental and 2652 (32%) were control, which gave percentage positivity of 2.96 and 1.41% respectively (Table 2). Statistical analysis indicated that

there was a significant difference in positivity in experimental and control containers ($p < 0.05$).

Table 3 reveals that a total of 1,37,380 eggs were collected from positive containers, out of which 99758 (73%) were in experimental and 37,622 (27%) were in control containers. Statistical analysis indicated that there was a significant difference in number of eggs in experimental and control containers ($p < 0.05$). A total of 1510 larvae were collected from positive containers, out of which 1051 (70%) were in experimental and 459 (30%) were in control containers. Also there was a significant difference in number of larvae collected from experimental and control containers ($p < 0.05$). No pupa was recorded from experimental containers and the difference was found statistically significant from control containers ($p < 0.05$).

Seasonal prevalence of positivity, number of eggs, larvae and pupae in both experimental and control containers in Bengaluru are given in Fig. 1, which indicated June and July as the peak prevalence months.

Delhi

A total of 2,74,332 containers were placed, out of which 3789 containers were found positive in which 1710

Table 2. Positivity and percent positivity of containers in study sites

Locality	Containers	Phase-1		Phase-2		Phase-3		Total	
		Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control
Bengaluru	Checked	155755	155764	22223	22223	10040	10040	188018	188027
	Positive	4144	1762	1013	551	414	339	5571	2652
	% Positive	2.66	1.13	4.56	2.48	4.12	3.38	11.34	6.99
Delhi	Checked	111324	111324	12829	12829	13013	13013	137166	137166
	Positive	1203	1746	423	286	84	47	1710	2079
	% Positive	1.08	1.57	3.30	2.23	0.65	0.36	5.02	4.16
Alappuzha	Checked	52978	53010	15941	15941	15300	15300	84219	84251
	Positive	10792	11360	5171	4291	3154	2365	19117	18016
	% Positive	20.37	21.43	32.44	26.92	20.61	15.46	73.42	63.81

Table 3. Number of eggs, larvae and pupae collected from immature stages of mosquito in study sites

Locality	Immature stages of mosquito	Phase-1		Phase-2		Phase-3		Total	
		Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control
Bengaluru	Egg	59174	17380	24202	9112	16382	11130	99758	37622
	Larvae	–	–	447	288	604	171	1051	459
	Pupae	–	–	–	–	0	138	0	138
Delhi	Egg	20788	30811	26918	9748	3877	1584	51583	42143
	Larvae	–	–	219	1248	499	594	718	1842
	Pupae	–	–	–	–	0	426	0	426
Alappuzha	Egg	121198	64047	146994	81213	77757	39466	345949	184726
	Larvae	–	–	0	3013	271	7510	271	10523
	Pupae	–	–	–	–	0	5529	0	5529

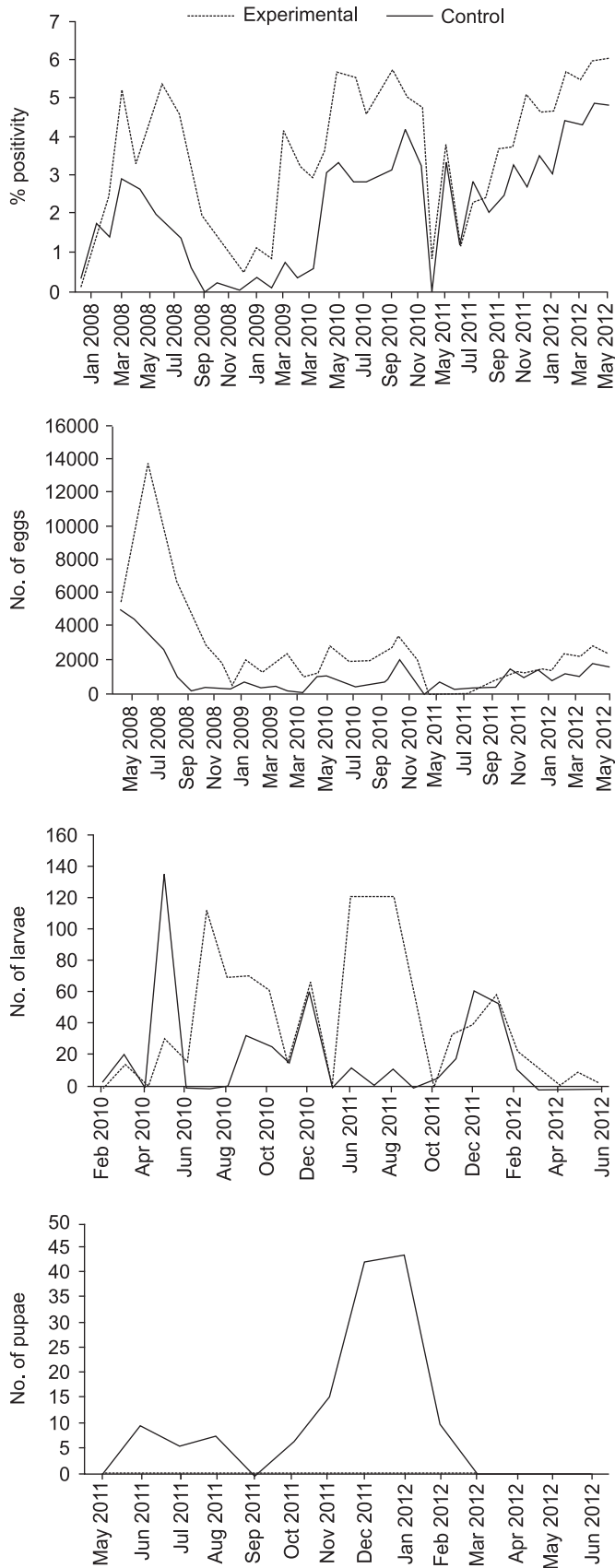


Fig. 1: Month-wise percent positivity, eggs, larvae and pupae in Bengaluru.

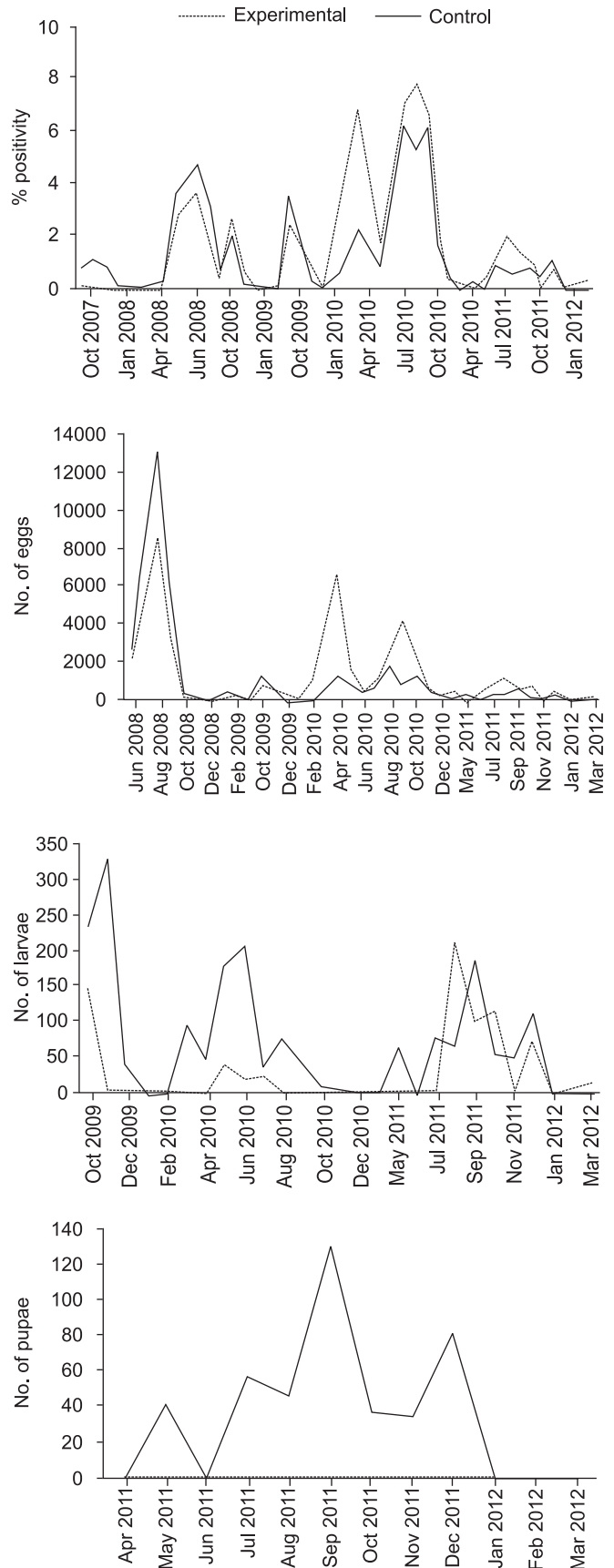


Fig. 2: Month-wise percent positivity, eggs, larvae and pupae in Delhi.

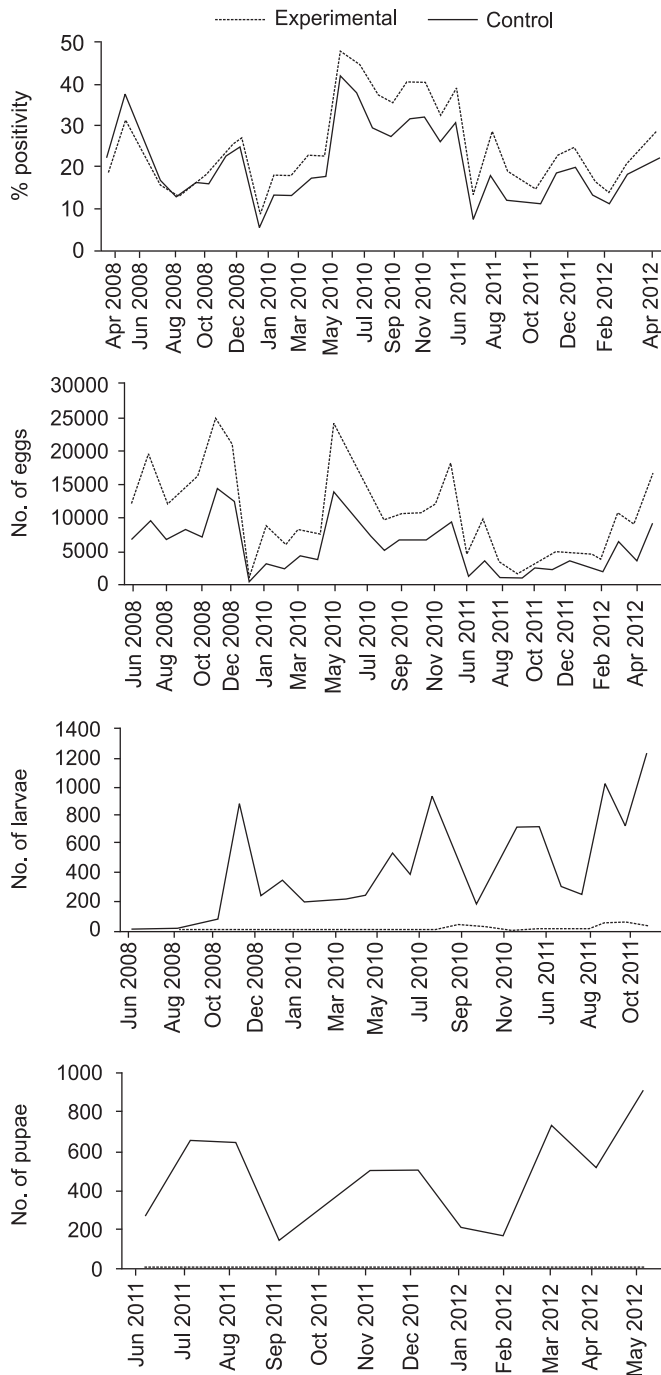


Fig. 3: Month-wise percent positivity, eggs, larvae and pupae in Alappuzha, Kerala.

(45%) were experimental and 2079 (55%) were control which gave percentage positivity of 1.25 and 1.52% respectively (Table 2). Statistical analysis revealed that there was no significant difference in positivity of experimental and control containers ($p>0.05$).

Table 3 reveals that a total of 93,726 eggs were collected from positive containers out of which 51,583 (55%) were in experimental and 42,143 (45%) were in control. Though more number of eggs were laid in experimental

containers but difference was not found statistically significant ($p>0.05$). A total of 2560 larvae were collected from positive containers, out of which 718 (28%) were in experimental and 1842 (72%) were in control containers. Significant difference was found in number of larvae collected from control in comparison to experimental containers ($p<0.05$). No pupa was collected from experimental containers which indicated significant difference in number of pupae in control in comparison to experimental containers ($p<0.05$).

Seasonal prevalence of positivity, number of eggs, larvae and pupae in both experimental and control containers in Delhi are given in Fig. 2, which indicated August and September as the peak prevalence months.

Alappuzha, Kerala

A total of 1,68,470 containers were placed, out of which 37,133 containers were found positive in which 19,117 (51%) were experimental and 18,016 (49%) were control which gave percentage positivity of 22.70 and 21.38% respectively (Table 2). Statistical analysis indicated that there was a significant difference in positivity of experimental and control containers ($p<0.05$).

Table 3 reveals that a total of 5,30,675 eggs were collected from positive containers, out of which 3,45,949 (65%) were in experimental and 1,84,726 (35%) were in control. Statistical analysis indicated that there was a significant difference in number of eggs in experimental and control containers ($p<0.05$). A total of 10,794 larvae were collected from positive containers, out of which 271 (3%) were in experimental and 10,523 (97%) were in control containers. Significant difference was found in number of larvae collected from control in comparison to experimental containers ($p<0.05$). No pupa was collected from experimental containers indicating difference being statistically significant from control containers ($p<0.05$).

Seasonal prevalence of positivity, number of eggs, larvae and pupae in both experimental and control containers in Kerala are given in Fig. 3, which indicated May as the peak prevalence month.

DISCUSSION

Similar to specialized containers used in the study, studies conducted using ovitraps (water-filled devices to attract female mosquitoes to lay eggs) in other parts of the world provided useful results. Ovitrap surveillance of dengue and chikungunya vectors helped to determine the distribution and abundance of both *Ae. aegypti* and *Ae. albopictus* for planning anti-*Aedes* campaign in several

suburban residential areas of Peninsular Malaysia²⁰. Ovitrap had been used to provide useful data for *Aedes* control operations in a Texas coastal county and Paya Lebar International Airport of Singapore²¹⁻²².

In the present study, it was observed that maximum container positivity was found in Kerala (22.04%) followed by Bengaluru (2.19%) and Delhi (1.38%). It was also observed that the positivity of experimental containers was significantly higher than control in Bengaluru ($p < 0.05$) and Alappuzha, Kerala ($p < 0.05$) while in Delhi, positivity of control containers was higher than experimental but the results were non-significant ($p > 0.05$). Number of eggs collected was higher in experimental containers than control at all locations including Delhi but the difference was non-significant ($p > 0.05$) at Delhi. Another observation was that the number of larvae collected was significantly higher in control at all the study sites except in Bengaluru. In experimental containers, there was no formation of pupa whereas in control, late instar larvae and pupae were found, hence, there was no emergence from the experimental containers, while from control, adults emerged. It indicated that IGR present in attractant played role in inhibiting the development of larvae in experimental containers.

The attracticide used in the study was found luring the adult *Aedes* mosquitoes for egg laying and no formation of pupa was found at all the three sites. However, variations in the results were found in terms of positivity, egg laying and larval formation due to the other factors, like availability of alternative breeding sites in vicinity, dissimilar physical surroundings of the containers in houses, change in temperature, rainfall and humidity, etc. for which further studies are required. The strength of the present study stems from the community who participated whole heartedly in the experiment and allowed the surveillance workers to keep containers inside their houses, provided water for cleaning and preparing the containers.

CONCLUSION

The overall results of the study indicate that attracticide can play an important role in surveillance of *Ae. aegypti* and *Ae. albopictus*, the vectors of chikungunya and dengue; and their management and control on long-term basis, by reducing the next generation mosquitoes. The operational difficulties were faced due to liquid formulation of C21 with regard to transportation, storage, measurement and implementation of dose, etc. To get better results, it is recommended that delivery system of the attracticide formulation need to be improved for easy dis-

persing in field and preferably tablet formulation would be a better option.

Conflict of interest

The study was funded by Ministry of Health and DRDE, Gwalior. However, they have neither interfered in designing nor influenced the execution of the study.

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