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Breeding ecology of Asian tiger mosquito (*Aedes albopictus)* in rubber plantation area of central Kerala

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Abstract
The Asian tiger mosquito, *Aedes albopictus* acts as vector of many diseases. It is widely distributed in Kerala and has served as a primary vector of Dengue Fever and Chikungunya. Vector control is the most effective solution for the prevention of mosquito-borne diseases. Any vector control measure should be based on a sound knowledge of the breeding ecology of vector species. An intensive survey was carried out for the breeding sites of vector mosquito in the selected study area. About 2093 potential breeding sites were observed during the survey, out of which 652 were found to be positive for *Aedes albopictus.* The main habitat exploited by *Aedes albopictus* for breeding was the latex collecting cups. Highest Breeding Preference Ratio was observed in discarded tyres (2.1). The overall House Index (56.01), Container Index (28.13) and Breteau Index (150.93) were also calculated.

Keywords: *Aedes albopictus,* house index, container index, breteau index

Introduction
Many species of mosquitoes act as vectors of various diseases. The Asian tiger mosquito, *Aedes albopictus* is reported to be a competent vector for about 22 arbo-viruses (Gratz, 2004) [10]. The species, originally considered a secondary vector of Dengue virus, has been shown to play a significant role in the transmission of Chikungunya virus and Dengue virus in several countries (Gratz, 1999, 2004, WHO, 1999; Jupp and McIntoch, 1988, Paupy *et al.,* 2009) [9, 10, 31, 20, 23]. The recent episode of Dengue Fever (DF) and Chikungunya (CG) outbreaks have brought to light the importance of *Ae. Albopictus* as primary vector in many parts of the world (Paupy *et al.,* 2009) [23]. The available literature shows that *Ae. Albopictus* is widely distributed in Kerala and has served as a primary vector of DF and CG. *Ae. Albopictus* is indicated as the major vector of various arbo-viruses in Kerala (Kalra and Prasittisuk, 2004; ICMR, 2006) [21, 18].

Vector control is the most effective solution for the prevention of mosquito-borne diseases. Breeding source reduction is recognised as the best vector control method. So any vector control measure in an area should be based on a sound knowledge of the larval ecology of vector mosquito in that area. *Ae. Albopictus* is originally a forest species, where it principally breeds in tree holes, leaf axils or in rock pools and similar sites (Barraud, 1934; Hawley, 1988) [2, 12]. Widespread deforestation, climate change and increase in global trade have forced this mosquito to adapt itself to breeding in domestic and semi-domestic artificial container habitats (Gubler *et al.,* 2001; Delatte *et al.,* 2008) [11, 6]. The habitat review reveals that *Ae. Albopictus is* an opportunistic breeder. It has been found to breed in any available habitat which contains some amount of water. Availability of breeding habitat in an area is closely associated with social practices, agriculture practice and ecology of that area. The present study has arisen largely because of the emergence of various Aedes-borne diseases, the inadequate knowledge on larval ecology of *Ae. Albopictus* and the agricultural practices in the study area.

Materials and Methods
Study area
Mundakkayam Panchayat (latitude 9°33' N a longitude 76 °53' E), belonging to Kottayam district was selected for the present Study. Selection of study area was purposive, based on the previous history of vector borne diseases. In Kerala Dengue Fever was first reported from this Panchayat. Heavy incidence of CG was experienced in 2006 and 2007. Panchayat has an area

of 82 sq.km. As of 2011 India census, the Panchayat has a population of 38,445 and has an average literacy rate of 94%. Rubber plantation is the main agriculture. Besides some estates (above 20 hectare), rubber plantation in this area is mainly owed by small scale farmers (mostly below 2 hectares per owner). They live in the midst of the small-scale rubber plantation they own. (Mundakkayam Panchayat, 2012) [22].

Design of study

Two year sample survey was carried out from February 2009 to January 2011 to understand the breeding ecology of *Aedes albopictus*. Each calendar year was divided into three seasons such as pre-monsoon (February to May), monsoon (June to September) and post-monsoon (October to January).

Samples were drawn from three randomly selected wards of Mundakkayam panchayat. A house was considered as a unit for the survey and every 10th house was selected for the study. The nearby house was considered in case of any inconvenience. Eighteen houses were sampled in each month (six houses each from each ward) and a total of 72 houses were sampled in a season per year

Fig 1: Map of Kottayam showing the study area (in circle)

Sampling technique

In each survey, peri-domestic areas of houses were searched for breeding places of *Aedes*es per the guidelines of WHO, (1999) [31]. All the habitats were enumerated up to 15 m and classified according to the type such as latex collecting cups, coconut shells, tree holes, plant stumps, plastic containers, metallic containers, leaf axils, mud pots, flower pots, tyres, cement tanks, water storage containers/tanks and discarded containers. Minor habitats such as coca pod, grinding stones, rubber fruit shells, other discards, etc. were grouped together as 'others' for the analysis. Numbers of both the potential and positive habitats (habitat with Aedes larvae) were recorded. Larvae were collected from each positive breeding habitat in properly labelled containers, separately. The collected larvae were transported to laboratory and reared to adulthood. The emerged adults were identified using standard light microscopy and relevant taxonomic references Huang, 1979 [16]; Das *et al.*, 1990 [5]; Das and Kaul, 1998 [4]. Observations were recorded on a data sheet.

Analysis of Data

Raw data were analysed using descriptive statistics (SPSS). Standard larval indices like House Index (HI), Container Index (CI) and Breteau Index (BI) were calculated by the standard procedure (WHO, 1999) [31]. Following formula was used for calculating the various indices.

$$HI = \frac{\text{No of houses positive for Aedes larvae}}{\text{No of houses inspected}} \times 100$$

$$CI = \frac{\text{No.of containers positive for Aedes Larvae}}{\text{No.of containers inspected}} \times 100$$

$$BI = \frac{\text{No.of containers positive for Aedes larvae}}{\text{No of houses inspected}} \times 100$$

The container preference of *Ae. Albopictus* breeding was assessed by calculation of breeding preference ratio (BPR) (Sharma, 2002) [26]. Observations were tabulated and suitable graphical depictions were prepared using Excel software for Microsoft Office.

Results

Breeding ecology

The potential breeding habitat observed during the study include latex collecting cups, plastic containers, coconut shells, metallic containers, mud pots, plant pots, tyres, tanks, leaf axils, tree holes and other discards, etc. (Table 1).

Table 1: Breeding habitats and breeding preference ratio (BPR) of *Ae. Albopictus* in the study area

Breeding sites	Observed	(X %)	Positive	(Y %)	BPR(Y/X)
Latex collecting cups*	759	36.3	237	36.3	1
Discarded Coconut shells	206	9.8	40	6.1	0.6
Plastic containers	245	11.7	67	10.3	0.9
Metallic containers	151	7.2	54	8.3	1.1
Mud pots	70	3.3	41	6.3	1.9
Plant pots	98	4.7	10	1.5	0.3
Tyres	57	2.7	37	5.7	2.1
Tank	81	3.9	21	3.2	0.8
Leaf axils**	112	5.4	18	2.8	0.5
Tree holes	73	3.5	36	5.5	1.6
Others***	241	11.5	91	14	1.2
Total	2093	100	652	100	

*plastic cups, coconut shells, metallic tin fastened to rubber trees
**pineapple, plantain, areca nut, etc.
***grinding stones, rubber fruit shells, coco pods and other discards.
X%-Percentage of potential habitats; Y%- Percentage of positive habitats for Aedes.

A total of 2093 water holding containers were observed during the period of study, out of which 652 were positive. Latex collecting cups were the most commonly occurring water holding container constituting about 36.3% of the total containers observed, followed by plastic containers (11.7%), coconut shells (9.8%), metallic containers (7.2%) and so on (Table 1).

Of the 2093 water holding containers observed 652 (31%) were positive for *Ae. albopictus*. By number latex collecting cup was the major breeding sites positive (237), followed by plastic containers (67), metallic containers (54), mud pot (41) and discarded coconut shells (40). Other breeding sites include the discarded grinding stones, tree holes, leaf axils, fallen leaves of coconut and areca plant, cocopods, etc. (Table 1).

In the present study the breeding habitat preference of *Ae. Albopictus* was analysed and presented in terms of Breeding Preference Ratio (BPR) (Table 1). Among all the habitats, highest breeding preference was recorded in tyre (2.1), followed by mud pots (1.9) tree holes (1.6), coconut shell (1.1), latex collecting cups (1) and plastic container (0.9) (Fig 3).

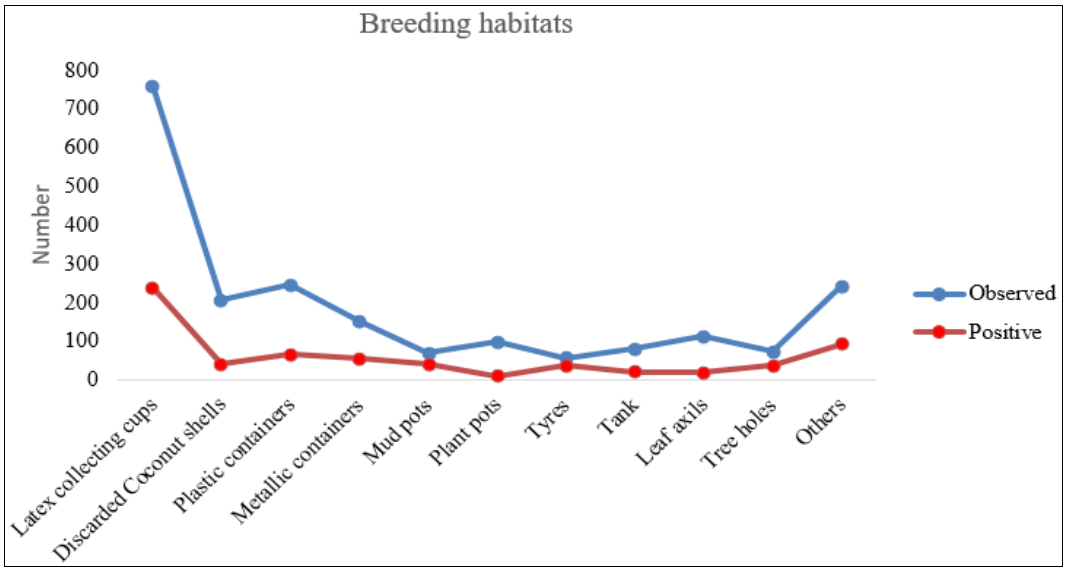

Fig 2: Number of habitat observed vs habitat positive for *Ae. Albopictus*

Larval Indices

A total of 432 houses were surveyed for *Ae. Albopictus* breeding. Breeding was observed in 242 houses with an overall House Index of 56. HI was found to be high during monsoon season (June to September), followed by pre-monsoon (February to May) and post monsoon (October to January) (Table 2).

CI also varied from season to season. A high over all CI of 36.90 was recorded during monsoon, followed by pre-monsoon (28.55) and post-monsoon (18.85) (Table 2). The overall BI was estimated as 150.93. Seasonal BI varied from 38.20 to 211.80. Highest BI was observed during pre-monsoon season (211.80), followed by monsoon (202.8) and post monsoon (38.2) (Table 2).

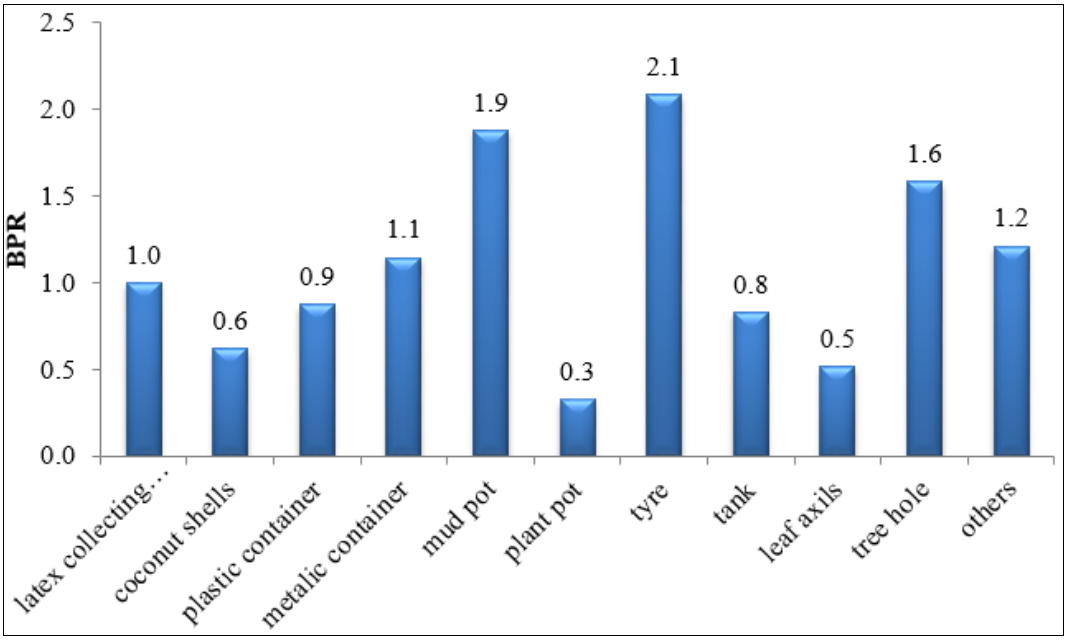

Fig 3: Breeding Preference Ratio for *Ae. Albopictus*

Table 2: Larval Indices of *Ae. Albopictus* in different seasons

Season	HI (Average)	CI (Average)	BI (Average)
Pre-monsoon	63.20	28.55	211.80
Monsoon	80.55	36.90	202.80
Post-monsoon	24.30	18.85	38.20
Over all larval Indices	56.01	28.10	150.93

Discussion

The present study gives the extent of invasion of *Ae. Albopictus* with respect to breeding.

Breeding ecology

The selection of breeding sites by mosquitoes is a critical factor for mosquito survival and has important implications for mosquito control. Diverse potential breeding habitats were observed in the study area and Aedes species exhibited high degree of adaptive flexibility to various breeding habitats (Table 2). *Ae. Albopictus* exploited all types of available potential breeding habitats (Fig. 2). The main habitat exploited by *Ae. Albopictus* for breeding was the latex collecting cup (36.3%), followed by 'others' (other discards) (14.5%), plastic container (10.3%) and so on (Fig 3).

Present observation supported previous finding that *Aedes* mosquitoes breed in small natural and artificial water holding containers (Hiriyan *et al.*, 2003) [14]. *Ae. Albopictus* is primarily a forest-fringe species breeding in natural habitat like tree holes, leaf axils, cut bamboo stumps, rock pools (Juliano and Lounibos, 2005; Hawley, 1988) [18, 12]. In the present study breeding of *Ae. Albopictus* was observed in leaf axils of many plants *viz.*, pineapple, banana, primary rachis of coconut palm and fallen leaves of areca palm. Study conducted by Eapen (2010) [8] in different districts of Kerala showed the breeding potential of *Ae. Albopictus* in leaf axils of plants such as pineapple, banana, coconut palm etc. They reported maximum breeding in leaf axils of pine apple (80%), followed by flowering plants (7.8%), screw pine (5%), coconut palm (5%) and banana plants (1.45%). Profuse breeding of *Ae. Albopictus* in shed leaf sheath of areca nut palms was reported earlier (Regu *et al.*, 2008) [23]. Breeding in cocopods was also reported from Kerala (Hirian and Tyagi, 2004) [13]. The breeding of Aedes in leaf axils and tree holes poses a serious challenge to vector control.

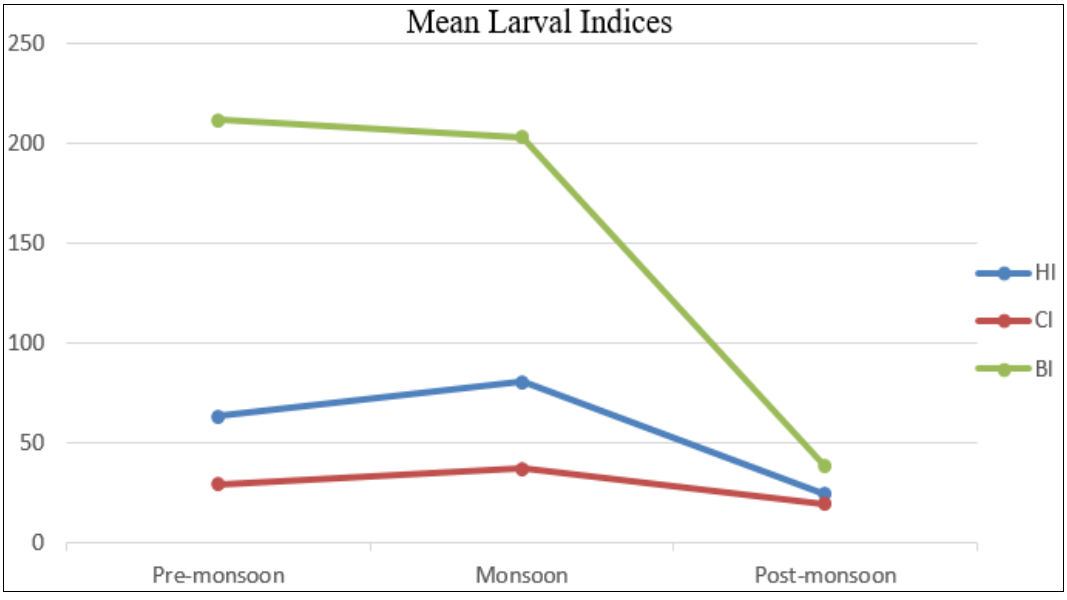

Fig 4: Larval Indices of *Ae. Albopictus* in different seasons

The vector was well-adapted for breeding in artificial containers as well. Latex collecting cups and discarded plastic cups were the key breeding habitats of *Ae. Albopictus*. Breeding of *Ae. Albopictus* in containers and latex collecting cup of rubber plantation of Kerala is well documented (Thenmozhi *et al.,* 2007; Sumodan, 2003) [27, 26]. Hiriyan *et al.,* (2003) [14] reported breeding of the mosquito in plastic cups around tea vendor shops in Ernakulum city Kerala. Increased usage of plastics the world over has created innumerable non-biodegradable habitats for this ecologically adaptive mosquito. Lack of proper disposal mechanisms for plastic waste, and indiscriminate dumping practices; aggravate the problem in developing countries. Replacing plastics with eco-friendly materials, recycling and discouraging their use by proper health awareness programs should be helpful in reducing breeding sites.

The vector was also capable of breeding in discarded coconut shells and tyre. Coconut shells discarded or dumped around the house collect water during rainy season and form an ideal ground for Aedes breeding (Tyagi and Das, 2006) [28]. The role of tyre in the dispersion of Aedes across the world was well studied. Present study recorded the breeding of *Ae. Albopictus* in cow dung pit and drains. Other breeding sites recorded in the study area were glass, egg shells, grinding stones, etc.

In the study, cups used for collecting latex in rubber plantation were found to be the dominant breeding habitat (Table 1). However discarded tyre had the highest positivity for *Ae. Albopictus*, followed by mud pot, tree hole etc. (Table 1). A positive rate of one was seen in latex collecting cups. Results of the present study supported previous finding that *Ae. Albopictus* breeds in all available water holding containers.

Larval Indices

Table 2 shows the various Aedes larval indices recorded during the study period. The CI, HI and BI were found to be higher than the normally accepted limit (WHO, 1999) [31]. HI for *Ae.albopictus* in all three seasons were greater than 10%, which indicated high risk of aedes-borne disease transmission in these areas. In the study area, larval indices like HI and CI were found to be high during monsoon season. Availability of breeding habitats in peri-domestic area and rubber plantations resulted in higher larval indices during rainy season. Present results correlated with the findings of various researchers (Christophers, 1960) [3]. In the present study, contrary to many previous observations all the larval indices of pre-monsoon season were found to be higher than that of post-monsoon season (Fig.4) the rise in larval indices during the pre-monsoon season was due to the intermittent summer rain that occurred during February- May months. Discarded and, or unprotected containers especially latex collecting cups collect rain water and forms breeding sites for mosquitoes. In many rubber plantations taping is traditionally suspended during February /March months. So that water collected in latex collecting cups may remain long for the completion of many life cycles of vector mosquito. People are lazier in source reduction during pre-monsoon as there is no serious consequence of vector borne diseases at that time. Serious biting nuisance, reports of outbreaks of vector borne diseases and awareness or source reduction programme of governments prompt many people to practice source reduction.

According to WHO (1999) [31], an area can be treated as a 'High Risk Transmission Place' for Dengue virus /Chikungunya virus when the HI and CI are higher than 5 and BI higher than 20. In the study area all the three larval indices are higher than the threshold level, during most of the months. So study area can be treated as a high risk transmission place for Dengue virus /Chikungunya virus, provided the vector *Ae. Albopictus* is viraemic.

Conclusion

Present study indicated the spread and depth of infestation of *Ae. Albopictus* in the study area. Extensive cultivation of rubber plants and pineapple in central Kerala provide suitable situation for proliferation of *Ae. Albopictus*. There were no reports on the existence of *Ae. albopictus* in Kerala till 1980's (ICMR, 2003; Tyagi *et al.,* 2006) [16, 28]. Environmental changes, including urbanization, widespread deforestation, agriculture practices, culture of people, improved mode of transportation etc., are considered the most important factors in the spread of *Ae. Albopictus* (Dutta *et al.,* 1998; Alto and Juliano, 2001; Gubler *et al.,* 2001: Vora, 2008; Roiz *et al.,* 2011) [7, 1, 11, 29, 24]. Larval indices of *Aedes albopictus* have been recorded above the critical levels and it implies their potential for future outbreaks. In this context one can't reject the possibility of occurrence of any arboviral diseases, even Yellow Fever, West Nile disease, etc. which are present in other parts of the world. In order to contain the occurrence of Aedes-borne diseases, entomological surveillance should be undertaken effectively in the state and the information should be utilized to forecast the possibility of future outbreaks of *Aedes albopictus*-borne arboviral diseases, so that necessary control measures could be undertaken to avoid any *Aedes albopictus* –borne arboviral outbreak in future.

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