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SEASONAL VARIATIONS AND PHYSICOCHEMICAL CHARACTERISTICS OF THE HABITATS IN RELATION TO THE DENSITY OF DENGUE VECTOR *AEDES AEGYPTI* IN THANJAVUR, TAMIL NADU, INDIA

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ABSTRACT
Nadu, India. In each study, the breeding habitats such as coconut shells, waste buckets and tires were examined for the presence of mosquito larvae. The surveys were carried out twice in each month from January, 2013 to December, 2013 and the larval density was correlated with the habitat characteristics. The physicochemical characteristics of breeding water such as pH, conductivity, salinity, total dissolved solids, turbidity and dissolved oxygen were measured. One way analysis of variance $F_{(2,21)} = 3.49$, gives p value 0.045, i.e., $p > 0.05$. From this observation of the value p, it was clear that there is no significant difference between the larval densities among the three habitats. The correlation between the physicochemical characteristics and the larval density of container- breeding mosquitoes indicated that the pH, calcium and the phosphate showed positive correlation with the larval density, the correlation coefficients being 0.2, 0.99 and 0.22 respectively. Total dissolved solids, electrical conductivity, total hardness, chloride, and sulphate were negatively correlated with larval abundance in the containers, with correlation coefficients of – 0.65, – 0.65, – 0.50, – 0.003 and – 0.66 respectively. Rainfall is an important environmental factor associated with *Aedes* breeding at the study sites.

KEY WORDS: *Aedes aegypti***,** physicochemical characteristics, Rainfall

INTRODUCTION
The occurrence of dengue has grown dramatically around the world in recent decades. Some 2.5 billion people – two fifths of the world's population – all now of risk from dengue. WHO currently estimates there may be 50 million dengue infections worldwide every year (WHO 2012). In the Indian scenario, almost due to favorable ecological conditions. *Aedes aegypti* L., a vector of dengue that carries the arbovirus responsible for these diseases, is widely distributed in the tropical and subtropical zones. The only way to prevent dengue virus transmission is to combat the disease carrying mosquitoes. The global resurgence and expanded distribution of vector borne diseases, such as dengue fever and chikungunya infection has generated a renewed interest in the biology and control of *Aedes* (Stegomyia) mosquitoes in recent years. *Aedes aegypti* (Linnaeus) is a principal vector of these diseases in several countries. This mosquito usually breeds in natural habitats especially in tree holes, leaf axils, rock pools and similar sites. The container habitats have unique ecological properties and these habitats could be natural such as tree holes and leaf axils or artificial such as tyres, plastic cups and water tanks (Service, 1995). The household wastes such as earthen, porcelain, plastic and coconut shells were as larval habitats of the dengue vectors (Soumyajit Banerjee *et al*., 2012). The population of *Aedes aegypti* fluctuates with temperature, rainfall and humidity. Dengue infectious was generally encountered during or after rain along with the rise in the vector *Aedes aegypti* population (Pandya *et al*., 1982; Ananya Bar and Andrew, 2012). In extremes of weather in winter and summer *Aedes aegypti* larvae die because of low and high temperature. Widespread deforestation, climate change and increase in global trade has forced this mosquito worldwide to adapt to breeding in domestic and semi domestic artificial container habitats (Gubler *et al*, 2001; Delatte *et al*, 2008). The species composition of the container-breeding mosquitoes and their habitat characteristics need to be studied in the context of the emergence of dengue and chikungunya in this part of the country. The information on the ecological factors influencing mosquito biology such as the physicochemical properties of breeding water could help in better implementation of the vector management programmes (Rao *et al*., 2011; Reji Gopalakrishnan *et al*., 2013). Hence, the present study was carried out to understand the Seasonal variations and physicochemical characteristics of the habitats in relation to the density of dengue vector *Aedes aegypti* in Thanjavur, Tamil Nadu, India.

MATERIAL & METHODS
Study area
The studies on the mosquito breeding habitats were conducted in and around Thanjavur District of Tamil Nadu, India.
Larval surveys
In each study, the breeding habitats such as coconut shells, waste buckets and tires were examined for the presence of mosquito larvae. The surveys were carried out twice in each month from January, 2013 to December, 2013.
Larval density
The mosquito larvae were collected from the breeding habitats using a net (6cm. width). Incidences of Aedes

aegypti larval collections were recorded and the larval density was calculated using the following formula:

Larval density= Number of larvae collected / Number of dips made

Physicochemical characteristics

Water samples were collected from the breeding habitats, which showed the presence of mosquito larvae. The breeding water characteristics were pH, conductivity (μS/cm), salinity (ppt), total dissolved solids (mg/l), turbidity (NTU) and dissolved oxygen (%) were recorded using Orion 5-star portable multipara meter (Thermo Scientific). The dissolved micronutrients such as, Calcium, Magnesium, Iron, Ammonia, Nitrite, Nitrate, Chloride, Fluoride, Sulphate and Phosphate was estimated by standard methods APHA (2006).

Data analysis

Means and standard deviations (SD) were used for summarizing the physicochemical factors. The mean larval density among the readings of each month of 2013 was compared using one-way ANOVA. The relationships between the mean values of the habitat characteristics and the larval densities were derived by Pearson's correlation. The statistical analyses were carried out using IBM SPSS 19 statistical software.

RESULTS

Aedes aegypti population density in Coconut shell, waste bucket and tires in and around Thanjavur District is considerably more in the collected mosquito larval samples. *Aedes aegypti* larvae are found in water collections throughout the year. The larval collections made from the places during January 2013 to December 2013 (Figure 1, Table 2) show the variation in the larval density from 1.66±0.57 to 18.00±1.00 from the monthly collections. From January to June, the larval index is considerably low. It ranges from 1to 8. *Aedes aegypti* larval development is found very slow in this season.

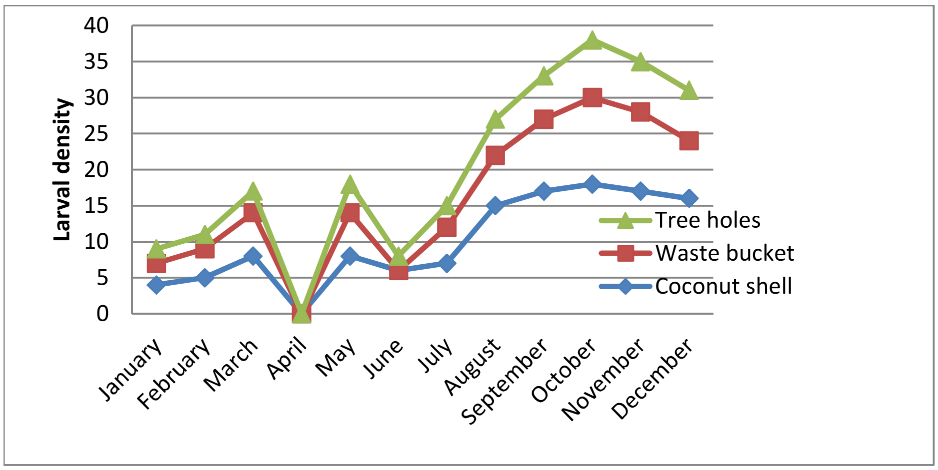

FIGURE 1. Larval density of *Aedes aegypti* in 2013

TABLE 1. Physicochemical parameters of different breeding habitats

Physicochemical parameters	Coconut Shell	Waste Bucket	Tires
Turbidity NTU	29.66 ±1.52	20.66±2.08	25.33±1.52
Total Dissolved Solids mg/l	511.33±1.52	79.83±1.75	693.00±0.5
Electrical Conductivity mho/cm	730.16±0.76	115.3±0.36	989.9±0.73
pH	6.73±0.67	6.38±0.38	6.50±0.02
Total Hardness(as CaCo3)mg/l	40.23±0.68	22.06±0.60	83.83±0.76
Calcium mg/l	55.93±1.10	40.26±1.02	20.16±1.25
Magnesium mg/l	9.93±0.63	3.10±0.36	21.80±0.62
Iron mg/l	1.62±0.22	00	0.28±0.2
Ammonia mg/l	0.13±0.005	0.5±0.01	0.18±0.01
Nitrite mg/l	8.67±0.05	0.62±0.03	11.54±0.20
Nitrate mg/l	50.00±1.52	6.9±0.63	50.0±1.00
Chloride mg/l	159.66±1.52	2.93±0.60	208.16±7.52
Fluoride mg/l	0.38±0.44	0.3±0.1	0.7±0.16
Sulphate mg/l	32.7±0.40	24.00±1.00	99.76±1.36
Phosphate mg/l	4.77±0.07	00	4.31±0.47

TABLE 2. The survey of *Aedes aegypti* larvae from January 2013 to December 2013 in Kannan Nagar, Thanjavur

Monthly catches	Mean sample of mosquito larvae 10 dipping / larval density (±)			Average Rainfall (mm)
	Coconut Shell	Waste Bucket	Tires	
January	3.66±1.52	3.00±1.00	1.66±0.57	4
February	5.33±1.52	4.66±2.08	2.33±1.52	6.5
March	6.66±1.52	5.33±2.51	3.00±2.00	13.6
April	7.00±1.00	6.00±1.00	4.33±1.52	00
May	6.00±1.00	4.33±0.57	2.66±0.57	7.7
June	6.66±0.57	5.33±0.57	3.33±1.52	24.6
July	14.66±0.57	6.66±1.52	4.00±1.00	5
August	16.66±1.52	10.16±0.76	5.66±.52	26.9
September	18.00±1.00	11.66±1.52	8.5±0.50	22.9
October	17.33±0.57	11.00±1.00	7.00±1.00	17.71
November	16.33±0.57	7.66±0.57	7.33±0.57	15.23
December	15.33±0.57	6.66±1.52	5.66±1.52	16.66

TABLE 3. Correlation coefficient between *Aedes aegypti* larval density and physicochemical parameter of breeding habitats

Parameters	Correlation coefficient	*p*-value
pH	0.80	0.20
Total Dissolved Solids	-0.065	0.47
Electrical conductivity	-0.065	0.47
Total hardness	-0.505	0.33
Calcium	0.952	0.99
Chloride	-0.003	0.49
Sulphate	-0.661	0.27
Phosphate	0.22	0.42

The maximum larval index was noticed in 2013 in the month of August and September (16-18) in all three breeding habitats. During this time, the average rainfall was high 26.9 and 22.9 and Dengue cases were also prevalent in Thanjavur. The larval index remained high till December (15.33) and it again decreased considerably in January. In March a decline in larval index (6.00) occurred and less rainfall (3.6) also was noticed. Due to extreme summer *Aedes aegypti* larval improper development and mortality was noticed. The density of container breeding mosquitoes (mean ± SE mean) was the highest in Coconut shell (18.00±1.00) followed by waste bucket (11.66±1.52) and Tree holes (8.5±0.50). Analysis of the physicochemical characteristics of water in the mosquito breeding sites indicated that the pH ranged from 6.38 ± 0.38 in waste bucket to 6.73 ± 0.67 in Coconut shell whereas the conductivity (mho/cm) ranged from 115.3 ± 0.38 in waste bucket to 989.9 ± 0.73 in Tree holes. The Total dissolved solids (mg/l) was the lowest in waste bucket (79.83 ± 1.75) and the highest in Tree holes (693.00 ± 0.5) whereas the total hardness (mg/l) was the highest in Tree holes (83.83 ± 0.76) and the lowest in waste bucket (22.06 ± 0.60). The turbidity of breeding water ranged from 20.66 ± 2.08 in waste bucket to 29.66 ± 1.52 in Coconut shell whereas the Calcium (mg/l) ranged from 55.93 ± 1.10 in coconut shell to 20.16 ± 1.25 in Tires (Table 1). One way analysis of variance (ANOVA) $F_{(2, 21)} = 3.49$, gives p value 0.045, i.e., p.> 0.05. From this observation of the value p, it was clear that there is no significant difference between the larval densities among the three habitats. The correlation between the physicochemical characteristics and the larval density of container- breeding mosquitoes indicated that the pH, calcium and the phosphate showed positive correlation with the larval density, the correlation coefficients being 0.2, 0.99 and 0.22 respectively. Total dissolved solids, electrical conductivity, total hardness, chloride, and sulphate were negatively correlated with larval abundance in the containers, with correlation coefficients of – 0.65, – 0.65, – 0.50, – 0.003 and – 0.66 respectively. (Table 3).

DISCUSSION

The selection of breeding sites by mosquitoes is a critical factor for mosquito survival and population dynamics and has important implications for mosquito control. Site may be affected by chemical and physical factors: attractants and deterrents. A deterrent causes insects to avoid that area, inhibiting egg laying. Sharma *et al* (2008) demonstrated the repellent effect of some fatty acid esters on oviposition of mosquitoes. Oviposition site selection is important in the life cycle of mosquitoes. Stimuli for an ovipositional flight are linked with some environmental factors, such as rainfall, relative humidity, temperature and wind speed; the selection of a breeding site involves visual, olfactory, and tactile responses (O'Gower, 1963; Bentely and Jonathan, 1989). *A. aegypti* exhibits a great deal of specialization in breeding site selection and consequently the distribution of this species is limited by those sites. Female mosquitoes have adapted to laying eggs in artificial containers, especially discarded tires, which resist desiccation, the eggs then hatch after being transported in used tires from Asian countries to Western countries (Medlock *et al*, 2006; Straetemans, 2008). It is possible species that breed in containers, both natural and artificial, may rely on some sort of visual analysis for site location and ensuing ovipositon. *Ae. albopictus* females may utilize smell and contact chemoreception for choosing breeding sites and prefer water with low illumination and dark color (Gubler, 1977). In a study in Delhi (Seghal and Pillar, 1970), *A. aegypti* and *Ae. albopictus* were found to favor identical breeding sites in regard to turbidity, pH, alkalinity, chloride and phosphate levels. However, they did not prefer the same water conditions. *Ae. albopictus* preferred less turbid waters with moderate alkalinity whereas *Culex* and *Anopheles* mosquitoes preferred more turbid water with high alkalinity and less oxygen content. The sulphate content was lower in the *Culex* and *Anopheles* breeding sites than the *Aedes* breeding sites, but the chloride content was higher in the *Culex* an *Anopheles* breeding sites and lower in the *Aedes* breeding sites. The phosphate content was low in *A. aegypti* breeding sites. The authors concluded mosquitoes exhibited species specific preferences in oviposition in regard to the chemical composition of water. Various physical and chemical factors of larval habitats contribute to mosquito breeding site selection, such as temperature, pH, ammonia, nitrate, sulphate, phosphate and dissolved solids (Oyewole *et al*, 2009; Oleymi *et al*, 2010). In our study we found a correlation between factors investigated and larval prevalence. Coconut shells were chosen more often by mosquitoes for breeding sites than other containers, with more *A. aegypti* eggs found in these habitats. Most of the coconut shells were discarded after use or had fallen from a tree and had been gnawed open by a rodent. Coconuts are rich in calcium, potassium, sodium, sulphur and magnesium forming an ideal breeding ground for *A. aegypti*. They have a small orifice and are dark inside, making it an ideal mosquito breeding site. The pH of water has an impact on mosquitoes, influencing osmoregulation and oxygen transportation (Umar and Donpedro, 2008). In this study of *A. aegypti* breeding, an increase in pH resulted in a significant decrease in larval density. The container sites sampled had a comparable pH levels. This demonstrates that *A. aegypti* mosquitoes favor a specific pH range for breeding. This could be an important factor in breeding site selection and larval survival. A pH variation outside the range of 7-8 could be used as a tool for management of this vector. Manipulation of the pH of preferred breeding sites, such as coconut shells, tires and plastic containers could be used to control this mosquito where breeding site reduction is not possible due to local factors. Further studies regarding this aspect need to be carried out. Spraying biopesticides, such as neem oil with a pH >8 could be useful for this purpose. Neem oil, a natural product, is environmentally safe and may be less harmful than synthetic insecticides. Salinity is another important factor that can have a repelling or attracting effect on oviposition. Navarro *et al* (2003) found increasing salinity in the laboratory decreased oviposition. However, our findings showed *A. aegypti* was tolerant to variations in salinity, possibly leading to further geographical expansion of this mosquito's breeding sites. Previous studies found *Aedes* prefers to breed in less turbid waters, similar to that found in plastic containers and tires; however, the high turbidity of the coconut shell breeding sites contradicts those findings. The rich organic content of coconut shells could be a reason for this. Carbonate was not detected in any of the samples we analyzed. Our study determined that larvae were present in the Coconut shells, Waste buckets and Tree holes. This indicates that larval density was associated with physicochemical parameters of breeding sites. During field surveys of mosquito breeding sites in the Garhwal region in the state of Uttaranchal, India, several environmental factors that could be quantified were found to be promising as predictors of mosquito occurrence (Pemola and Jauhari 2005). The present study showed that the physicochemical parameters, Nitrate, Sulphate, Salinity, Iron, Calcium, pH and temperature was affect the *A. aegypti* larval density. Various chemical properties of the larval habitat in relation to vegetation, optimum pH and temperature, concentration of ammonia, nitrate and sulphate have been reported to affect larval development and survival (Pal, 1945; Mutero *et al.*, 2004; Thangamathi *et al*., 2011).

In India Dengue infections generally occur during or after rain along with the rise in the vector *Aedes aegypti* population (Pandya, 1982). In Agra, *Aedes aegypti* larval density is high from July (17) to October (24.5) in 2009 and from July (20.7) to October (20) in 2010. In the neighboring city Delhi, *Aedes aegypti* larval density was high during July to October (Katyal *et al*., 2003), whereas in Gorakhpur it was during June to October (Rao, 1967). In the neighboring states, Rajasthan and Madhya Pradesh the larval density was also high during July to October (Pandya, 1982). In Vellore of the state of Tamilnadu *Aedes aegypti* larval density was high from September to December (Rao, 1967). In coastal plains of Orissa, Rohilkhand and Avadh plains and Haryana the larval density is high in wet season whereas in Assam valley in dry season the larval population is high (Kalra *et al*., 1997). The rise in dengue incidence during the monsoon and the post-monsoon season is due to the increased larval density and in the increased number of potential breeding sites due to water loggings. In India, all dengue/DHF outbreaks are associated with a higher container index of more than 20 *Aedes Aegypti* larvae (Sharma *et al*., 2005). *Aedes aegypti* larval density in Agra reach to a peak in the month of September in 2009 and in the months of July and October in 2010. In Gurgaon the maximum larval density was observed in the month of August in 1991 and in May in 1992 (Sharma, 1997). In Delhi the maximum larval density was noticed from August and September (Sharma *et al*., 2005), and July to September (Pramanik *et al*., 2007). In Gorakhpur (Rao, 1967) the maximum larval density was observed during August, in Vellore] during September and in Kolkata during August and November.

Because of the variation in the incidence of temperature and rainfall, fluctuation in larval density happens. In the months of October in 2009 and November in 2010 the larval density again decreases. Pandya in 1982 described that one of the possible reason for the seasonal aberration could be the fluctuating breeding habitat of *Aedes aegypti* in different types of containers e.g. dumped and moist tyres or earthen-wares used for storing water during summers (Ananya Bar and Andrew 2012). Maximum larval density above 20 was observed in the months of September and October in 2013. Prevalence of the maximum larval density of *Aedes aegypti* in Thanjavur city had a direct impact over the incidence of Dengue cases. In conclusion, rain fall and physicochemical characteristics exert a significant influence on mosquito breeding site selection among *A. aegypti*. Coconut shells were the preferred breeding site for this mosquito in our study, followed by plastic containers and tree holes. These preferences could be exploited to develop novel techniques to deter oviposition.

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