

Research Article

Survey of *Aedes* Mosquito Breeding Sites, Density and Pattern of Distribution: An Approach to Manage Dengue Outbreaks in Bhawanipatna Town, Odisha, India

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ABSTRACT

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According to the surveillance data, the state Odisha now contributes nearly 10-15% of total dengue cases of the country although the population of Odisha forms 3.47 percent of India as per the 2011 census. In the present study, the newly evolved educational hub of western Odisha town, Bhawanipatna Municipal Corporation, located around the Kalahandi university campus was chosen for field collections of adults and larvae. Adults of Ae. aegypti and Ae. albopictus were collected from different areas covering the entire areas of the town, both outdoors and indoors monthly for periods of one year from January 2019 to December 2019. Larval collections were made from indoor and outdoor water containers every month for the entire period of study and the larvae so collected were reared in the laboratory to identify them after hatching to the adult stage. Five different types of breeding sites were surveyed from 94 fixed houses covering the entire Bhawanipatna once a month during study period. A total of 528 water containers were examined during the entire period of study. Out of the total number of breeding sites examined from indoors, 224 containers contained larvae of Aedes mosquitoes. The Breeding Preference Ratio (BPR) was calculated from the data as maximum in an earthen pot (0.96) followed by the

plastic container (0.94). It was minimum in a plastic bucket (0.23). From outdoor survey a total of 1191 breeding sites were screened for the presence of *Aedes* larvae and 523 of them were positive for larvae of this genus. The BPR was calculated and was found to be maximum in a plastic container (1.33) followed by an earthen pot (0.98). No Aedes larvae could be collected from metal drums and plants axils. The house index (HI %) was maximum (50.58%) in July and was followed by 48.78% in June. The house index was minimum in November, which was 18.75%. Similarly, the container index (CI %) was maximum (54.35%) in July, and which was minimum (8.5%) in April. The Breteau index (BI %) was maximum (249.41%) in July, and it was followed by 227.77% in August. The BI was minimum (18.08%) in April. The different larval indices and the number of adults (Ae. aegypti and Ae. albopictus) were collected from indoor (ID) and outdoor (OD) at Bhawanipatna. The Pearson's correlation was made between HI% with total Aedes adult mosquito's data (MHD), the Pearson's correlation coefficient (r) value was 0.6, and the strength of the relationship between HI% and abundance of adult Aedes mosquito was moderate. The r-value of CI% and MHD were 0.3; here the strength of the relationship was low. Again, the r-value of BI% and MHD were 0.5, so the relationship between BI% and abundance of adult Aedes mosquitoes was moderate. The present study is the first in Bhawanipatna to explore the potential high risk dengue transmission.

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INTRODUCTION

Development of vaccines against dengue and chikungunya is far from reality now. Even if the vaccines are produced predicting their safety in different sub-sets of the population is difficult. The only alternative left, therefore, at the moment is the control of the vectors of such diseases. Although different types of synthetic insecticides have been tried in the past, they have been proved to be ineffective due to the development of resistance by the vector species. The best alternative, therefore, has been the destruction of stagnant waters where the mosquitoes breed like containers, trash cans, broken vessels, coconut shells, discarded tires, etc. to check the breeding of the Aedes aegypti and Ae. albopictus which are efficient vectors for dengue, chikungunya, and zika [1]. The estimated annual dengue burden in the globe is nearly 390 million cases from 120 countries^[2]. Aedes mosquito gradually occupies urban, semi-urban, and rural areas; also oviposit in a variety of natural habitats (like leaf axils, small ponds, tree holes, etc.) and different artificial habitats (such as discarded containers, earthen pots, tyres, gutters, and cement tanks, etc.) [3-5]. It is however, a herculean task and needs intensive campaign an against mosquitoes. It is important to study the biology, ecology, behaviour, and breeding preference of the Aedes mosquitoes in the

laboratory and outdoors to formulate strategies aimed at abating the growth of the vector population. Therefore, the use of Aedes larval indices with well-defined threshold values, considering the microlevel dynamics of dengue vectors, can result in successful entomological management of dengue, through the provision of reliable pre alerts on probable epidemics [6-8]. Therefore, the development of reliable threshold values for the entomological management of dengue vectors is essential to control dengue in developing countries like India. Aedes larval indices that focus on the immature stages of dengue vectors. Aedes larval indices (House Index [HI], Breteau Index [BI], and Container Index [CI]) are widely used in many developing countries, due to their convenient applicability, and restrictions in human and financial resources [9]. Since the mid-1990s, dengue epidemic episodes in India have grown rapidly and have become more frequent [10]. Currently, India is endemic for both dengue fever (DF) and dengue hemorrhagic fever (DHF). Initially, the infection was geographically limited to a few states, which later expanded to most of the states in the country [11]. Odisha, an Eastern state of the country historically did not have any reported cases of dengue till 2009, but now there is wide reporting from all the districts [12]. According to the

surveillance data, the state now contributes nearly 10–15 % of total dengue cases of the country [13] although the population of Odisha forms 3.47 percent of India as per the 2011 census.

In the present study, the newly evolved educational hub of western Odisha Bhawanipatna Municipal town, Corporation, located around the Kalahandi university campus was chosen for field collections of adults and larvae. Adults of Ae. aegypti and Ae. albopictus were collected from different areas covering the entire areas of the town, both outdoors and indoors monthly for periods of one year from January 2019 to December 2019. Larval collections were made from indoor and outdoor water containers every month for the entire period of study and the larvae so collected were reared in the laboratory to identify them after hatching to the adult stage.

From the larval collection data from indoor and outdoor containers, the breeding preference ratio (BPR) was calculated for *Aedes* mosquito for Bhawanipatna town under survey. In Bhawanipatna, earthen pots, indoors and plastic containers outdoors were the most preferred sites for breeding. No larva of either species could be observed in the water collected in plant axils and metal drums.

Based on larval collection data, the house index (HI), containers index (CI), and Breteau index (BI) were calculated and attempts were made to correlate the HI, CI, and BI with man per hour density (MHD) based on the adult. The HI, CI, and BI at Bhawanipatna were the highest in July and were followed in August.

The present study was conducted to determine the breeding preferences habitats of Aedes mosquito in a form of breeding preference ratio (BPR) and larval indices of *Aedes* mosquitoes by conducting the larval survey and man per hour density (MHD) by conducting the adult survey in Bhawanipatna city, Odisha. The results obtained are summarized in the present study for further use of the data to formulate strategies for the control and pre alerts on probable epidemics of Aedes vectors. There mosquito are no entomological parameters of the Aedes mosquito to manage dengue outbreaks to date in Bhawanipatna.

MATERIALS AND METHODS

The State of Odisha is located on the east coast of India between the parallels of 17° 48′ and 23° 34′ N latitudes and meridians of 80° 24′ and 87° 29′ E longitudes. The State came into existence on 1st April 1926. Orissa is bordered by Jharkhand in the north, Chhattisgarh in the west, West Bengal in the northeast, and

Andhra Pradesh in the south. On the east, it has the Bay of Bengal.

During the pre-independence period, the State Odisha occupied an area of 83682 km² and comprised of 6 districts. Following the merger of provincial states after independence, the State acquired a total area of 155707 km² and was divided into 13 districts. Further, at a later stage, the State has been divided into 30 different districts. The of Balasore, districts Bhadrak. Kendrapara, Jagasingpur, Khurda, Puri, and Ganjam are situated along the Bay of Bengal, and the coastline is about 450 km long. The coastal region contains extensive alluvial tracts between the hills on the west and the salty tracts on the east. The State of Odisha has been divided into 5 major morphological regions – coastal plain in the east, middle mountainous and highland region, central plateaus, western rolling uplands, and major flood plains. The total population of Odisha as per the 2011 census is 41,974,218 of which males and females are 21,212,136 and 20,762,082 respectively.

Study Areas: The collection of data was made from different locations of Bhawanipatna Municipal Corporation, the headquarter of the district of Kalahandi. Bhawanipatna is located at 19.9°N 83.17°E, has a tropical wet and dry climate, and the annual average rainfall is

1300mm. The municipality has a population of 69,045 of which 35,506 are males while 33,539 are females residing in around 16,500 houses as per a report released by census India 2011.

Collection: Adult Adult Aedes mosquitoes were surveyed month-wise from the selected human dwelling, both inside and outside the house or premises of Bhawanipatna Municipal area from January 2019 to December 2019 to determine human per hour density (MHD). Adult mosquitoes were collected with the help of a manual aspirator tube and a torch light [14]. Immediately after collection, the mosquitoes were transferred into test tubes at the rate of 8-10 mosquitoes per tube. The date, place, and time of collection were marked on each test tube. The mosquitoes were anesthetized and identified under a binocular stereo zoom microscope in the laboratory-based on the standard morphological keys [15]. Indoor collections were made during the morning hour between 6 am and 10 am.

Larval Collection: Monthly Larval collections were made at random from 5 indoor (earthen pot, cement tank, plastic container, flower pot, plastic bucket) and 11 outdoor (earthen pot, cement tank, old vehicle, tree hole, coconut shell, bamboo clump, metal drum, plant pot, plastic container, discarded tire, and plant axils) breeding sites from January 2019 to

December 2019. The location (indoor or outdoor), date and time, type of habitat, and the number of larvae collected were recorded. The immature stages were collected with the help of a glass dropper and transferred to the laboratory in plastic containers, for development into mature stage and identification of mosquito at the species level. Larger water containers were sampled by dipping a fishnet in the water starting at the top and continuing to the bottom in a swirling motion that sampled all edges of the containers [16; 17, 18]. House Index [HI], Breteau Index [BI], and Container Index [CI] were calculated following the WHO guidelines [19].

The house index (HI) i.e., percentage of houses positive for *Aedes* larvae calculated by the formula

$$HI = \frac{\text{Number of infested house}}{\text{Total no. of house inspected}} X100$$

The container index (CI), i.e., the number of positive containers with *Aedes* larvae per 100 containers was calculated by the formula

$$CI = \frac{\text{Number of infested containers}}{\text{Total no.of Containers inspected}} X100$$

The Breteau index (BI), i.e., number of positive containers with *Aedes* larvae per 100 houses was calculated by the formula

$BI = \frac{\text{Number of infested containers}}{\text{Total no.of house inspected}} X100$

All the colonies were maintained in the insectary at a temperature $25 \pm 1^{\circ}$ C and relative humidity of $65 \pm 5\%$ with 12 hour light and dark periods. The adults were fed on cotton pads soaked in 10% glucose solution and soaked raisins. The larvae were reared in white enamel trays on yeast tablets obtained from Alembic, India Ltd., and dog biscuit at a ratio of 1:3.

RESULT AND DATA ANALYSIS

Adult collection: Monthly surveys were conducted in Bhawanipatna municipal during the period January through December 2019. Indoor and outdoor collections of adult mosquitoes were made and the results are shown in the Table 1.

Table 1 Man per hour density (MHD) of *Aedes aegypti* and *Aedes albopictus* collected from different random human dwelling indoor and outdoor collection during January 2019 to December 2019

	Indoor						Outdoor					
Month	Ae. aegypti			Ae. albopictus			Ae. aegypti			Ae. albopictus		
	3	\$	Total	3	\$	Total	3	\$	Total	3	\$	Total
January	0	0	0	0	3	3	0	0	0	4	3	7
February	0	1	1	2	3	5	0	2	2	3	3	6
March	1	1	2	1	4	5	2	2	4	4	4	8
April	1	3	4	2	1	3	3	5	8	7	8	15
May	2	2	4	2	3	5	3	6	9	6	10	16
June	1	2	3	5	6	11	4	5	9	9	10	19
July	2	2	4	4	6	10	2	5	7	9	13	21
August	1	2	3	5	9	14	4	5	9	7	8	15
September	0	2	2	5	5	10	3	2	5	7	7	14
October	1	1	2	5	6	11	2	3	5	7	8	15
November	0	0	0	2	3	5	1	2	3	5	6	11
December	0	0	0	1	2	3	0	0	0	3	4	7

In Bhawanipatna town adult mosquitoes were collected from different random human dwellings. A maximum of 4 *Ae. aegypti* was collected from the indoor, each in April, May and July followed by 3 each in June and August. On the other hand, a maximum of 14 adults of *Ae. albopictus* were collected in August

followed by 11 adults each in June and October. For Ae. aegypti no adult available mosquitoes were in the collections made during January, November, and December, although Ae. albopictus adults were available in the catches during all the months (Figure 1 and 3).

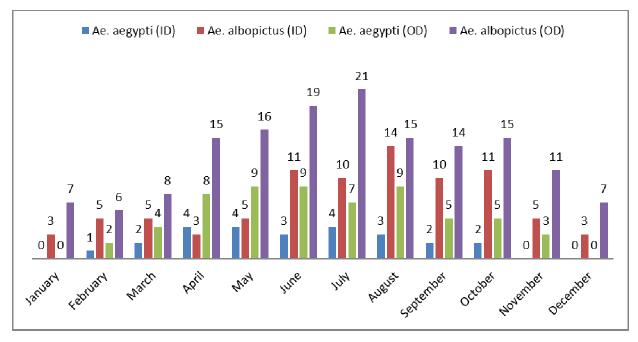


Figure 1 Number of adult collections from indoor and outdoor during January 2019 to December 2019 in Bhawanipatna.

Outdoor collections were made around the houses from where the adults were collected monthly during indoor collections and the data are represented in the Table 1. The maximum number of 9 adults of *Ae. aegypti* were collected each in May, June, and August followed by 8 in April. No adults could be collected in January and December. For *Ae. albopictus*, a maximum of 21 adults was collected in July, followed by 19 in June.

Larval Collection: Table 2 represents the details of larvae collected from 5 different types of indoor breeding sites once every

month by making house visits from 94 fixed houses covering the entire town of Bhawanipatna. A total of 528 containers containing water were examined during the entire period of study. Out of the total number of breeding sites examined from indoors, 224 containers contained larvae of mosquitoes. The Breeding Aedes Preference Ratio (BPR) was calculated from the data as maximum in an earthen pot (0.96) followed by the plastic container (0.94). It was minimum in a plastic bucket (0.23).

Table 2 The Breeding Preference Ratio (BPR) for different types of containers (indoors and outdoors) by *Aedes* mosquitoes at Bhawanipatna

	Ei1	X%	Ae. Larvae	Y%	BPR =			
	Examined	A%0	present	Y %0	Y/X			
INDOOR								
Earthen Pot	56	10.60	23	10.26	0.96			
Cement Tank	45	8.52	18	8.03	0.44			
Plastic Container	230	43.56	156	69.64	0.94			
Flower Pot	86	16.28 16		7.14	0.43			
Plastic Bucket	111	21.02	11	4.91	0.23			
Total	528		224					
OUTDOOR								
Earthen Pot	58	4.86	25	4.78	0.98			
Cement Tank	82	6.88	22	4.20	0.61			
Old Vehicles	21	1.76	02	0.38	0.21			
Tree Holes	19	1.59	05	0.95	0.60			
Coconut Shell	25	2.09	02	0.38	0.18			
Bamboo clumps	41	3.44	07	1.33	0.38			
Metal Drum	31	2.60	00	00	00			
Plant Pot	59	4.95	11	2.1	0.42			
Plastic Container	710	59.61	415	79.34	1.33			
Discarded Tyres	105	8.81	34	6.50	0.73			
Plant Axils	40	4.76	0	0	0			
Total	1191		523					

Table 2 contains the data on the number of containers examined outdoors and the number of containers positive for *Aedes* larvae and the BPR. A total of 1191 breeding sites were screened for the presence of *Aedes* larvae and 523 of them

were positive for larvae of this genus. The BPR was calculated and was found to be maximum in a plastic container (1.33) followed by an earthen pot (0.98). No *Aedes* larvae could be collected from metal drums and plants axils.

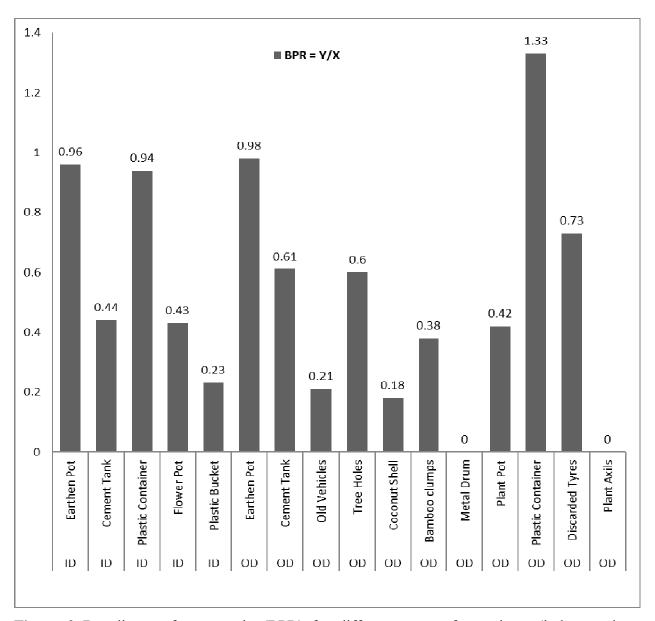


Figure 2 Breeding preference ratio (BPR) for different types of containers (indoor and outdoor) by Aedes mosquitoes at Bhawanipatna.

The month-wise house index (HI %), container index (CI %), and Breteau index (BI %) were calculated from the data collected from the houses visited and the containers surveyed in Bhawanipatna, and the data are summarized in the Tables 3.

Table 3 Month-wise containers screened in houses, the House Index (HI), Container Index (CI), and Breteau index (BI) in Bhawanipatna from January 2019 to December 2019

Sl.	Months	No. of	No. of	No. of	No. of			BI%
No.		House	House	Container	positive	HI%	CI%	
		visited	positive	screened	container			
1.	January	91	28	180	18	30.76	10	19.78
2.	February	85	26	200	20	30.58	10	23.52
3.	March	90	20	240	21	22.22	8.75	23.33
4.	April	94	18	200	17	19.14	8.5	18.08
5.	May	80	17	190	21	21.25	11.05	26.25
6.	June	82	40	180	22	48.78	12.22	26.82
7.	July	85	43	390	212	50.58	54.35	249.41
8.	August	90	38	400	205	42.22	51.25	227.77
9.	September	91	35	350	182	38.46	52	200
10.	October	86	20	210	110	23.25	52.38	127.90
11.	November	80	15	200	87	18.75	43.5	108.75
12.	December	81	17	190	65	20.98	34.21	80.24

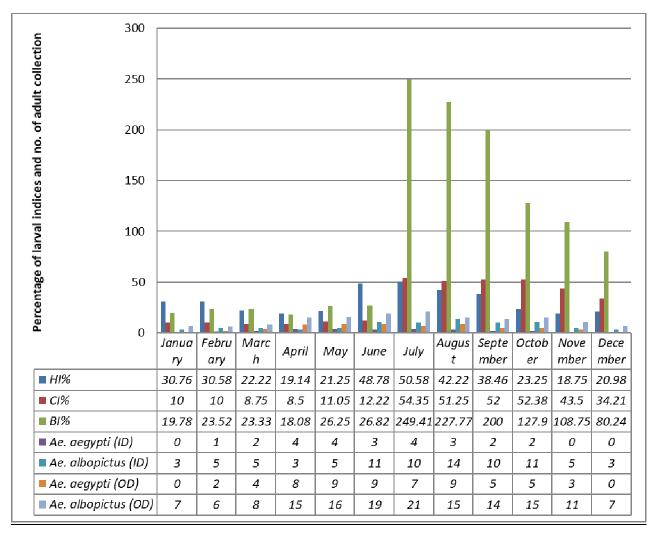


Figure 3 Month wise larval indices and MHD of adult Aedes (Indoor and Outdoor survey)

The house index (HI %) was maximum (50.58%) in July and was followed by 48.78% in June. The house index was minimum in November, which was 18.75%. Similarly, the container index (CI %) was maximum (54.35%) in July, and which was minimum (8.5%) in April. The Breteau index (BI %) was maximum (249.41%) in July, and it was followed by 227.77% in August. The BI was minimum (18.08%) in April. The different larval indices and the number of adults (*Ae. aegypti* and *Ae. albopictus*) were collected

from indoor (ID) and outdoor (OD) at Bhawanipatna are summarized in the figure 3 to compare each other.

An attempt was made to correlate the data of HI%, CI%, and BI% each with MHD of Aedes adult mosquitoes collected together from indoor (ID) and outdoor (OD) (Figure 4-6). The Pearson's correlation coefficient (r) was calculated individually to analyze the strength of the relationship between different larval indices with MHD. The Pearson's correlation was made between HI% with total *Aedes* adult mosquito's data (MHD), the Pearson's correlation coefficient (r) value was 0.6, and the strength of the relationship between HI% and abundance of adult *Aedes* mosquito was moderate (Fig. 4). The r-value of CI% and MHD was 0.3; here the strength of the relationship was low (Figure 5). Again, the r-value of BI% and MHD was 0.5, so the relationship between BI% and abundance of adult *Aedes* mosquitoes was moderate (Figure 6).

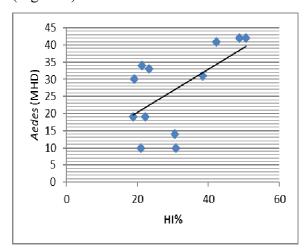


Figure 4 Correlations in between HI% and MHD

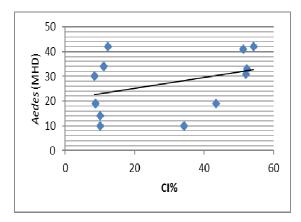


Figure 5 Correlations in between CI% and MHD

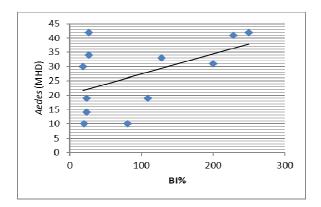


Figure 6 Correlations in between BI% and MHD

Discussion

Adult Collection: In the present study, it has been observed that the populations of Ae. aegypti and Ae. albopictus started increasing both indoors and outdoors in Bhawanipatna municipal area with the onset of rainfall in May after a long spell of dry weather. It was reported that the abundance of the vectors is associated with environmental factors like rainfall, temperature, and relative humidity [20, 21]. Ae. aegypti and Ae. albopictus adults were collected in the catches of all the months of the year of study. However, the present findings are in agreement with the reports made by Micieli, Campos [22], and Guindo-Coulibaly *et al.* ^[23]. In this present study, it was found that there was no strong relationship between larval indices and the density and distribution of Aedes adult mosquitoes. The present studies confirmed that the relationship between House Index (HI%) and Breteau Index (BI%) with abundance and distribution of adult Aedes mosquito is moderate because the correlation coefficient (r) value lie between 0.4 to 0.7. Whereas this study low relationship between shows a Container Index (CI%) and density of adult Aedes mosquitoes. Therefore, present studies confirm that larval indices are not related with the density of Aedes adult mosquitoes in Bhawanipatna; because hatching the mosquito after flew somewhere range about less than 200 meters [24] thus there was adult density (MHD) is less than the larval density (larval indices). Similarly, the hatching of eggs or development of adults was not 100% so there was more difference between the density and distribution of larvae of Aedes and adult Aedes mosquitoes. The quality of water, as well as the condition of water containers, seemed to contribute to an abundance of Aedes species in Bhawanipatna town; and the water chemistry of aquatic habitats may also play a critical role in determining the survival rate of mosquitoes. This was also earlier reported by Chen et al; for a university campus in Kuala Lumpur, Malaysia in the year 2009 [25].

Larval Collections: Ae. aegypti is an urban vector that has been adopted to utilize man-made containers like flower pots, discarded tyres, cans, etc. for breeding. It feeds primarily on human blood [26]. It is therefore highly adapted to

human settlements [27, 28] and rests in secluded locations inside homes, under beds, inside closets, and on curtains [29].

Ae. albopictus is more cosmopolitan in its feeding habit and, therefore, rests both inside and outside human dwellings. It breeds both in manmade and natural containers like coconut shells, bamboo clumps, tree holes, etc. [30]. Since this species is more cosmopolitan and can breed in all possible containers, it is more difficult to control Ae. albopictus than Ae. aegypti. Larval habitats are of determinants adult important distribution and abundance [31].

The World Health Organization has recommended the use of three measured indices like House Index (HI), container index (CI), and Breteau Index (BI) for estimation of vector density for dengue and related fevers [32]. To predict the risk of dengue transmission [33] the above indices are traditionally used to characterize *Aedes* infestation levels [34]. However, Tun-Lin et al. [35], and Focks and Alexander [36] have argued that these indices are not always correlated with the abundance of adults and thereby disease transmission. The main limitation to BI is that it fails to account for adults produced from containers [37].

In order to find out the breeding preference of the two species of *Aedes* mosquitoes in different water containers, 5 types of indoor containers were screened during the period of study from different localities of Bhawanipatna. From the survey, it has been clear that the Aedes mosquitoes preferred earthen pots followed by plastic containers for egg-laying indoors. During the outdoor container surveys Bhawanipatna municipal area, the plastic container was most preferred followed by the earthen pot and discarded tyres. No larvae could be observed in plant axils and metal drums. The similar study conducted by panigrahi et al. [18] in Berhampur, Odisha, earthen pot appeared to be the most preferred breeding sites by Aedes. On the contrary, Singh et al. [38] have reported the first preference for coconut shells followed by discarded tyres by Aedes females for egg-laying in Ranchi. In another study conducted by Sharma et al. [39] in Rourkela, Orissa, coconut shells and discarded tyres appeared to be the most preferred breeding sites by Aedes mosquitoes. It is concluded that Aedes mosquito alter their breeding habitats (both natural and artificial) on the availability of water containing containers. In this study plastic containers were most preferred for oviposition by Aedes; the reason may be nonavailability of suitable natural breeding habitat in Bhawanipatna town.

CONCLUSIONS

This study revealed that the Aedes larval indices (HI, CI and BI) were greater than

10% most of the months in the year, which represent a dengue epidemiological risk according to WHO [40]. The elimination of artificial and natural containers or alternation of breeding sites in Bhawanipatna, should be taken into consideration since the presence of water in plastic containers outdoor and earthen pot indoor were probably the most important factor in determining the breeding of *Aedes* mosquitoes. Especially plastic container management should be taken up as a priority by the Bhawanipatna municipal. The study is the first in Bhawanipatna to explore the potential high risk dengue transmission on basis of larval indices (BI, CI and BI) and adult indices (MHD). There was no strong relationship between larval density and adult density of Aedes. So, an effective control strategy should be made for eradication of larval habitats as well as vector control.

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