

A comprehensive study on the larvae of *Aedes* species in Angul district of Odisha, India: An approach to determine their habitat and prevalence in association with the ecology.

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Abstract: Currently, the disease 'dengue viral infection' was endemic in Angul district, Odisha in India, transmitted by the vectors of *Aedes* species. This study was designed to analyze the prevalence of larvae of *Aedes* species and its relationship with climatic factors during different seasons. During the study, monthly climatologically data was obtained and analyzed; and mosquitogenic sites were surveyed and larvae samples of *Aedes* species were collected and reared and identified. Out of the total larvae collected, *Ae. aegypti* and *Ae. albopictus* were predominant; whereas, *Ae. vittatus* was found very lesser % in these localities. Larval density of *Aedes* species coincided mainly with the post-monsoon period of subnormal rainfall. *Aedes* index, container index and breteau index were found significantly higher in November during post-monsoon period. This prospective study highlighted rain, temperature and relative humidity as the major and important climatic factors, which could alone or collectively be responsible for the larval development. More studies in this regard could further reveal the correlation between the climatic changes and the prevalence of larvae of *Aedes* species, which would help in making the strategies to forecast any epidemic or outbreak and also early possible measures are taken to control the vector density.

Keywords: Dengue infection (DI), Larval density, *Aedes* Index (AI), Container Index (CI), Breteau Index (BI).

I. Introduction

Mosquito can be found all over the world and commonly known to pose a significant threat to public health. The biodiversity of mosquitoes is very evident, with many genera having worldwide distribution and some genera with limited or endemic distribution [1]. The common fear for mosquitoes is their role as 'vectors' that can spread vector borne diseases such as Dengue, Malaria, Filariasis, Yellow fever, and Japanese encephalitis etc.

Dengue virus infection (DENV) is amongst the most important emerging viral diseases transmitted by mosquitoes to humans, in terms of both illness and death [2]. In the tropics and subtropics, infections from the dengue virus (DENV) are transmitted by the vectors of *Aedes* species which belong to the family 'Culicidae and order Diptera', circulates as four distinct serotypes of the dengue virus such as DENV-1, DENV-2, DENV-3, and DENV-4 [3-5]. *Aedes* mosquitoes occur around the world and there are over 950 species; amongst *Ae. aegypti* was the most potential vector, but other species such as *Ae. albopictus*, *Ae. vittatus*, *Ae. polynesiensis* and *Ae. niveus* have also been incriminated as secondary vectors.

As the effective vaccine for dengue is not yet available, dengue control is limited to a reduction of the vector population [6]. The worldwide large-scale reappearance of dengue for the past few decades has turned this disease into a serious public health problem and also the dengue fever (DF) has gradually become one of the leading causes of morbidity and mortality in tropical and subtropical areas throughout the world [7]. Overall, two-fifth of the world population is living in areas, at risk for dengue [8-10]. It is estimated that 52% of the global population are at the risk of contracting Dengue fever (DF) or dengue hemorrhagic fever (DHF) lives in the South East Asian Region (SEAR). In most of the countries, dengue epidemics are reported to occur, during the warm, humid and rainy seasons, which favor the abundant mosquito growth and shorten the extrinsic incubation period as well [11-14]. In Malaysia the outbreak of dengue cases is one of the major problems which seemed to be a global issue as well. According to Skae, F.M., the first outbreak occurred in Penang from 1901-1902 [15]. In India, DENV was first isolated in 1946 and many outbreaks have been reported [16-19]. DF was first reported in Calcutta, West Bengal in 1963 [20]. Overpopulation has consequently led to poor sanitary conditions and water logging at various places. A major epidemic of DHF from Delhi was last reported in the year 1996 after which DI became a notifiable disease and a number of policies were formulated to bring the DI as well as its vector under control [21-22]. Although this region, ecological and climatic factors are reported to influence the seasonal prevalence of the dengue vector, *Aedes aegypti*, on the basis of which, countries in this region are divided into four zones with different DF/DHF transmission potential [23].

Mosquitoes can thrive in a variety of habitats with fresh water, brackish water, or any water (clear, turbid or polluted) except in marine habitats with high-salt concentration [1]. Mosquito breeding places in and around houses can be divided into two main types, breeding sites with clean water: mainly rain-filled receptacles in humid tropical areas which are suitable breeding sites for some *Aedes* species. These habitats are favored by *Aedes aegypti*, *Aedes albopictus* and *Aedes vittatus* which can act as a vector of dengue and by, also a dengue vector referred as the Asian tiger mosquito by Americans. Urbanization is one of the factors that increase the number of habitats suitable for *Aedes* mosquitoes, especially *Aedes aegypti* [24]. While *Aedes aegypti* commonly found inside houses, *Aedes albopictus* is more common in outside areas, in open spaces with shaded vegetation and suitable breeding sites such as car tyres and garbage dumps [25]. Breeding places in the city rise from the neglected features of the construction sites, stagnant drain water collections, tanks, coolers and receptacles of rain water collections. These species also breed in containers that are used to store water for drinking or washing.

In Southeast Asia, a strong association between dengue vectors and rainfall has been well established [26, 27]. The population of *Aedes aegypti* fluctuates with temperature, rainfall and humidity. Dengue infections were generally encountered during or after rain, as an outcome of the rise in the vector population [28]. The optimal temperature for *Aedes aegypti* larva is 28°C. Above this the rate of development is high and below 18°C the growth gets prolonged [29]. Above 36°C larval development is not complete [30]. Extremely hot and dry weather may kill most of the eggs [31] and render adult vectors inactive [32]. *Aedes aegypti* population is higher in the rainy season and lower in extreme hot weather. During rainy season the risk of virus transmission by the vector is greater. Control measures of *Aedes aegypti* larva are necessary as Dengue fever (DF) and Dengue haemorrhagic fever (DHF) cases increase proportionally with the larval density [33].

Particularly, Odisha state is considered as an endemic for infection of DENV. In the state, DENV infection was first documented in 1998; and several epidemics due to DENV precipitated after 2005, causing an increase of the case fatality rate (CFR =total mortality X100/total positive cases of dengue) [34]. In the year 2011, the region of Angul district in Odisha, India had experienced one of the wettest monsoons in 25 years, which led to a spate of mosquito growth creating an alarming situation of mosquito borne diseases in many districts. For an instance in 2011, of the total 1846 detected dengue cases in Odisha, 33 death cases occurred with the epicenter at the Talcher coalmine area of Angul district; the maximum number of dengue positive cases (33.7%), with the CFR at 66.7% had been reported alone in the Angul district [34]. In the rural, semi-urban and urban areas in Angul district fall in the deciduous, dry and wet climatic zone [34].

There are numerous scientific reports on investigation of prevalence (composition and distribution) of *Aedes* species (larvae and adult), dengue fever (DF) and dengue haemorrhagic fever (DHF), from various parts of the Indian subcontinent [35-40] and studies conducted in the countries like Brazil, Indonesia and Venezuela, where DI is present either in epidemic or endemic form have suggested a correlation between the weather and prevalence (composition and distribution) of *Aedes* species (larvae and adult). Rain, temperature and relative humidity are suggested as important factors attributing towards the growth and dispersion of this vector and potential of dengue epidemic or outbreaks [41-43].

However, systematic studies on the association of climatic conditions with composition and distribution of *Aedes* species and the pattern of DI from this geographical region in Angul district of Odisha, India has yet not been attempted, either in epidemic or in sporadic dengue outbreaks in Odisha, Indeed, except recording of prevalence/ epidemiological trends of both diseases in databases, there is no systematic study [34]. Therefore, the study was conducted to find out the composition and distribution of larvae of *Aedes* species and also to find out the relationship of the prevalence of larvae of *Aedes* species in and around of Angul district of Odisha, India during different seasons with climatic factors such as the rainfall, temperature and relative humidity from different water collections was sampled during the year (January to December, 2013). This paper presents a comprehensive surveillance report on the correlation between prevalence of *Aedes* species larvae with climatic factors such as the rainfall, temperature and relative humidity in the Angul district of Odisha and also to mapping the prevalence of *Aedes* species larvae presently conquered in this region.

II. Materials And Methods

The present study was conducted retrospectively for a period of one year during January to December, 2013 in Angul district of Odisha, India. The study population comprised larvae of *Aedes* species.

2.1. Study area:

Centrally located Angul district with area 6232 sq. km (20° 31' N and 21° 40' N latitude and 84° 15' E and 85° 23' E longitude) in the state of Odisha has the total population of 1,271,703 with males 654,898 and females 616,805, as per 2011-census. With a brief winter, the climate of the district is sub-tropical with the temperature range, 6 to 47° C (Figure 1).

2.2. Entomological studies:

Entomological studies were conducted on *Aedes* species in the study area periodically from January to December, 2013. A demographic map of the localities in rural, suburban and urban areas was prepared and houses to be examined in each survey were marked.

2.2.1. Larvae Survey:

Aedes larvae surveys were carried out in different parts of the study area periodically during the study period; both types of instar stages of larvae were collected from different breeding habitats using standard dippers (250 ml capacity) method (about 5–20 dippers were taken from each breeding habitat according to its area) and the samples were brought to the laboratory and reared at room temperature (25°C) with the help of thermostat. Larvae and adults were preserved and identified [44]. Incidences of *Aedes* species larval collections were recorded and the larval density was calculated using the following formula:

$$\text{Larval density} = \frac{\text{Total number of larvae}}{\text{Number of dip}}$$

To evaluate the distribution and density of the *Aedes* mosquito species in the study area, the parameters of three larval indices: *Aedes* Index (AI), Container Index (CI), and Breteau Index (BI) were calculated as per standard WHO guidelines 1999.

- House/ *Aedes* Index (AI): percentage of houses infested with larvae.

$$\text{House/ } Aedes \text{ Index (AI)} = \frac{\text{Number of houses infested}}{\text{Number of houses inspected}} \times 100$$

- Container Index (CI): percentage of water-holding containers infested with larvae.

$$\text{Container Index (CI)} = \frac{\text{Number of positive containers}}{\text{Number of containers inspected}} \times 100$$

- Breteau Index (BI): number of positive containers per 100 houses inspected.

$$\text{Breteau Index (BI)} = \frac{\text{Number of positive containers}}{\text{Number of house inspected}} \times 100$$

2.3. Analysis of metrological data:

Monthly details of total rainfall, temperature and relative humidity for all the months of the year, 2013 were obtained from the 'Meteorological Department of Angul district, Odisha' and retrospectively analyzed in relation to the total number of dengue infection positive cases. According to the intensity of the rainfall, weather data was divided into three periods, namely; pre-monsoon period: from February – May, monsoon period: from June – September and post monsoon period: from October – January.

2.4. Analysis of data:

During the study (January to December, 2013), the statistical data's of the prevalence of dengue infection with climatic factors such as the rainfall, temperature and relative humidity were entered in Excel format by given codes as per the requirement and the data was analyzed by using the computer program Excel-2003 to find out the composition and distribution of *Aedes* sp. larvae and also to find out the relationship of the prevalence of *Aedes* species larvae in and around Angul district of Odisha, India during different seasons with climatic factors such as the rainfall, temperature and relative humidity from different water collections in comparison to p value ($p < 0.05$). The statistics were used in this study as the following: percentage (%), mean and standard deviation (SD). The larval index at various months of the year were recorded and analysed. Kruskal-Wallis one way analysis of variance (ANOVA) was employed to determine the significant difference among the readings of each month along with Standard deviation (SD). The statistics graph was designed by the chart wizard in this study as the following: pie chart wizard and line column in 2 axes chart wizard.

Figure 1:

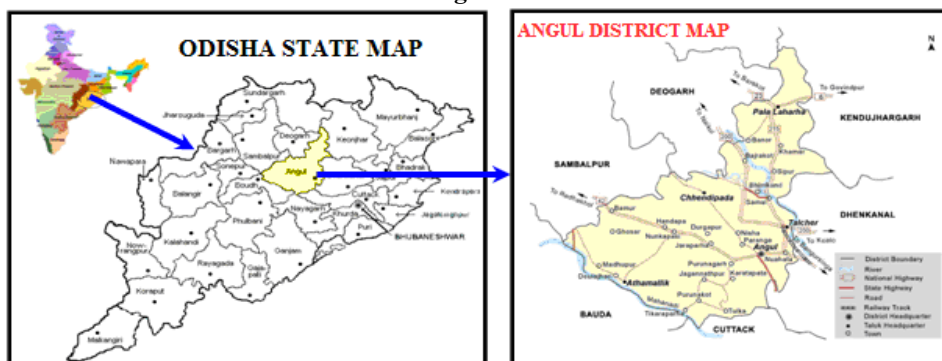


Figure 1: Map showing the study area of Angul district, Odisha.

III. Results

The larval compositions of *Aedes* species were presented (Figure 2). The result revealed that, three types of *Aedes* mosquito larvae species were found such as *Ae. aegypti*, *Ae. albopictus* and *Ae. vittatus*. Amongst, *Ae. aegypti* (50.0%) and *Ae. albopictus* (49.1%) larvae were mostly found; whereas, a very less number of larvae percent of *Ae. vittatus* (0.9%) was found in these localities during the study.

The *Aedes aegypti* population density in the study area was significantly found more amongst the collected *Aedes* mosquito larval samples. *Aedes aegypti* & *Aedes albopictus* larvae were found in water collections throughout the year. *Ae. aegypti* species larvae was found in all types breeding habitats i.e. natural breeding habitats (tree holes), artificial breeding habitats (water coolers, flower pots, storage tanks and unused well) and discard habitats (plastic containers, glass bottles, drum, tyres, Others); whereas, *Ae. albopictus* species larvae was also found in all types breeding habitats i.e. natural breeding habitats (streams & tree holes), artificial breeding habitats (water coolers, flower pots, and unused well) and discard habitats (plastic containers, glass bottles, drum, tyres and Others) but *Ae. vittatus* only breeds in natural breeding habitats (streams & riverbeds). However, larvae of *Aedes* species were not found in irrigation canals, ponds and water collection near the pumps (natural breeding habitat) during the study were presented (Table 1).

Monthly wise distribution of *Aedes* larval density in the study area of Angul district, Odisha during January to December, 2013 was presented (Figure 3). The result revealed that, the variation in the larval density ranges from 7.3 to 36.2 from the monthly collections. The larval density was considerably lower during the pre monsoon period; it ranges from 7.3 to 13.3. During monsoon the larval density gradually increases; it ranges from 14.8 to 33.5; whereas, in months; October and November the larval density was significantly found high (34.3 and 36.2) respectively in comparison to all the months of the year and its again decreased considerably in December and January (27.3 and 23.4) accordingly and a fluctuation in larval prevalence was noticed during post monsoon. The mean larval density of *Aedes* mosquito species was 20.7 in the study area during the study period.

Month-wise distribution of larvae density of *Aedes* species in the study area of Angul district, Odisha during January to December, 2013 was also presented (Table 2). The result was shown that, the variation in the *Ae. aegypti*, *Ae. albopictus* and *Ae. vittatus* larval density range, 5.0 - 17.8, 3.5 - 18.1 and 0.06 - 0.26 respectively from the monthly collections. During the pre monsoon season the larval density of *Ae. aegypti*, *Ae. albopictus* and *Ae. vittatus* was considerably lower and development was found very slow due to extreme summer; whereas, the larval density of *Ae. aegypti*, *Ae. albopictus* and *Ae. vittatus* was gradually increases during monsoon i.e. the rainy season and in humid weather, but in months; October and November the larval density was significantly found high in comparison to all the seasons of the year 2013 and its again decreased considerably in December and January and a fluctuation in larval prevalence was noticed during post monsoon. The mean larval density and SD was recorded in *Ae. aegypti*, *Ae. albopictus* and *Ae. vittatus* was (10.4, 5.0), (10.1, 5.1) and (0.18, 0.1) respectively.

The larval indices of *Aedes* mosquito by month wise in the study area of Angul district, Odisha during Jan-Dec, 2013 were presented (Table 3). The results was shown that, the average Stegomyia indices were house index (HI) 26.1, container index (CI) 16.0 and breteau index (BI) 43.7. Its density was lowest during May (pre monsoon period) when HI, CI and BI were 7.3, 12.4 and 12.0 respectively; whereas, highest in November (post monsoon season) when HI, CI and BI were 46.7, 30.5 and 83.3 respectively during the period the density also recorded higher i.e. 36.2. The larval indices was decreased during the hot season (March to June) but increased with peak abundance during the rainy seasons (July to November).

The climatologically status of 2013 in the Angul district of Odisha, India was presented (Table 4). It was observed that, the annual rainfall of the district was recorded as 1304 mm and the average annual rainfall

was 108.7 mm. The highest rainfall was recorded (354.6 mm) in the month of September; whereas, the lowest rainfall was (5.4 mm) in the month of November & December. May was the hottest month with a mean daily maximum temperature of 46 degree Celsius. With the onset of monsoon, early in the month of June the day temperature drops appreciably. December was usually the coldest month of the year with a mean daily minimum temperature of 14° Celsius. The highest maximum temperature was recorded in Angul district (46° Celsius) in the month of May; whereas, the lowest minimum temperature was recorded (13° Celsius) in the month of January. The humidity of the air was generally high and the high humidity was recorded (90%) in the month of July, especially during the South West monsoon and post monsoon months; whereas, the lowest humidity was 60% in the month of January. The afternoons were comparatively drier throughout the year, the relative humidity varies between 62 to 90 percent and the average annual humidity was recorded 74.7%.

Month wise distributions of the prevalence of *Aedes* larval density and climatologically (rainfall, temperature and relative humidity) condition in Angul district, Odisha during January to December, 2013 were presented (Figure 4 and 5). The result indicates that, the prevalence of *Aedes* larval density coincided mainly with the post monsoon period of subnormal rainfall (Cumulative rainfall = 25.9 mm) from October to December, 2013 and was followed by relatively heavy rainfall (Cumulative rainfall = 266.7 mm) during the post monsoon period; from June to September, 2013 (Figure 4). The difference between relative humidity during the three periods was not significant. The mean relative humidity was 74.3% during the pre monsoon period. It increased during the monsoon period to 82.8% and decreased during the post monsoon period to 67.0% (Figure 4). The divergence in the rainfall and temperature between three seasonal periods was found to be important ($p < 0.05$) (Figure 4 and 5). The mean ambient temperature was 32.5°C during the pre monsoon period (Mean temperature from February to May), which fell to 29.8°C during the monsoon period (Mean temperature from June to September); the period preceding peak the prevalence of *Aedes* larval density and decreased to 23.9°C (Mean temperature from October to December) during the post monsoon period; the period high prevalence of *Aedes* larval density was observed (Figure 5).

Figure 2:

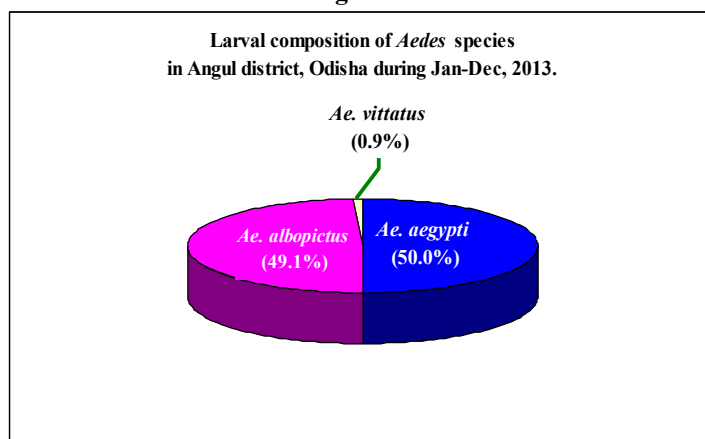


Figure 2: Larval composition of *Aedes* species in Angul district, Odisha during Jan-Dec, 2013.

Table 1: *Aedes* mosquitoes larval collection and identification of breeding habitat in the study area of Angul district, Odisha during Jan-Dec, 2013.

Sl. No.	Breeding habitat	Type of habitat	Types of mosquito species emerge from larvae
1	Irrigation canals	Natural BH	Larvae absent
2	Ponds	Natural BH	Larvae absent
3	Water collection near the pumps	Natural BH	Larvae absent
4	Streams	Natural BH	<i>Ae. vittatus</i> and <i>Ae. albopictus</i>
5	Riverbeds	Natural BH	<i>Ae. albopictus</i> and <i>Ae. vittatus</i>
6	Tree holes	Natural BH	<i>Ae. aegypti</i> and <i>Ae. albopictus</i>
7	Water coolers	Artificial BH	<i>Ae. aegypti</i>
8	Flower pots	Artificial BH	<i>Ae. aegypti</i> and <i>Ae. albopictus</i>
9	Storage tanks	Artificial BH	<i>Ae. aegypti</i>
10	Unused well	Artificial BH	<i>Ae. aegypti</i> and <i>Ae. albopictus</i>
11	Plastic containers	Discards BH	<i>Ae. aegypti</i> and <i>Ae. albopictus</i>
12	Glass bottles	Discards BH	<i>Ae. aegypti</i> and <i>Ae. albopictus</i>
13	Drum	Discards BH	<i>Ae. aegypti</i> and <i>Ae. albopictus</i>
14	Tyres	Discards BH	<i>Ae. aegypti</i> and <i>Ae. albopictus</i>
15	Others	Discards BH	<i>Ae. aegypti</i> and <i>Ae. albopictus</i>

Figure 3:

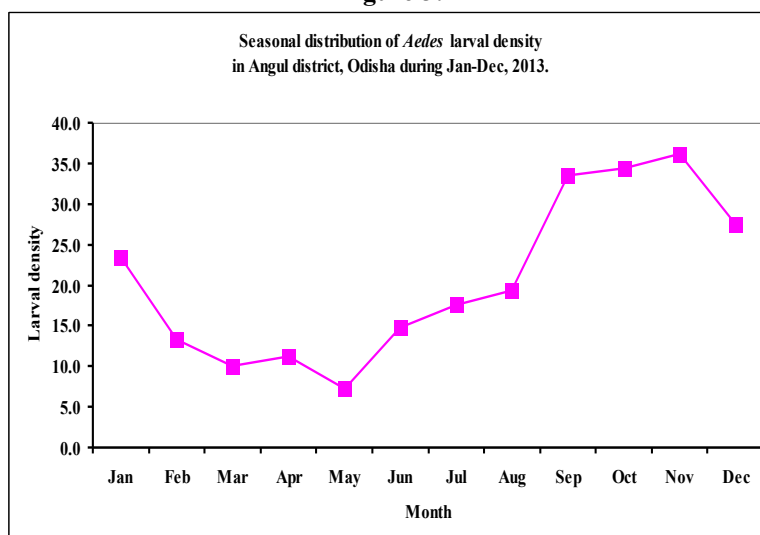


Figure 3: Seasonal distribution of *Aedes* larval density in Angul district, Odisha during Jan-Dec, 2013.

Table 2: Distribution of *Aedes* larvae according to species and larval density by month in the study area of Angul district, Odisha during Jan-Dec, 2013.

Month	No. of dips	Larvae collected		Identified <i>Aedes</i> larvae					
		No	Larval Density	<i>Ae. aegypti</i>		<i>Ae. albopictus</i>		<i>Ae. vittatus</i>	
				No	Larval Density	No	Larval Density	No	Larval Density
January	200	4670	23.4	2347	11.7	2298	11.5	25	0.13
February	200	2652	13.3	1417	7.1	1215	6.1	20	0.10
March	200	2012	10.1	1008	5.0	986	4.9	18	0.09
April	200	2243	11.2	1113	5.6	1098	5.5	32	0.16
May	200	1453	7.3	745	3.7	696	3.5	12	0.06
June	200	2960	14.8	1466	7.3	1446	7.2	48	0.24
July	200	3501	17.5	1718	8.6	1731	8.7	52	0.26
August	200	3866	19.3	1920	9.6	1900	9.5	46	0.23
September	200	6700	33.5	3337	16.7	3311	16.6	52	0.26
October	200	6868	34.3	3428	17.1	3382	16.9	58	0.29
November	200	7232	36.2	3568	17.8	3612	18.1	52	0.26
December	200	5493	27.5	2789	13.9	2684	13.4	20	0.10
Total	2400	49650	20.7	24856	10.4	24359	10.1	435	0.18
Mean	200	4137.5	20.7	2071.3	10.4	2029.9	10.1	36.3	0.18
SD	0.0	2024.9	10.1	1001.1	5.0	1014.5	5.1	16.6	0.1

Table 3: The larval indices of *Aedes* mosquito by month in the study area of Angul district, Odisha during Jan-Dec, 2013.

Month	House investigated			Container investigated			House/ <i>Aedes</i> Index (HI/AI)	Container Index (CI)	Breteau Index (BI)
	No	Positive		No	Positive				
		No	%		No	%			
January	150	34	22.7	410	60	14.6	22.7	14.6	40.0
February	150	20	13.3	410	29	7.1	13.3	7.1	19.3
March	150	22	14.7	410	30	7.3	14.7	7.3	20.0
April	150	29	19.3	410	42	10.2	19.3	10.2	28.0
May	150	11	7.3	410	18	4.4	7.3	4.4	12.0
June	150	24	16.0	410	32	7.8	16.0	7.8	21.3
July	150	40	26.7	410	69	16.8	26.7	16.8	46.0
August	150	44	29.3	410	75	18.3	29.3	18.3	50.0
September	150	58	38.7	410	118	28.8	38.7	28.8	78.7
October	150	68	45.3	410	114	27.8	45.3	27.8	76.0
November	150	70	46.7	410	125	30.5	46.7	30.5	83.3
December	150	49	32.7	410	75	18.3	32.7	18.3	50.0
Total	1800	469	26.1	4920	787	16.0	26.1	16.0	43.7
Mean	150	39.1	26.1	410	65.6	16.0	26.1	16.0	43.7
SD	0.0	19.2	12.8	0.0	37.4	9.1	12.8	9.1	24.9

Table 4: Climatologically status of Angul district of Odisha, India during Jan- Dec, 2013).

Month	Temperature (°C)		Relative humidity (%)	Rain fall (MM)
	Maximum	Minimum		
January	32	13	62	22.1
February	38	14	67	20.8
March	39	22	70	10.2
April	43	29	75	12.6
May	46	29	85	90.2
June	43	24	75	220.4
July	38	22	90	190.9
August	34	22	86	300.9
September	34	21	80	354.6
October	32	20	78	70.6
November	32	17	64	5.4
December	31	14	64	5.4
Mean	36.8	20.6	74.7	108.7
SD	5.1	5.4	9.4	125.2

Figure 4:

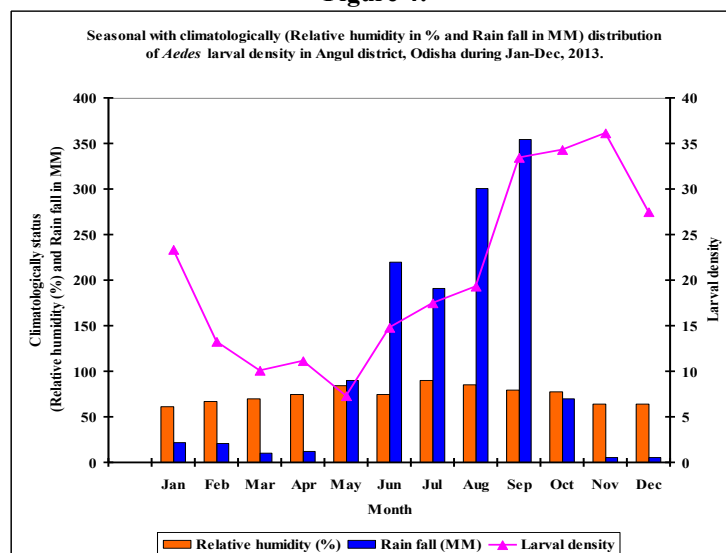


Figure 4: Seasonal with climatologically (relative humidity in % and rain fall in MM) distribution of *Aedes* larval density in Angul district, Odisha during Jan-Dec, 2013.

Figure 5:

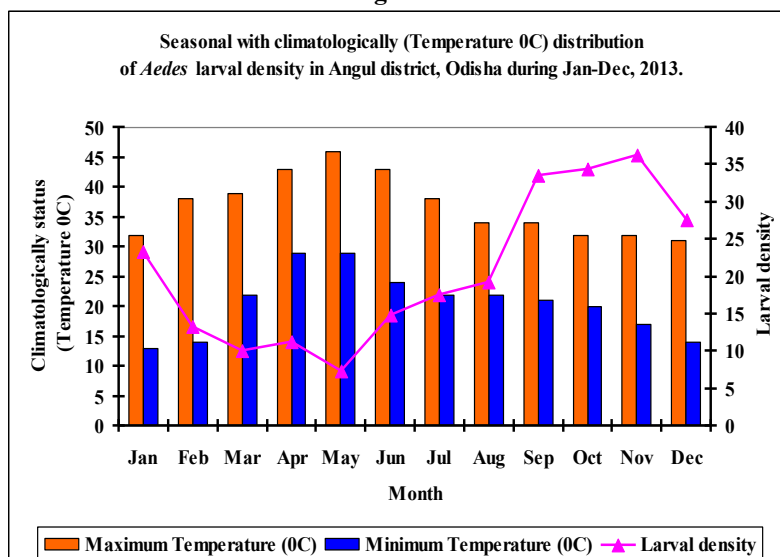


Figure 5: Seasonal with climatologically (temperature⁰C) distribution of *Aedes* larval density in Angul district, Odisha during Jan-Dec, 2013

IV. Discussion

Currently, the disease 'dengue viral infection (DI)' was endemic in all continents except Europe. In India, DENV was first isolated in 1946 and many outbreaks have been reported [16–19]. DI was first reported in Calcutta, West Bengal in 1963 and the first dengue outbreak occurred in Calcutta, India in 1963 with 30% DHF cases [20]. In India Dengue infections generally occur during or after the rain along with the rise in the vector *Aedes* Sp. population [3]. Particularly, Odisha state was considered as endemic for dengue infection. In the state, DENV infection was first documented in 1998; and several epidemics due to DENV precipitated after 2005, causing an increment of the case fatality rate (CFR) [34]. In the year 2011, Odisha in India had experienced one of the wettest monsoons in 25 years, which led to a spate of mosquito growth creating an alarming situation of mosquito borne diseases in many districts. There are numerous scientific reports on investigation of prevalence (composition and distribution) of larvae of *Aedes* species, dengue fever (DF) and dengue haemorrhagic fever (DHF), from various parts of the Indian subcontinent [36–41] and studies conducted in the countries like Brazil, Indonesia and Venezuela, where DI is present either in epidemic or endemic form have suggested a correlation between the weather and prevalence (composition and distribution) of larvae of *Aedes* species. Rain, temperature and relative humidity are suggested as important factors attributing towards the growth and dispersion of this vector and potential of dengue epidemic or outbreaks [42–44]. Most of vector borne diseases exhibit a distinctive seasonal pattern and climatic factors such as rainfall, temperature and other weather variables affect in many ways both the vector and the pathogen they transmit [45]. Worldwide studies have proposed that ecological and climatic factors influence the seasonal prevalence of larvae of *Aedes* species. In the rural, semi-urban and urban areas of Angul district, the vectors *Aedes* occur throughout the year [34]. The vector mainly responsible for the spread of DI is present at the basal level all the year around in Angul district of Odisha. However, systematic studies on the association of climatic conditions with composition and distribution of *Aedes* species from this geographical region in Angul district of Odisha, India has yet not been attempted, either in epidemic or in sporadic dengue outbreaks in Odisha. Indeed, except recording of prevalence/epidemiological trends of both diseases in databases, there is no systematic study [34]. This study was planned to carry out the month wise detailed analysis of the composition and distribution of larvae of *Aedes* sp. and also to find out the relationship of the prevalence of larvae of *Aedes* sp. in and around Angul district of Odisha, India during different seasons with climatic factors such as the rainfall, temperature and relative humidity from different water collections was sampled and studied during the year (January to December, 2013).

Studies on the distribution and relative abundance of mosquitoes in rural, urban/suburban housing areas indicated that *Culex quinquefasciatus* (Say), *Aedes albopictus* (Skuse) and *Aedes aegypti* (Linnaeus) were the most abundant [46]. During one year of study in Angul district of Odisha, a total of 469 (26.1%) houses were found of positive for larval of *Aedes* species from the total of 1800 houses investigated; whereas, a total of 787 (16.0%) containers were found positive for larval of *Aedes* species from the total of 4920 containers investigated, 49650 *Aedes* mosquito larvae species were collected and from this total, 24856 (50.0%) were *Aedes aegypti* larvae, 24359 (49.1%) of *Aedes albopictus* larvae and 435 (0.9%) of *Aedes vittatus* larvae. *Aedes albopictus*, is known to be a container breeder and mostly found in outdoor areas as supported by studies done by Bhaskar [47] who found that *Aedes albopictus* is a container breeder and it breeds in both natural and man-made habitats. While *Aedes aegypti* commonly breeds and feed inside houses, *Aedes albopictus* is more common outside, in open spaces with shaded vegetation and suitable breeding sites such as car tyres and garbage dumps [25]. *Ae. albopictus* is more likely to be found in natural containers or outdoor man-made habitats containing a greater amount of organic debris [48]. All containers containing *Aedes albopictus* were found outdoors, while three out of four containers positive for *Aedes aegypti* were also found outdoors [49].

The study has been conducted in urban, suburban and rural areas of Angul district, Odisha; it was found that the main breeding habitats for *Aedes* species in these areas were not significantly different. The *Aedes aegypti* & *Aedes albopictus* larvae were found in water collections throughout the year. According to Isaacs, in tropical countries, anything that retained water would be potential breeding sites for *Aedes* mosquitoes, which were within human dwellings. Containers that retained water for long periods of time will make good or suitable breeding habitats for mosquitoes such as the artificial containers found in these locations [50].

This study also indicated that, *Aedes aegypti* and *Aedes albopictus* was found to be predominant in the study area of Angul district, Odisha. While *Aedes aegypti* commonly found inside home dwellings, *Aedes albopictus* and *Aedes vittatus* is more common in outside areas. The *Aedes albopictus* was a container breeder, and it breeds in both natural and man-made habitats. *Aedes aegypti* breeds and feed inside houses, *Aedes albopictus* was more common outside, in open spaces with shaded vegetation and suitable breeding sites such as car tyres and garbage dumps. *Ae. albopictus* was more likely to be found in natural containers or outdoor man-made habitats containing a greater amount of organic debris. *Ae. aegypti* species larvae was found in all types, breeding habitats i.e. natural breeding habitats (tree holes), artificial breeding habitats (water coolers, flower pots, storage tanks and unused well) and discard habitats (plastic containers, glass bottles, drum, tyres,

Others); whereas, *Ae. albopictus* species larvae was also found in all types breeding habitats i.e. natural breeding habitats (streams & tree holes), artificial breeding habitats (water coolers, flower pots, and unused well) and discard habitats (plastic containers, glass bottles, drum, tyres and Others) but *Ae. vittatus* only breeds in natural breeding habitats (streams & riverbeds). Previous work had shown that *Aedes aegypti* larvae was always found in indoor conditions, in contrast with what is revealed in this current work where *Aedes aegypti* was found mostly in indoors and outdoors with *Aedes albopictus*. This finding is supported by [51] where *Aedes aegypti* was found breeding in natural receptacles such as tree holes, but always near human habitation. A similar result was found by [52] where both species *Aedes albopictus* and *Aedes vittatus* was found to breed outside, rather than inside home dwellings.

In this study, the result revealed that, the variation in the larval density ranges from 7.3 to 36.2 from the monthly wise sampling. The larval density was coincided mainly with the post-monsoon period of subnormal rainfall; from October to December, 2013 and was followed by relatively heavy rainfall during the monsoon period; from June to September, 2013; whereas, the larval density considerably lower during the pre monsoon period; from February to May, 2013. Similarly, the larval density of *Ae. aegypti*, *Ae. albopictus* and *Ae. vittatus* was considerably lower and development was found very slow during the pre-monsoon period due to extreme summer; whereas, the larval density of *Ae. aegypti*, *Ae. albopictus* and *Ae. vittatus* was gradually start increasing during monsoon i.e. the rainy season and in humid weather, but in months; October and November the larval density of *Aedes* species was significantly found high in comparison to all the seasons of the year 2013 and its again decreased considerably in December and January and a fluctuation in larval prevalence was noticed during post monsoon. In Angul district, in the month of May and June a fluctuation (reduction in larval density) was noticed in *Aedes* sp. larval population due to extreme heat in the summer months irrespective of the availability of coolers and other water collections. 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. The divergence in the rainfall and temperature between three seasonal periods was found to be important ($p < 0.05$). Monthly weather data showed that temperature variations were more amongst different months during the pre monsoon and post monsoon period as compared to the monsoon period. Even though, the monsoon season began in mid- June, there was no respite from the heat as there was not much difference in the temperature during the last month of pre monsoon; May and beginning of monsoon in the June. Unusually heavy rainfall subsequently led to decrease in temperature during the later part of monsoon period. The temperature showed a decline during the months of July and August, continuous heavy rainfall subsequently led to a further decrease in the temperature during the month of September to 34°C. The mean ambient temperature was 32.5°C during the pre monsoon period, which fell to 29.8°C during the monsoon period; and further decreased to 23.9°C during the post monsoon period. Relative humidity increased during the rainy season and remained high for several weeks. The difference between relative humidity during the three periods was not significant. The mean relative humidity was 74.3% during the pre monsoon period. It increased during the monsoon period to 82.8% and decreased during the post monsoon period to 67.0%. An in-depth analysis of these three factors, thus led to a proposal that optimum temperature with high relative humidity and abundant stocks of fresh water reservoirs generated due to rain developed optimum conditions conducive for mass breeding and propagation of vector and transmission of the virus. This study supported the proposal that all the three climatic factors studied could be playing an important role in creating the contributing condition required for breeding and propagation of this vector, the basal level of which is present all round the year. This prospective study was therefore highlighted the major important factors, which could alone or collectively be responsible for an epidemic or outbreak. In the city Delhi, *Aedes aegypti* larval density was high during July to October [53], whereas in Gorakhpur it was during June to October [54]. In the neighboring states, Rajasthan and Madhya Pradesh the larval density was also higher during July to October [28]. In Vellore of the state of Tamilnadu *Aedes aegypti* larval density was high from September to December [54]. In the coastal plains of Rohilkhand and Avadh plains and Haryana the larval density is high in the wet season, whereas in the Assam valley in dry season the larval population is high [55]. In Gurgaon the maximum larval density was observed in the month of August in 1991 and in May in 1992 [56]. In Delhi the maximum larval density was noticed from August and September [27], and July to September [57]. In Gorakhpur [54] the maximum larval density was observed during August, in Vellore [54] during September and in Kolkata [54] during August and November. Maximum larval density above 20 was observed in 2009 in the months of September and October and in 2010 in July, September and October. Prevalence of the maximum larval density of *Aedes aegypti* in Agra city had a direct impact over the incidence of Dengue cases. In extremes of weather in winter and summer *Aedes aegypti* larvae die because of low and high temperature. The larval development also varies at different temperatures in different larval stages. *Aedes aegypti* is sensitive to low temperature [58]. Some growth takes place at 10°C but development is not complete. Above 14°C growth becomes increasingly rapid with raising temperature reaching the optimum at 32°C [59]. Whereas, Christopher; 1960 described the optimal temperature for *Aedes aegypti* larva is 28°C.

Observations on the seasonality were based on a single year's data, the *Aedes* Index (AI) was higher in the month of November with 46.7%, followed by 45.3% in October during post-monsoon period; whereas, the AI was lower in the month of May with 7.3% followed by 13.3% in February during pre-monsoon period. Container index (CI) was higher in the month of November with 30.5%, followed by 27.8% in October during post-monsoon period; whereas, the CI was lower in the month of May with 4.4% followed by 7.1% in February during pre-monsoon period. Similarly, the Breteau index (BI) was higher in the month of November with 83.3%, followed by 78.7% in September during the end of monsoon and start of post-monsoon period; whereas, the AI was lower in the month of May with 12.0% followed by 19.3% in February during pre-monsoon period. In India, all dengue/DHF outbreaks are associated with a higher container index of more than 20 *Aedes aegypti* larvae [27]. Katyaj et al., 2003 also observed that in Delhi, the container index reduced in August which is lower than July and September [53]. Sharma et al., 2005 studied the prevalence of *Aedes aegypti* in defense area of Delhi Cantt. The data show that the total containers positive in August are lower than July and September. Higher rates of adult mortality causing severe reduction of mosquito densities have frequently been associated with a rise in temperature. Above 36°C the larval development is not complete [30]. Extremely hot and dry weather may kill most of the eggs [31] and render adult vectors inactive [32]. In *Aedes aegypti* reduction in egg production and variation in oviposition was observed with an increase in temperature [60]. In Vellore the larval density is lower in March in 1964-1965 and in Kolkata in May the density is very low [57]. In Gurgaon in the year 1991 during June, July and August, in 1992 during April the larval density was nil [56]. 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 reasons 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 [28].

In this study, we have observed that temperature tends to decrease towards the end of monsoon period, specially remains moreover constant during the later months of rainy season. Angul district is a very popular tourist and coal mine area, situated close to the capital city Bhubaneswar, Odisha, India fall in the deciduous, dry and wet climatic zone. The temperature remains high during the pre monsoon period. It is continuous rain pouring for a couple of days that brings down the temperature during the monsoon period, which may also be responsible for an increase in the relative humidity and decrease in the evaporation rate thus maintaining secondary reservoirs containing rain water. The prevalence of *Aedes* sp. ultimately resulting in causing dengue is mainly due to the pressure of urbanization, improper management of the domestic and other neglected water collections and water coolers. They are the causative factors in the budding up of enormous breeding sites of *Ae. aegypti*, *Ae. albopictus* and *Ae. vittatus* larvae.

During the study (January to December, 2013), it was sampled, studied & analysis the composition and distribution of larvae of *Aedes* sp. and the relationship of the prevalence of larvae of *Aedes* sp. in and around of Angul district of Odisha, India during different seasons with climatic factors such as the rainfall, temperature and relative humidity from different water collections and the outcome of the study is good and satisfactory.

V. Conclusion

This prospective study highlighted rain, temperature and relative humidity as the major and important climatic factors, which could be playing an important role in creating the contributing condition required for breeding and propagation of this vector, the basal level of which is present all round the year. During this time, Dengue cases were also prevalent in Angul district of Odisha due to the pressure of urbanization, improper management of the domestic and other neglected water collections occur. Construction sites in the city and excessive use of water coolers in summer also are the sources of *Aedes* species breeding. The readings of the three indices were influenced by the awareness of the residents and environmental factors such as rainfall, humidity and temperature which could contribute to the dynamic fluctuations of indexes. More studies in this regard could further reveal the correlation between the climatic changes and the prevalence (composition and distribution) of larvae of *Aedes* species and control measures that can be applied for the mosquito breeding prevention. Organizing literacy programs and making awareness among the people regarding the need of the control of vectors and vector borne diseases is the primary need in mosquito control. The public participation and change of habits in minimizing the breeding sites by elimination of the unused containers within the vicinity of their houses, drainage clearing and maintaining the garden areas. Provide a proper waste management system for all housing areas. The health education is one of the important ways to educate residents. Residents should be alert and concerned about their housing areas, especially when these can contribute to mosquito breeding. The larvivorous predators like Gambusia, Guppy, Dragon fly nymph and other predators also die from the water collections in the extremes of temperatures. Artificial rearing of predators and their release during larval prevalent seasons can be an alternative for *Aedes* control. This would help in making the strategies and plans to

forecast any endemic, epidemic or outbreak in the future well in advance and also early possible measures are taken to control the vector density.

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