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The Physical Parameters of the Septic Tank Provide Evidence for the Existence of a Novel Ecosystem for the *Aedes* sp. Mosquito

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Abstract

Aedes sp**.** mosquito is a dreaded vector and carrier of pathogens of different life-threatening diseases causing global community health problems. At present its formal life cycle is with some controversy as it has been reported by many authors that the ecosystem of *Aedes* sp. needs to be investigated in different ways, based on that philosophy an innovational hypothesis has been framed on a new ecosystem of *Aedes* sp. in the water reserve of multi-chambered septic tank. A year-round study and survey have been carried out on the physical parameters and vector population (pupa/adult stages) of both the Septic Tank Environment (STE) and the Ambient Atmospheric Environment (AAE). The acquired data were evaluated using the ANOVA and produced significant results. The F-Values for STE and AAE were 177.2 and 4.58527, respectively. The corresponding P-values were <0.0001 and <0.5. The Vector Population Mean for STE and AAE was 271.42 and 122.08, respectively, across all three values. Other values are also highly significant as well supportive in favor of the STE environment for *Aedes* sp., leading to a condition of water reserve of septic tank can effectively be utilized as a "new and alternative ecosystem by *Aedes* sp. Vector".

***Key words*:** *Aedes* sp., Eco-system, Septic tank environment, Community health, Physical parameters

A niche suitability survey in regards to physical parameters for *Aedes* sp. mosquito in the water reserve of the Septic Tank environment in the light of smooth growth and development of the famous villain vector is the basic view of this study. In the present study, part of a comprehensive Doctoral Thesis, niche suitability of different immature stages, larva/pupa ultimately to adult mosquito, with respect to physical parameters has been attempted to establish the hypothesis of a 'New and Alternative Ecosystem of *Aedes* sp. mosquito', study on other important parameters have been considered independently. *Aedes* sp**.** Mosquito (Diptera: Culicidae) is a notorious and dreaded vector, causing community health problems all over the world. "Before 1970, only nine countries had experienced severe dengue epidemics. The disease is now endemic in more than 100 countries. Africa, the Americas, the Eastern Mediterranean, South-east Asia, and the Western Pacific are affected out of which South-east Asia and the Western Pacific regions are the most seriously affected" [1].

A report in the "NATURE" expressed that, "Dengue is a systemic viral infection transmitted between humans by *Aedes* mosquitoes. For some patients, dengue is a life-threatening illness. There are currently no licensed vaccines or specific therapeutics, and substantial vector control efforts have not stopped its rapid emergence and global spread. The contemporary worldwide distribution of the risk of dengue virus infection and its public health burden is poorly known" [2]. Regarding the growth and development of *Aedes sp.* there are so many views, as per WHO theory followers "*Aedes sp.* mosquito can't develop in the dirty water, it bred and develop only in clean and clear water" [3]. As per "WHO comprehensive guidelines for prevention and control of dengue and dengue haemorrhagic fever" – Page No-71: "Throughout most of South-East Asia, Ae. aegypti oviposit almost entirely in domestic and man-made water receptacles. (y) These include a multitude of receptacles found in and around urban environments (households, construction sites, and factories) such as water-storage jars, saucers on which flowerpots rest, flower vases, cement baths, foot baths, wooden and metal barrels, metal cisterns, discarded tires, bottles, tin cans, polystyrene containers, plastic cups, discarded wet-cell batteries, glass containers associated with "spirit houses" (shrines), drainpipes and ant-traps in which the legs of cupboards and tables are often rested" [4]. But different views are exposed in the domain with strong pace and pieces of evidence in favour of alternate ecosystem, moreover, Scholars and scientific workers are not getting the desired result by following those concepts as reported here "Association between dengue cases and larval indices, BI/CI/ HI/ OI, and meteorological parameters were not significant. Migration of mosquitoes and patients could be considered as possible factors affecting the absence of a significant relationship" [5].

It is ascertained by the different authors that maximum and minimum temperature, and Relative Humidity are very

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much important factors for the growth and development of *Aedes* sp. "The effect of temperature and relative humidity on dengue virus propagation in the mosquito as one of the possible contributing factors to dengue hemorrhagic fever (DHF) outbreaks" [6]. AC temp between 24°C and 28°C can lead to dengue mosquitoes breeding in your home, climate expert warns. Pune News, Updated on Apr 23, 2018 02:50 PM IST". The ideal temperature for mosquito breeding is in the range of 24-28 degrees Celsius and many times, air conditioners inside houses are set at a temperature of 24 degrees Celsius, which helps in breeding and survival of the dengue-transmitting *Aedes* mosquitoes," [7]. Literature surveys also reveal various information regarding the specific range of maximum and minimum temperatures and relative humidity under which the growth and development of *Aedes* sp. mosquitoes are not only optimum rather beyond that range it is fatal for the different stages of the life cycle of *Aedes* sp. [8-12]. A meta-analysis by some authors on the "effects of temperature on the development and survival of the *Aedes* sp. mosquito reveals so many significant observations. The *Aedes* mosquito species, which are the vectors for the transmission of the dengue virus (DENV) to humans, are becoming increasingly susceptible to the formidable effects of influential factors, especially temperature" [13]. In a study on "Impact of climate change on abundance, distribution, and survival of *Aedes species*: Systematic review it has been concluded that climate components like rainfall, temperature, humidity, wind velocity, and season affect the distribution of Aedes species Among all the components, the one that has the most effect on the mosquito density are the rainfall and temperature." [14]. As per Sood, 2016 [15] some important life cycle changes are coming into knowledge regarding *Aedes* sp. but there is no report in favour of such type of unorthodox thinking or review. Considering all the above-mentioned information, some effective measures and studies have been followed for making the relation between physical parameters and niche suitability of two different ecosystems causing ecological problems leading to a condition of severe community health problems and *Aedes* sp. mosquito growth and development in respect of a new ecosystem.

MATERIALS AND METHODS

The study and survey have been carried out at Asansol and Burnpur belongs to the state of West Bengal, India. The coordinates of the areas are 23.68890N, 86.96610, and 23.6445°N, 86.9326°E respectively.

Sampling

Year around (April 2021 to March 2022) a total of 96 sample data were recorded based on Maximum and Minimum Temperature by following partly modified Diurnal Extrema Timing (DET) [16] and Relative Humidity simultaneously in both Ambient Atmosphere and Underground Septic tank environment by following the partially modified method [17-18] and vector population by following WHO, 2016 [21].

All the studies have been carried out under the supervision and guidance of the University Department of Life Science (Zoology), BBMK University, Dhanbad, Jharkhand, India along with some other associated laboratories [19-20]

Fig 1 Adult mosquitoes trapped into the cage for 48 hours

Fig 2 Different instar of pupa collected for a period of 48 hours for subsequent counting in the collecting jar, pupas are mingled with the larva of *Aedes* sp.

Method of data collection

Partial Diurnal Extrema Timing (DET) process has been followed, in the present study [16], month-wise diurnal Extrema temperature has been recorded. Data was recorded once every month, preferably 15th day of the month. For measuring the Diurnal Extrema Timing (Month) date and temperature, the ready thermometer (Minimum-Maximum Thermometers - 2 in number) was kept fitted for a period of 24 hrs. Around the cycle of the complete diurnal period, The Initial starting time was noon, finishing time was 11 AM. The used method was the same for measuring both the Ambient Atmosphere and the Underground Septic Tank environment. For measuring Relative Humidity, a Digital Hygrometer was used as mentioned above. The method was spot study, i.e. hygrometer was fitted and instantly recorded reading was collected both from the ambient environment and the Underground septic tank once in month.

Vector population productivity survey

Collection of the adult / pupal stage of *Aedes* sp. for the purposes has been carried out as follows. Vector Population has been collected from two quite different habitats from where the same stage of the life cycle of *Aedes* sp. was not possible due to positional and technical limitations. It has been ascertained by Eisen, 2014 [8] and also clearly mentioned that "the relationship between water temperature and development rate is similar for eggs, larvae, and pupae", so the stage of the life cycle is not a factor for productivity frequency calculation. As per WHO, 2016, "For reasons of practicality and reproducibility, the most common survey methodologies employ larval (active immatures, including pupae) sampling procedures rather than egg or adult collections. Funnel traps are used for sampling *Aedes* species and other container-breeding organisms in sites with poor or difficult access" [21]. So, for practicality and habitat specificity, adult mosquito is trapped, in a Septic Tank Environment, by fitting a mosquito trapping cage (Fig 1) in the outlet port of the septic tank environment and preserved by following the modified methodology [22] for subsequent counting and further proceeding.

For the Ambient Atmosphere, the Collection of pupa was carried out by following the modified funnel trap method by WHO, 2016 (Fig 2) For this study an abandoned water reservoir considered, situated in the open ambient atmosphere, was nearly equal volume to the selected septic tank for the study.

Statistical analysis

The Windows EXCEL 2016 software was used to do all the statistical analysis.

RESULTS AND DISCUSSION

From the Year around (April - 2021 to March -2022) survey following data have been collected and produced in tabular form along with nos. of vector trapped /captured. Table 1 shows the Physical Parameters of the Ambient Atmosphere with Collected Vector (Pupa stage of *Aedes* sp.). Table 2 shows the Physical Parameters of the Atmosphere inside the Septic Tank with Collected Vector (Adult stage of *Aedes* sp.)

Table 1 Physical parameters and pupa growth in ambient atmosphere

Period (April-2021 to March -2022)	Maximum Temperature (Celsius)	Minimum Temperature (Celsius)	Relative humidity (%)	Pupa of *aedes* sp. collected
April	42	28	20	NIL
May	40	25	50	NIL
June	38	27	60	NIL
July	36	27	90	197
August	38	21	90	361
September	33	25	90	370
October	35	24	95	373
November	32	18	79	155
December	25	11	53	NIL
January	25	16	53	NIL
February	35	16	28	NIL
March	39	23	50	9

Table 2 Physical parameters and adult stage of *Aedes* sp and atmosphere inside septic tank

Period (April-2021 to March -2022)	Maximum Temperature (Celsius)	Minimum Temperature (Celsius)	Relative humidity (%)	Pupa of *aedes* sp. collected
April	35	24	60	219
May	35	24	65	205
June	33	25	65	211
July	32	29	95	345
August	32	30	95	333
September	32	31	95	365
October	29	27	95	341
November	26	24	85	305
December	23	20	65	254
January	22	*19*	70	231
February	24	22	60	225
March	33	28	65	223

Significant ANOVA results were generated for both parameters but the septic tank environment is more suitable for the vector population, adult-stage, formation of *Aedes* sp, (F= 177.2, p<0.0000) (Table 6). The physical parameters of the atmosphere inside the Septic Tank are more suitable for the multiplication of *Aedes* sp mosquito. In the Tukey HSD test for pairwise mean comparison, (Table 3-6), the vector population development rate is 222.329% higher in the septic tank environment in comparison to the ambient atmosphere. The temperature extrema (both low and high) of the Septic tank environment are more favorable than the environment of the ambient atmosphere. In Tukey's HSD analysis (Table 4-7), p-values are more significant in the Septic Tank Environment (p< 0.01), the p-value is significant <0.01 level for physical parameters and < 0.001 for vector population, denoting means are significantly different and the mentioned events affected by their condition. If the p-value is less than the chosen significance level (often 0.05), then we reject the null hypothesis in favor of the alternative hypothesis. This suggests there is a statistically significant difference in mean stress levels between the three exercise types" [23]. The septic tank environment provides more favorable conditions for the development and multiplication of Aedes sp mosquitoes compared to the ambient atmosphere.

Table 3 ANOVA test for physical parameters of ambient atmosphere with collected vector (Pupa stage of *Aedes* sp.)

Source	SS	df	MS	F value	p-value
Between group	99469.8333	4	24867.4583	F = 4.58527	0.002879.
Within-group	298283.5	55	5423.3364		
Total	397753.3333	59			

Grand Mean 49.667 CV 148.28

Table 4 Pairwise comparisons of post hoc Tukey HSD test for physical parameters of ambient atmosphere with collected vector (Pupa stage of *Aedes* sp.)

	Maximum Temperature (Celsius)	Minimum Temperature (Celsius)	Relative humidity (%)	Pupa of *Aedes* sp.	Period
Max. temp. (Celsius)		0.9923	0.879	0.04078 *	0.879
Min. temp. (Celsius)	0.6154		0.6444	0.0127 *	0.9863
Relative humidity	1.333	1.948		0.2992	0.3375
Pupa of *Aedes* sp.	4.104	4.72	2.771		0.002811**
Period	1.333	0.7173	2.666	5.437	

P= 0.05, Standard error for comparison= 30.065, *<0.5, **<0.01

Table 5 Tukey test for pairwise mean comparisons for physical parameters of ambient atmosphere with collected vector (Pupa stage of *Aedes* sp.)

Variable	Mean	Homogeneous groups
Max. temp. (Celsius)	34.833	B
Min. temp. (Celsius)	21.750	B
Relative humidity	63.167	AB
Pupa of *Aedes* sp.	122.08	A
Period	6.5000	B

Critical Q Value= 3.989. The critical value for comparison = 84.806

There are 2 groups (A and B) in which the means are significantly different from one another.

Table 6 ANOVA test for physical parameters of the atmosphere inside the septic tank with collected vector (Adult stage of *Aedes* sp.)

Source	SS	df	MS	F value	p-value
Between group	570854	4	142713	177.2	0.0000
Within group	44285.1	55	805.183		
Total	615139	59			

Grand Mean 81.817 CV 34.68

Table 7 Pairwise comparisons of post hoc Tukey HSD test for physical parameters of atmosphere inside the septic tank with collected vector (Adult stage of *Aedes* sp.)

	Maximum Temperature (Celsius)	Minimum Temperature (Celsius)	Relative humidity (%)	Adult stage *Aedes* sp.	Period
Max. temp. (Celsius)		0.9954	0.001611*	<0.0001**	0.2798
Min. temp. (Celsius)	0.5392		0.000463**	<0.0001**	0.4922
Relative humidity	5.687	6.226		<0.0001**	<0.0001**
Adult of *Aedes* sp.	29.51	30.05	23.83		<0.0001**
Period	2.828	2.289	8.515	32.34	

P= 0.05, Standard error for comparison= 30.065, *<0.1, **<0.001

Table 8 Tukey test for pairwise mean comparisons for physical parameters of atmosphere inside the septic tank with collected vector (Adult stage of *Aedes* sp.)

Variable	Mean	Homogeneous groups
Max. temp. (Celsius)	29.667	A
Min. temp. (Celsius)	25.250	B
Relative humidity	76.250	C
Adult stage of *Aedes* sp.	271.42	C
Period	6.5000	C

Critical Q Value= 3.989. The critical value for comparison = 32.677
There are 3 groups (A, B, and C) in which the means differ significantly among A, B and C

Data depicted in (Table 5-8) show the mean difference of various parameters of the Ambient Atmosphere with Collected Vector (Pupa stage of *Aedes* sp.) and the parameters of the Atmosphere inside the septic tank with collected vector (Adult stage of *Aedes* sp.), where different letters expressing the mean is different. Tukey's HSD analysis showed that there was a significant difference between the means of the number of Pupa of *Aedes* sp. and Max temp./ Min. temp. Therefore, we can conclude that the number of pupae produced depends on the temperature, whereas the temperature and humidity and the physical factors are more suitable for mosquito generation in septic tanks [24-25]. The correlation of vector population with humidity is highly positive in both conditions (Table 9-10). The physical parameters minimum temperature and vector transformation r- value in the Septic Tank Environment are more favorable than the values of the environment of the Ambient Atmosphere. A few parameters are neutral and others are negatively correlated with each other [26].

Table 9 Correlations (Pearson) for physical parameters of ambient atmosphere with collected vector (Pupa stage of *Aedes* sp.)

	Max temp. (Celsius)	Min. temp. (Celsius)	Relative humidity	Pupa of *Aedes* sp.	Period
Max temp. (Celsius)					
Min. temp. (Celsius)	0.2078				
Relative humidity	-0.4550	0.2273			
Pupa of *Aedes* sp.	-0.3039	0.1711	0.8848		
Period	0.2582	-0.3354	-0.8938	-0.7020	

Table 10 Correlations (Pearson) for physical parameters of atmosphere inside the septic tank with collected vector (Adult stage of *Aedes* sp.)

	Max temp. (Celsius)	Min. temp. (Celsius)	Relative humidity	Pupa of *Aedes* sp.	Period
Max temp. (Celsius)					
Min. temp. (Celsius)	0.6500				
Relative humidity	0.1612	0.7866			
Pupa of *Aedes* sp.	0.0777	0.7614	0.9644		
Period	-0.9504	-0.4456	0.0000	0.0927	

The present study is identical and supports the observation by Beserra [27], statistical analysis of the collected data clearly and specifically indicated that the septic tank environment is at par with optimal temperature, 22°C - 32°C, for the development of Aedes sp. Vector. It has been ascertained by Marinho [10] and Eisen [8] that "no embryonic development or survival of the larval stages occurred at 39° C, also the temperature extremes of 16ºC and 36°C decreased the number of eggs per female considerably and development drops dramatically". These statements also corroborate the result of statistical analysis of the present paper for Ambient Environment Atmosphere where vector development frequency is very poor.

CONCLUSION

Based on the analysis of the aforementioned data and results, it has been determined that the methodologies employed in this study effectively support the stated purpose. The present study yielded findings that indicate a positive correlation between *Aedes* sp. and various physical factors, including Maximum Temperature, Minimum Temperature, and Relative Humidity, within the water reserve of a multi-chambered Septic Tank. The growth of vectors might be regarded as a highly significant alternate habitat for *Aedes* sp. mosquitoes. Statistical analysis reveals that the mean value of vector development is F= 271.42 in the septic tank environment and F= 122.08 in the ambient atmosphere. Additionally, the F-value is 37.55500404 times higher in the septic tank environment compared to the ambient atmosphere. The growth and development of faunal creatures, particularly the sensitive immature stages of *Aedes* sp., are primarily influenced by physical parameters such as temperature and relative humidity. Therefore, both the raw data and the statistical analysis confirm that the septic tank environment is increasingly favorable for the production of *Aedes* sp. vector population in all physical parameters. The water reserve within a sewage tank has been found to effectively serve as an alternative habitat for *Aedes* sp., a medically significant mosquito species, thereby creating a new ecological niche.

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