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# Effects of leaf litter diversity on the pupal development of stream puddle mosquitoes (Culicidae: Insecta)

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**Abstract**
The leaf litter input provides the dietary source for aquatic insect communities in streams, yet the consequences of diversity of plant material have not been studied in stream puddles important for the production of mosquitoes. To study the influence of leaf litter and the distribution of mosquito larvae in stream puddles and how diversity in leaf litter affects container-inhabiting mosquitoes, we examined the field survey in Nilgiri biosphere reserve of Southern Western Ghats and tested the effects of dominant riparian species on the development of mosquito larvae, *Aedesaegypti*. In the field survey, six species in two genera of mosquitoes were collected. *Aedes pesudotaniatus* was predominant species in stream puddles. The statistical analyses revealed that riparian cover, leaf litter and elevation were significant factors for the distribution of mosquitoes. The laboratory result indicates that the combination of three leaves of *Spondias pinnata*, *Acacia caesia* and *P. avettaindica* was reinforced the fast larval growth and huge production of *Ae. aegypti*. Overall results showed that leaf diversity does have a different effect on the development of mosquitoes in stream puddles.

**Keywords:** leaf litter, *Aedes*, stream puddle, mosquito larvae, India

## Introduction

The water flow delivers every facet of existence for life in lotic habitats and provides shelter or medium to each habitat, which access to an entry of stream organisms. The lotic organisms have grown in diverse and interesting ways in the different environments of streams [1]. For lotic organisms, the riparian vegetation is the major source of food. The entry of freshly fallen leaves from riparian species into stream is subjected to physical abrasion, microbial degradation and invertebrate fragmentation [2]. The rate of decomposition and invertebrate assemblages are correlated with nutritional quality of leaf species [3, 4].

In a typical stream, the variety of stream substrates like bedrock, boulders, pebbles and sand are available and these substrates offer variety of aquatic invertebrate assemblages. When the water flow decreases or increases, the holes/cavities in bedrock and large cobbles filled by water and they are referred to as instream puddles. These puddles are ideal habitats for breeding mosquitoes [5]. The main source of nutrition in stream puddles is derived from leaf litter [6]. The mosquito larvae obtain food by two ways: from the microbial communities associated with leaf detritus and by fine particulate organic matter from detritus dissolved in the water column [7, 8].

The results of laboratory experiments revealed that mosquito larval development is influenced by leaf species identity and ratio of leaf species combinations [9-11]. At the same time, some studies reflect that certain leaf species contain toxic chemicals (tannins and lignins) act at inhibitory activity to the development and survival of aquatic communities [12, 13]. Although relationship between leaf litter and larval development of mosquito is clearly known in tree-hole mosquitoes in forest areas [14], this pattern is lacking in in stream puddle associated mosquitoes. Hence, the present study is focused to study the relationship between leaf litter and mosquito larvae in stream puddles of Nilgiri biosphere reserve of South India and to analyze the effects of leaf litter species on the development of immature mosquitoes in the laboratory condition. Since, the wide distribution of mosquitoes, especially *Aedes* species in this reserve and tribal communities associated habitats attract for breeding of mosquitoes, the present study would be useful for controlling vector mosquito species and maintaining tribal health.

## Materials and methods

### Field Survey

The present study was carried out in streams of Nilgiri Biosphere Reserve of Southern Western Ghats. The geographic area of Nilgiri Biosphere Reserve lies between latitude: 10°50′ N - 12°16′ N and longitude: 76°00′ E to 77°15′ E and the annual rainfall ranges from 500 to 7000 mm. A pilot study conducted in nine streams of Nilgiri Biosphere Reserve between December 2019 and January 2020. In each stream, the instream puddles of bedrock pool, boulder cavities and sand pools were observed for investigating the presence of mosquito larvae. The physico-chemical characters of temperature, pH, total dissolved solids, salinity and conductivity of water in stream puddles were measured with the help of PCS tester 35 (Eutech instruments, India). The puddle depth and diameter were measured using a measuring tape. The floating and submerged leaf litter species were identified and their density was also noted.

The physical and chemical parameters of water in stream puddles from nine sampling sites of Nilgiri biosphere reserve were measured. Of nine sampling streams, six sites were bedrock pools and three sites (Kallar, Conoor A and

Kottacombai) were sand pools. The latitude and longitude ranged from 11º 19ˈ to 11º 23ˈ N and 76º 48ˈ to 76º 56ˈ E, respectively. The sampling sites were between 400 and 1600 m. The higher water temperature (26 ºC) was observed in Kunjapaarai stream and lower temperature (18 ºC) at Conoor stream A. The bedrock pools diameter were ranged from 15 to 60 cm and sand pools from 13 to 40 cm. The mean water depth and pH of puddle was 8 cm and 7.5, respectively. The conductivity was between 70 and 309 µSec. The average total dissolved solids and salinity 135 ppm and 93 ppt. The riparian cover ranged from 40 to 80% and leaf litter density occurred between 10 and 30%.

In each sampling site, the presence of mosquito larvae in stream puddles was observed. With the presence of larvae in puddles in each sampling site, mosquito larvae were collected from puddle's water by using circular dip net (10 cm in diameter x 10 cm depth). The dip ranged from 5 to 10 times in stream puddles with 2 to 3 minutes interval, since mosquito larvae may not collect if it goes to benthic or entering inside the detritus in puddle (with 1 or two minutes interval, larvae moved from benthic/detritus to surface region for respiratory purpose). The collected larvae and pupae were placed in a plastic container (5 cm in diameter x 5 cm in deep) half filled with respective puddle's water. Then, all containers were carefully transported to the laboratory, where larvae and pupae were reared until the emergence of adults for the purpose of identification. The emerged adults were stored in a glass test tubes plugged with cotton individually.

**Laboratory Experiment**

In the sampling sites, the dominant riparian species nearer to the stream puddles were identified. Though, the sampling sites have variety of plants including shrubs, five riparian species were observed, which are common in the study area as *Spondias pinnata* (SP), *Cassia montana* (CM), *Tamarindus indica* (TI), *Acacia caesia* (AC) and *Pavetta indica* (PI). From the study area, the fresh fallen leaves were collected under the respective riparian species. The collected leaves were sorted as species-wise, stored in polythene bags and brought to the laboratory , where all bags were stored at 4ºC till the beginning of experiment. A laboratory experiment was undertaken into two steps determine differences in the development of mosquito larvae and pupation time among the five litter species. The first step, to form the microcosm and extract the nutrient of respective leaf litter, litter with water was kept for 8 days at adjust to study area temperature. The second step, introduction of mosquito larvae in microcosm of water for determining the development and pupation time.

The experimental design was (66 number = 5 leaf species x 3 replicates + 16 mixed leaf species x 3 replicates + 1 control x 3 replicates). Each of the 66 containers initially 100 ml of distilled water, 1 ml of filtered stream puddles water collected from study area and 1 g of dry weight of leaves. The containers (66) were separated into five sets: individual leaf species-5 (1 g of each species- SP, CM, TI, AC and PI), two mixed leaves-10 (0.5 g of one species + 0.5 g of another species – SP+CM, SP+TI, SP+AC, SP+PI, CM+TI, CM+AC, CM+PI, TI+AC, TI+PI and AC+PI), three mixed leaves-5 (0.33g of each species – SP+CM+TI, SP+AC+PI, CM+TI+AC, CM+TI+PI and TI+AC+PI), five mixed leaves-1 (0.2 g of each species –SP+CM+TI+AC+PI) and control -1 (without leaves). All containers were allowed to stand for 8 days for allowing fungal and bacterial growths and dissolving nutrients of leaves in water. Then, end of the 8$^{th}$ day, leaves were removed with a nylon sewer.

A day before (7$^{th}$ day), eggs of*Aedesaegypti* borrowed from Centre for Research in Medical Entomology (CRME), Indian Council of Medical Research (ICMR), Madurai were placed in a tray containing distilled water. The eggs were hatched out within 6-8 hours and added the powder of dog biscuit in the water surface and they were maintained for next day. On 9$^{th}$ day, 25 larvae (1$^{st}$ instar) were taken with the help of Pasteur pipette and introduced in each container. All containers were maintained at 23°C and were checked daily for newly formed mosquito pupae. The developed pupae were captured with the help of wide tip Pasteur pipette and they were stored in 70% ethanol. During the whole experimental study periods, chemical characters of pH, conductivity, total dissolved solids, temperature and salinity of water in each container were measured.

**Data analysis**

The data of physico-chemical parameters, faunal distribution and development of immature of mosquitoes from field as well as laboratory were organized in MS-Excel spreadsheet, where the arithmetic mean, standard deviation and percentile calculations were done. To show the abundance of species in each sampling site, matrix plot based on the distribution of total number of individuals were used. Pearson correlation coefficient were calculated for measuring relationship between abundance of larvae and environmental factors. In addition, Canonical Correspondence Analysis (CCA) was used to measure the relationship between environmental variables and species abundance and pupal development. All the above statistical analyses were calculated by using the statistical software of PAST version 4.01 [15]. The cohort production for immature development of mosquito was calculated based on Benke [16]. Production during the interval is calculated by using the formula of $P= \Sigma L \times \Delta P$, as $\Delta P$ times the mean density (L) between dates. Total cohort production (P) is the sum of production for all time intervals: P is the total number of pupae developed, N is the total number of larvae.

**Results**

**Field investigation**

In total of 708 mosquito larvae were collected under six species and two genera (*Aedes* and *Anopheles*) from nine sampling sites of Nilgiri biosphere reserve (Fig. 1). The individuals from the genus *Aedes* were predominantly (58%) present in stream puddles. The species-wise analysis indicates that *Aedespseudotanitaus* had the higher percentage (37%) than the other species. The habitat-wise distribution of immature mosquitoes showed that bedrock pool had five species in two genera whereas sand pool had the three species in genera. In the bedrock pools, *Aedesvitttaus and Anopheles stephensi* were dominated over the other species, while *Ae. pseudotaniatus* was occupied high percentage in sand pools.

Pearson correlation coefficient was calculated for determining the relationship between the larval abundance and environmental variables in stream puddles of sampling sites (Table 1). Results of this analysis showed that larval abundance of mosquito in stream puddles were significantly related with the factors of leaf litter, conductivity and total dissolved solids. To determine the respective environmental parameter with mosquito species abundance, the multi-parametric test of Canonical Correspondence Analysis (PCA) were measured. The result of CCA showed that eigen value was 0.023 in axis1 and 0.005 in axis 2 and the cumulative variance was 82.2% in axis 1 and 17.8% in axis 2. The loadings of CCA indicate that elevation was a significant factor rather than other environmental parameters. In axis 1, riparian cover and leaf litter were significantly associated with *An. dravidicus* and *An. stephensi* whereas elevation was significantly associated with the four mosquito species revealed in axis 2. Thus, riparian cover, leaf litter and elevation were an important factor the distribution of mosquito species in stream puddles (Fig. 2).

**Table 1.** Pearson correlation coefficient analysis between larval abundance of mosquitoes and environmental variables of sampling sites.

| | No. of sample | Test value (*r*) | Significance (*P*) |
|---|---|---|---|
| Latitude | 9 | -0.32 | 0.01 |
| Longitude | 9 | 0.22 | 0.01 |
| Elevation | 9 | -0.53 | 0.01 |
| Water temperature | 27 | 0.34 | 0.1 |
| Conductivity | 27 | 0.69 | 0.1 |
| TDS | 27 | 0.68 | 0.1 |
| pH | 27 | -0.15 | 0.1 |
| Salinity | 27 | 0.68 | 0.1 |
| Pool diameter | 32 | -0.20 | 0.1 |
| Water depth | 32 | 0.07 | 0.1 |
| Riparian cover | 27 | -0.44 | 0.1 |
| Leaf litter | 32 | 0.98 | 0.1 |

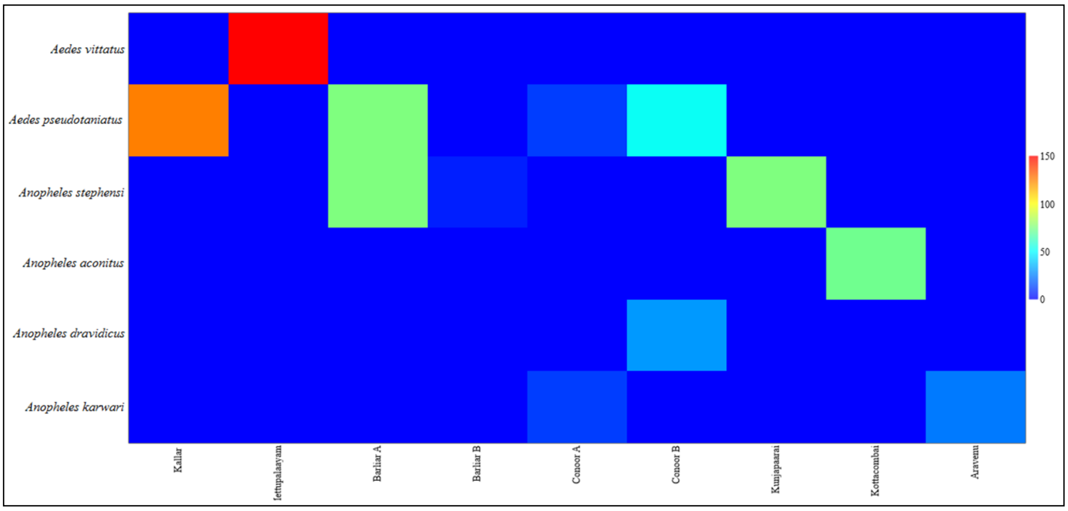


**Fig 1:** Distribution of mosquito larvae (no. of individuals) in stream puddles of nine sampling sites of Nilgiri Biosphere reserve of Western Ghats.

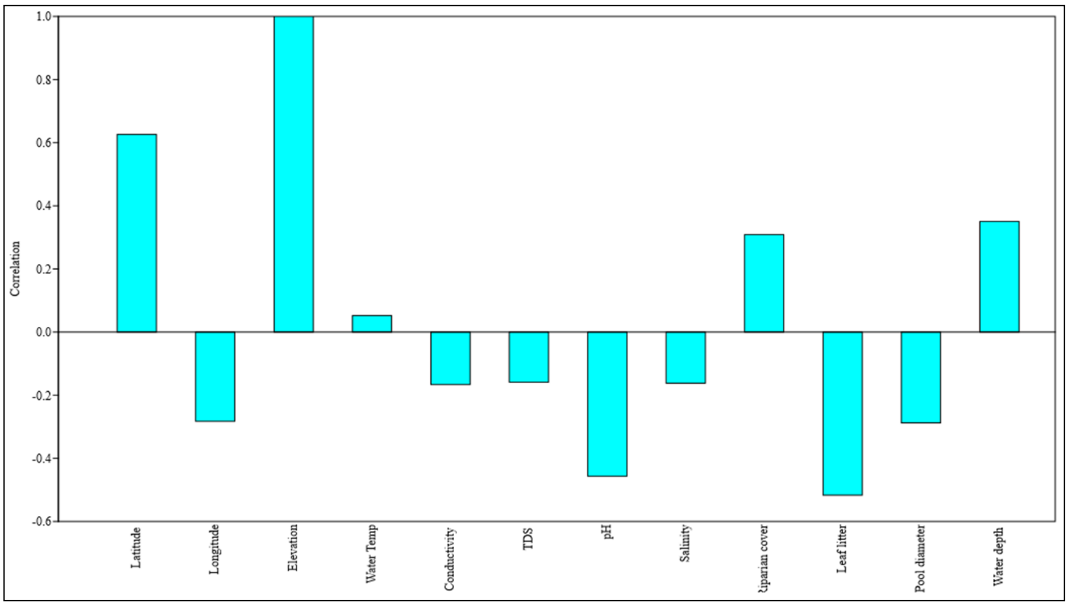


**Fig 2:** The ordination plot of Canonical Correspondence Analysis between mosquito species abundance and environmental variables of sampling sites.

**Laboratory Analysis**

The field investigation of sampling sites between larval abundance of mosquitoes and environmental variables in stream puddles revealed that elevation was a significant factor as well as leaf litter and associated variables of total dissolved solids, pH and conductivity were another important factors for the distribution of immature mosquitoes. Therefore, the laboratory experiment was conducted for determining the relationship between leaf litter and the development of mosquitoes. The larvae of *Aedesaegypti* were taken for laboratory analysis, since *Aedes* larvae were common and occupied percentage in the field.

The physico-chemical characters of water temperature, pH, conductivity, total dissolved solids and salinity were measured throughout experimental periods (Table 2). The experimental period is divided into two parts as leaf litter incubation (1-8 days) and larval developmental periods (9-16 days). During the leaf litter incubation and larval developmental periods, the mean water temperature and pH were ranged from 26.6 to 28.1 ºC and 7.1 to 8.4, respectively. The high conductivity, total dissolved solids and salinity were observed in water with two mixed leaves of TI-AC and low value found in control.

The rates of larval survival, mortality and pupal development of *Ae. aegypti* were measured and are given in Table 3. In the six days observation, the larval mortality was found from 1st day to 3rd day and they ranged from 8 to 68%. In control, there was no mortality was observed. The highest mortality was found in water with five mixed leaves (SP-CM-TI-AC-PI) and the lowest mortality in each water with AC and PI leaves. The mortality of larvae was absent from 4th day to final experimental periods. The development of pupae was observed at 4th day in all containers except control and five mixed leaves, whereas the pupal development in five mixed leaves container occurred on 5th day and control during 6th day. The higher number of pupae was found in water three mixed leaves (SP-AC-PI) on 4th day. About 90% of pupae were developed between 4th and 6th day except SP-TI, AC, TI, SP and control.

Nearly, 80% of pupal development was found in water with PI, SP-PI and SP-AC-PI leaves and two combinations of CM-PI and SP-CM-TI-AC-PI had the lowest percentage (36%) of pupal development (Fig. 3). The cohort production value indicates that five containers were significantly produced high percentage of pupae (PI-2%, AC-3%, TI-AC and TI-AC-PI-7% and SP-AC-PI-70%) when compared to control and the water (Table 4). To understand the relationship between chemical characteristics of water for different leaves and production value of tested mosquito species, Principal Component Analysis was used (Fig. 4). PCA result indicates that conductivity, total dissolved solids and salinity were significantly related with TI-AC-PI, AC and TI-AC, while production of mosquito larvae was associated with SP-AC-PI.

**Table 2:** The mean physico-chemical parameters of water with different leaves during experimental periods. (Temp -Water temperature, Cond –Conductivity, TDS –Total dissolved solids, Sal –Salinity and the leaf detail is given in methodology).

| Leaves | Incubation period (1 – 8 days) | | | | | Larval development period (9 – 14 days) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Temp | pH | Cond | TDS | Sal | Temp | pH | Cond | TDS | Sal |
| Control | 27.0 | 7.4 | 31.73 | 80.63 | 22.67 | 27.6 | 8.1 | 288.1 | 204.6 | 137.2 |
| SP | 26.9 | 5.9 | 187.6 | 192.7 | 131.3 | 27.8 | 7.1 | 343.4 | 241.2 | 164.2 |
| CM | 26.9 | 5.7 | 554.3 | 409.7 | 279.7 | 27.8 | 7.1 | 655.6 | 466.4 | 320.4 |
| TI | 26.9 | 5.8 | 684.7 | 474.3 | 330.2 | 27.8 | 8.1 | 777.8 | 552.1 | 381.1 |
| AC | 26.6 | 6.8 | 803.0 | 571.7 | 389.3 | 27.8 | 8.3 | 967.2 | 687.4 | 478.8 |
| PI | 26.7 | 7.1 | 358.0 | 255.3 | 173.7 | 27.8 | 8.2 | 635.2 | 449.1 | 308.2 |
| SP-CM | 26.8 | 6.1 | 412.3 | 291.3 | 196.3 | 27.6 | 7.8 | 586.6 | 399.4 | 273.8 |
| SP-TI | 26.6 | 6.8 | 438.1 | 294.3 | 212.3 | 27.6 | 8.2 | 590.4 | 418.2 | 286.6 |
| SP-AC | 27.0 | 7.3 | 715.7 | 516.3 | 391.3 | 27.9 | 8.3 | 997.1 | 711.8 | 491.6 |
| SP-PI | 27.0 | 7.0 | 357.3 | 253.3 | 191.1 | 27.7 | 8.1 | 543.8 | 384.6 | 263.6 |
| CM-TI | 27.0 | 6.2 | 616.3 | 438.3 | 338.1 | 27.7 | 8.1 | 931.4 | 655.8 | 455.8 |
| CM-AC | 26.8 | 6.7 | 758.7 | 538.7 | 303.3 | 27.5 | 8.1 | 1039 | 739.6 | 511.8 |
| CM-PI | 26.7 | 6.1 | 447.1 | 317.3 | 303.3 | 27.6 | 7.4 | 589.4 | 416.6 | 285.6 |
| TI-AC | 26.6 | 6.7 | 893.7 | 678.7 | 435.2 | 28.1 | 8.3 | 1205 | 856.1 | 600.8 |
| TI-PI | 26.8 | 6.8 | 657.7 | 468.7 | 334.1 | 27.6 | 8.3 | 904.6 | 640.8 | 445.4 |
| AC-PI | 27.0 | 7.1 | 869.3 | 607.1 | 351.1 | 27.8 | 8.4 | 1080 | 761.4 | 532.6 |
| SP-CM-TI | 26.7 | 6.4 | 418.7 | 296.3 | 212.7 | 27.9 | 8.0 | 704.6 | 499.4 | 343.4 |
| SP-AC-PI | 26.8 | 7.3 | 491.0 | 348.7 | 260.7 | 27.8 | 8.3 | 638.4 | 451.4 | 310.8 |
| CM-TI-AC | 27.0 | 6.8 | 607.7 | 432.1 | 276.3 | 27.8 | 8.2 | 848.8 | 601.1 | 415.6 |
| CM-TI-PI | 26.7 | 6.4 | 532.1 | 376.7 | 286.7 | 27.6 | 8.0 | 724.2 | 513.6 | 352.4 |
| TI-AC-PI | 27.0 | 6.5 | 694.1 | 489.7 | 337.3 | 27.7 | 8.4 | 835.8 | 586.1 | 410.1 |
| SP-CM-TI-AC-PI | 27.3 | 6.9 | 690.7 | 487.0 | 217.1 | 27.5 | 8.3 | 900.0 | 637.6 | 442.4 |

**Table 3:** Larval developmentpattern of *Aedesaegypti* in the water with different leaves during experimental period. (LA -Larvae (No.), LM -Larval mortality (%) and PU –Pupal development (No.)

| | Initial | | | 1st day | | | 2nd day | | | 3rd day | | | 4th day | | | 5th day | | | 6th day | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | LA | LM | PU | LA | LM | PU | LA | LM | PU | LA | LM | PU | LA | LM | PU | LA | LM | PU | LA | LM | PU |
| Control | 25 | 0 | 0 | 25 | 0 | 0 | 25 | 0 | 0 | 25 | 0 | 0 | 25 | 0 | 0 | 25 | 0 | 0 | 10 | 0 | 15 |
| SP | 25 | 0 | 0 | 25 | 0 | 0 | 24 | 4 | 0 | 22 | 12 | 0 | 21 | 0 | 4 | 17 | 0 | 0 | 5 | 0 | 12 |
| CM | 25 | 0 | 0 | 25 | 0 | 0 | 20 | 20 | 0 | 15 | 40 | 0 | 12 | 0 | 1 | 8 | 0 | 3 | 0 | 0 | 8 |
| TI | 25 | 0 | 0 | 25 | 0 | 0 | 15 | 40 | 0 | 13 | 48 | 0 | 10 | 0 | 6 | 4 | 0 | 4 | 2 | 0 | 2 |
| AC | 25 | 0 | 0 | 24 | 4 | 0 | 23 | 8 | 0 | 23 | 8 | 0 | 23 | 0 | 2 | 21 | 0 | 0 | 5 | 0 | 16 |

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PI | 25 | 0 | 0 | 25 | 0 | 0 | 23 | 8 | 0 | 23 | 8 | 0 | 22 | 0 | 2 | 10 | 0 | 10 | 2 | 0 | 8 |
| SP-CM | 25 | 0 | 0 | 24 | 4 | 0 | 21 | 16 | 0 | 18 | 28 | 0 | 11 | 0 | 7 | 0 | 0 | 4 | 0 | 0 | 0 |
| SP-TI | 25 | 0 | 0 | 25 | 0 | 0 | 25 | 0 | 0 | 22 | 12 | 0 | 20 | 0 | 1 | 17 | 0 | 2 | 4 | 0 | 13 |
| SP-AC | 25 | 0 | 0 | 25 | 0 | 0 | 16 | 36 | 0 | 15 | 40 | 0 | 13 | 0 | 3 | 3 | 0 | 7 | 0 | 0 | 3 |
| SP-PI | 25 | 0 | 0 | 22 | 12 | 0 | 20 | 20 | 0 | 18 | 28 | 0 | 19 | 0 | 7 | 6 | 0 | 6 | 0 | 0 | 6 |
| CM-TI | 25 | 0 | 0 | 25 | 0 | 0 | 18 | 28 | 0 | 15 | 40 | 0 | 14 | 0 | 8 | 1 | 0 | 5 | 0 | 0 | 1 |
| CM-AC | 25 | 0 | 0 | 24 | 4 | 0 | 20 | 20 | 0 | 15 | 40 | 0 | 12 | 0 | 7 | 1 | 0 | 4 | 0 | 0 | 1 |
| CM-PI | 25 | 0 | 0 | 25 | 0 | 0 | 15 | 40 | 0 | 10 | 60 | 0 | 9 | 0 | 9 | 0 | 0 | 0 | 0 | 0 | 0 |
| TI-AC | 25 | 0 | 0 | 25 | 0 | 0 | 22 | 12 | 0 | 20 | 20 | 0 | 18 | 0 | 12 | 0 | 0 | 6 | 0 | 0 | 0 |
| TI-PI | 25 | 0 | 0 | 25 | 0 | 0 | 25 | 0 | 0 | 15 | 40 | 0 | 13 | 0 | 9 | 0 | 0 | 4 | 0 | 0 | 0 |
| AC-PI | 25 | 0 | 0 | 25 | 0 | 0 | 25 | 0 | 0 | 20 | 20 | 0 | 17 | 0 | 5 | 6 | 0 | 6 | 0 | 0 | 6 |
| SP-CM-TI | 25 | 0 | 0 | 25 | 0 | 0 | 21 | 16 | 0 | 18 | 28 | 0 | 16 | 0 | 9 | 0 | 0 | 7 | 0 | 0 | 0 |
| SP-AC-PI | 25 | 0 | 0 | 25 | 0 | 0 | 24 | 4 | 0 | 21 | 16 | 0 | 19 | 0 | 14 | 4 | 0 | 1 | 0 | 0 | 4 |
| CM-TI-AC | 25 | 0 | 0 | 25 | 0 | 0 | 23 | 8 | 0 | 16 | 36 | 0 | 13 | 0 | 8 | 0 | 0 | 5 | 0 | 0 | 0 |
| CM-TI-PI | 25 | 0 | 0 | 25 | 0 | 0 | 19 | 24 | 0 | 17 | 32 | 0 | 13 | 0 | 6 | 3 | 0 | 4 | 0 | 0 | 3 |
| TI-AC-PI | 25 | 0 | 0 | 25 | 0 | 0 | 21 | 16 | 0 | 20 | 20 | 0 | 18 | 0 | 12 | 0 | 0 | 6 | 0 | 0 | 0 |
| SP-CM-TI-AC-PI | 25 | 0 | 0 | 25 | 0 | 0 | 22 | 12 | 0 | 8 | 68 | 0 | 8 | 0 | 0 | 1 | 0 | 7 | 0 | 0 | 1 |

**Table 4:** Calculation of cohort production for *Aedesaegypti* in the water with differentleaves. (D = days, C =control, Numbers 1 to 21 sequence of leaves as per table 3, P = production)

| | **Δ P** | | | | | | | **L** | | | | | | | **Δ P x L** | | | | | | | ***p*** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | |
| C | | 0 | 0 | 0 | 0 | 0 | 15 | | 25 | 25 | 25 | 25 | 25 | 18 | 0 | 0 | 0 | 0 | 0 | 0 | 263 | 263 |
| 1 | | 0 | 0 | 0 | 4 | 4 | 8 | | 25 | 25 | 23 | 22 | 19 | 11 | 0 | 0 | 0 | 0 | 86 | 76 | 88 | 250 |
| 2 | | 0 | 0 | 0 | 1 | 2 | 10 | | 25 | 23 | 18 | 14 | 10 | 4 | 0 | 0 | 0 | 0 | 14 | 20 | 40 | 74 |
| 3 | | 0 | 0 | 0 | 6 | 2 | 0 | | 25 | 20 | 14 | 12 | 7 | 3 | 0 | 0 | 0 | 0 | 69 | 14 | 0 | 83 |
| 4 | | 0 | 0 | 0 | 2 | 2 | 14 | | 25 | 24 | 23 | 23 | 22 | 13 | 0 | 0 | 0 | 0 | 46 | 44 | 182 | 272 |
| 5 | | 0 | 0 | 0 | 2 | 8 | 16 | | 25 | 24 | 23 | 23 | 16 | 6 | 0 | 0 | 0 | 0 | 45 | 128 | 96 | 269 |
| 6 | | 0 | 0 | 0 | 7 | 3 | 3 | | 25 | 23 | 20 | 15 | 6 | 0 | 0 | 0 | 0 | 0 | 102 | 17 | 0 | 118 |
| 7 | | 0 | 0 | 0 | 1 | 1 | 14 | | 25 | 25 | 24 | 21 | 19 | 11 | 0 | 0 | 0 | 0 | 21 | 19 | 147 | 187 |
| 8 | | 0 | 0 | 0 | 3 | 4 | 7 | | 25 | 21 | 16 | 14 | 8 | 1.5 | 0 | 0 | 0 | 0 | 42 | 32 | 11 | 85 |
| 9 | | 0 | 0 | 0 | 7 | 1 | 5 | | 24 | 21 | 19 | 19 | 13 | 3 | 0 | 0 | 0 | 0 | 130 | 13 | 15 | 157 |
| 10 | | 0 | 0 | 0 | 8 | 3 | 2 | | 25 | 22 | 17 | 15 | 8 | 0.5 | 0 | 0 | 0 | 0 | 116 | 23 | 1 | 140 |
| 11 | | 0 | 0 | 0 | 7 | 3 | 2 | | 25 | 22 | 18 | 14 | 7 | 0.5 | 0 | 0 | 0 | 0 | 95 | 20 | 1 | 115 |
| 12 | | 0 | 0 | 0 | 9 | 9 | 9 | | 25 | 20 | 13 | 10 | 5 | 0 | 0 | 0 | 0 | 0 | 86 | 41 | 0 | 126 |
| 13 | | 0 | 0 | 0 | 12 | 6 | 6 | | 25 | 24 | 21 | 19 | 9 | 0 | 0 | 0 | 0 | 0 | 228 | 54 | 0 | 282 |
| 14 | | 0 | 0 | 0 | 9 | 5 | 5 | | 25 | 25 | 20 | 14 | 7 | 0 | 0 | 0 | 0 | 0 | 126 | 33 | 0 | 159 |
| 15 | | 0 | 0 | 0 | 5 | 1 | 7 | | 25 | 25 | 23 | 19 | 12 | 3 | 0 | 0 | 0 | 0 | 93 | 12 | 21 | 126 |
| 16 | | 0 | 0 | 0 | 9 | 2 | 2 | | 25 | 23 | 20 | 17 | 8 | 0 | 0 | 0 | 0 | 0 | 153 | 16 | 0 | 169 |
| 17 | | 0 | 0 | 0 | 14 | 13 | 9 | | 25 | 25 | 23 | 20 | 12 | 2 | 0 | 0 | 0 | 0 | 280 | 150 | 18 | 448 |
| 18 | | 0 | 0 | 0 | 8 | 3 | 3 | | 25 | 24 | 20 | 15 | 7 | 0 | 0 | 0 | 0 | 0 | 116 | 20 | 0 | 136 |
| 19 | | 0 | 0 | 0 | 6 | 2 | -1 | | 25 | 22 | 18 | 15 | 8 | 1.5 | 0 | 0 | 0 | 0 | 90 | 16 | 1.5 | 108 |
| 20 | | 0 | 0 | 0 | 12 | 6 | 6 | | 25 | 23 | 21 | 19 | 9 | 0 | 0 | 0 | 0 | 0 | 228 | 54 | 0 | 282 |
| 21 | | 0 | 0 | 0 | 0 | 7 | 5 | | 25 | 24 | 15 | 9 | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 39 | 5 | 44 |

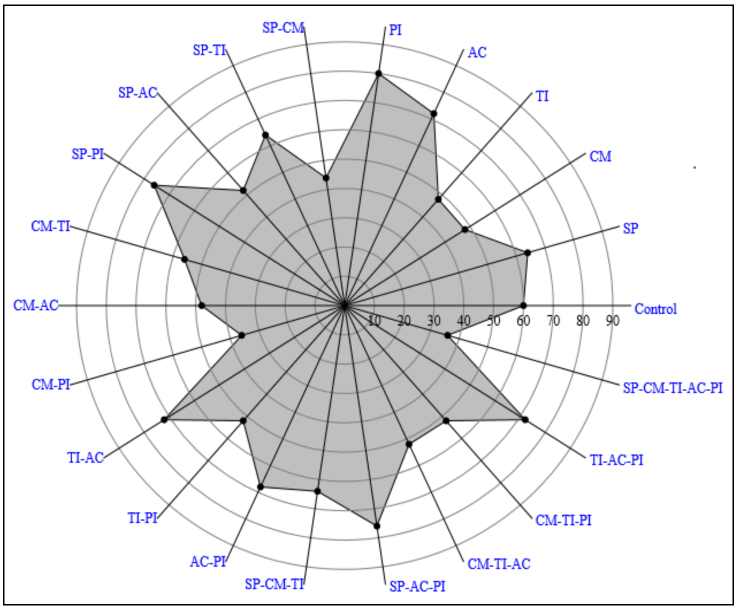


**Fig 3:** The radar chart showing the percentage of pupal development of*Aedesaegypti* in all water samples.

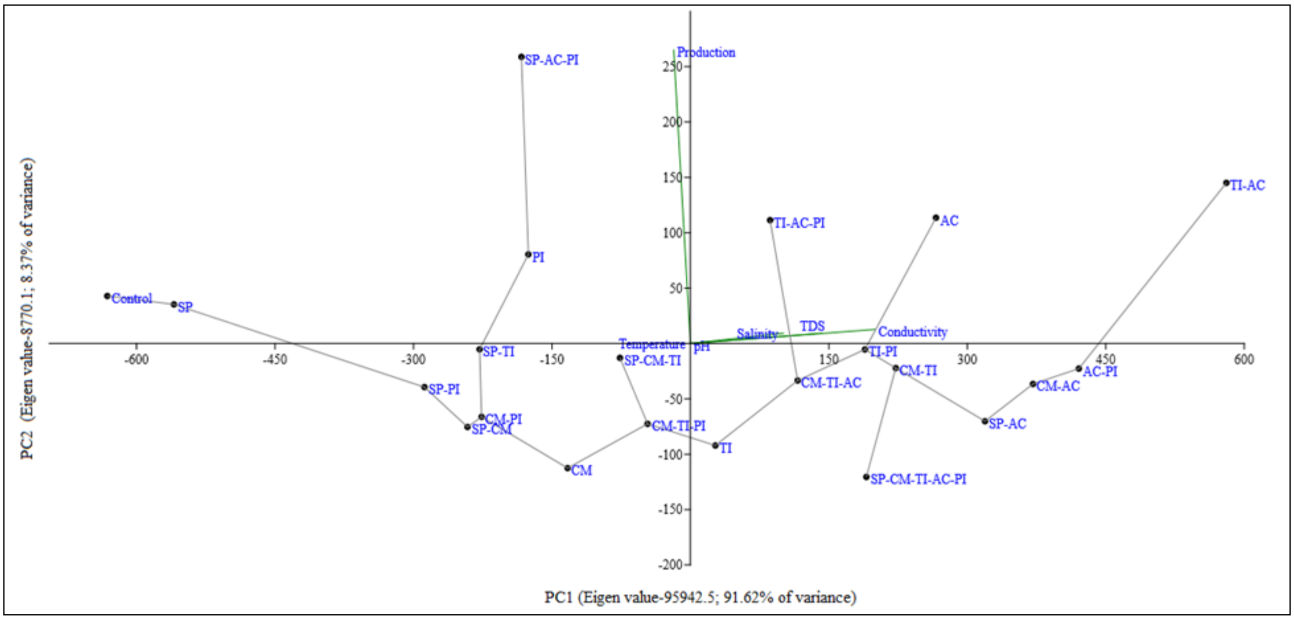


**Fig 4:** Biplot of Principal Component Analysis for chemical characters of water with different leaves and production of mosquitoes.

**Discussion**

Riparian vegetation in forest areas is an imperative factor when considering the stream habitats of benthic macroinvertebrates and they affects aquatic insect diversity and abundance [17]. In a riparian forest, leaf litter input in the stream plays various roles for the aquatic insect assemblage depending on the litter species [18]. The various study revealed to effects of litter species on the assemblage of aquatic insect streams, but lack of knowledge was found on the effect of litter species on immature mosquitoes in stream puddles. In the present study, field and laboratory studies concerning the relationship between leaf litter and mosquito larvae distributed in stream puddles were investigated.

In the field, six species under two genera of mosquito larvae were collected in stream puddles (bedrock and sand pools) of Nilgiri biosphere reserve. The individuals of *Aedes* were predominant in stream puddles than the genus *Anopheles*. This finding reflects that stream puddles containing natural water, which support the population of *Aedes* mosquito. *Aedes* can breed the twelve types of natural habitats including rock holes, tree holes, leaf axils, bamboo joints and coconut shells [19]. Among the larvae of *Aedes*, *Ae. pseudotaniatus* had the highest percentage in stream puddles of the present study. This may be due to stream puddles may provide an ideal habitat structure with sufficient nutrients for enhancing the production of *Ae. pseudotaniatus*.

Although the distribution of mosquito populations is occurred up to 1,500 m of elevation throughout the year, but mosquitoes may occur seasonally at higher elevations [20]. *Ae. taeniorhynchus* is present in dry seasons at higher elevations due to the availability of suitable breeding habitats for mosquitoes and conditions that favor abundance and attraction of breeding [21]. Similarly, elevation is a significant factor the distribution of mosquitoes in stream puddles revealed by statistical analyses. Since Niligiri biosphere reserve is protected by local government and maintaining the natural habitats, moreover this region is a familiar for tourism, the larval abundance of mosquitoes in stream puddles is significant and related with elevation.

Canonical Component Analysis showed that leaf litter, riparian cover and total dissolved solids were significantly related with the larval abundance of mosquitoes in the study area. The leaf litter input, especially decaying leaves and other plant matter is a vital resource for invertebrates in aquatic environments [22]. Many benthic macroinvertebrates directly consume leaf litter or feed the fine particulate organic matter dissolved in water and their production are affected by detritus quality and quantity [23, 24], which restrict the development of some aquatic insect species [25, 26].

For investigating the direct relationship between riparian species and the development of mosquito larvae, the laboratory experiment was done with five different dominant riparian species. The individual and combination of plant litter species in water were formed as nutrient microcosm within a week. Though each litter species and combinations provided diversified nutrient quality in water, the high conductivity, total dissolved solids and salinity were observed in water with combination of *Tamarindusindica* and *Acacia caesia*. The leaves of these two plants are having essential and non-essential amino acids with the presence of tannins and steroids [27, 28], moreover leaves of both species are tiny, which may easily decompose by bacteria and fungi and possible their nutrients may excessively dissolved in water than the other combination of leaves.

The result of the experiment showed that the highest mortality of *Ae. aegypti* larvae was observed in water with combination of five leaves species. It may be due to the accumulation of surplus nutrients in the water, which may be unfavorable for the growth of larva. Simultaneously, control had no mortality of larvae up to end of the experiment period. In the control, we added artificially prepared food, which favored the growth of larvae. In the all containers with different combination of leaves, no mortality of larvae was observed from 4$^{th}$ day. The rate of growth with different leaf species contains toxic chemicals (e.g., tannins and lignins) that have been detrimental to the development and survival of aquatic communities [29, 13].

The cohort production analysis and PCA resulted that the fast growth and high production value were found in the combination three leaves (*Spondiaspinnata*, *Acacia caesia* and *Pavettaindica*). The high production and rapid growth of *Ae. aegypti*larvae may be the presence of sufficient nutrients, essential growth factors and anti-microbial components in the water. The fruits and leaves of *S. pinnata* are having the excellent nutrients, bioactive compounds, as well as antioxidant compounds [30, 31], the leaves of *Acacia caesia* has an anti-microbial compounds and it is used for several microbial infectious diseases [27] and *Pavettaindica* leaves are good source of essential oils [32].

Overall, the high percentage of *Ae. aegypti* larvae was found in stream puddles of sampling sites. The environmental factors of riparian cover, leaf litter and elevation were significant factor for the distribution of mosquito species. Of these three factors, elevation was determining the most number of species revealed by statistical analyses. The laboratory experiment indicates the combination of three leaves of *Spondiaspinnata*, *Acacia caesia* and *Pavettaindica* was significantly supported the fast growth and large production of tested mosquito species (Ae. *aegypti*). This information would be helpful for understanding the stream puddle associated mosquito community and used for controlling mosquitoes if vector mosquito species is present. The present study suggests conducting blood-feeding behavior pattern of adult mosquitoes and identification of vector species in the study area.

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