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# Periphytic microalgae colonization in mosquito breeding stream puddles of Southern Western Ghats

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## Abstract

We examined the colonization of microalgae in mosquito breeding stream puddles of Southern Western Ghats, India. In addition, to study the relationship between microalgae and pupal development of mosquito (*Aedes aegypti*), the laboratory experiment was conducted. For this study, nine different sampling sites from Nilgiri biosphere reserve were selected, Tamil Nadu province, India. As a result, a total of 13 species of microalgae were observed from the field sampling taken for the study. The predominant members of microalgae found were belonging to the class Bacillariophyceae. Among the tested environmental factors, riparian cover influence the diversity and distribution of microalgae in stream puddles revealed by statistical analyses. In the laboratory experiment, six species of microalgae were observed in the container with different combination of leaves. Of these, the cyanobacterial species of *Macrocytis* had the highest percentage existence during experimental period. Overall, diatomic algae were high in mosquito breeding stream puddles; whereas, cyanobacteria were abundant in the laboratory experiment.

**Keywords:** Mosquitoes, bloodsucking behavior, microalgae colonization

## 1. Introduction

Mosquitoes are an important insect group with having conspicuous bloodsucking behavior. With respect of this habit, they transmit pathogens causing several infectious diseases like dengue, malaria, filariasis, and other fatal diseases. The immature mosquitoes that inhabit freshwater habitats, plays an important role in the ecological food chain. Mosquitoes can breed in variety of freshwater habitats such as ditches, ponds, swamps, permanent and temporary pools, rock and tree holes, artificial containers, and other habitats <sup>[1]</sup>. As mosquitoes are medically important group, vast research on their taxonomy, ecology, molecular and medical have been conducted <sup>[2-5]</sup> now and then. Little attention has also received on mosquito associated with stream puddles <sup>[6]</sup>.

In the forest ecosystem, any small water-filled habitat harbor a particular species of mosquitoes and other insects that share variety of food sources including leaf detritus <sup>[7]</sup>, for example, tree-hole ecosystems are mainly reinforced by decomposition of leaf detritus, which develops microorganisms and dissolved organic matters in water that are utilized by immature mosquitoes <sup>[8]</sup>. The litter diversity also affects the growth performance of mosquito larvae <sup>[9]</sup>. The relationship of leaf detritus and litter diversity effects on the growth of mosquito larvae in stream puddles remains poorly understood.

Mosquitoes are divided into substrate scrapers or water or water surface film feeders <sup>[10]</sup>. The microbial film exposed on leaf surfaces can be swiftly grazed by immature mosquitoes <sup>[11]</sup>. The experimental evidence showed that microalgae separated from biofilm or scraped from leaf litter, is having high nutrition and they strongly influence the food chain <sup>[12]</sup>. The colonization of microalgae is varied with different riparian cover <sup>[13]</sup> and substrates <sup>[14]</sup>. In streams, the pool microalgae exhibits significant intra-annual variation in density and physiological condition <sup>[15]</sup>. Nevertheless, interaction and role of microalgae in stream puddles has not been well studied.

The purpose of this study to find the relationship between microalgae and immature mosquito development in stream puddles. For this study, we surveyed stream puddles from Nilgiri Biosphere reserve of Southern Western Ghats, India. As microalgae is enormously present on the surface of leaf litter [16], litter relationship and diversity effects on the colonization of microalgae, in turn the pupal development of mosquitoes were investigated in the laboratory.

## 2. Materials and methods

### 2.1 Field analysis

The Western Ghats has an area of 140,000 square kilometres in a stretch of 1,600 kilometres and the hills are crossing the six provinces of India along the west coastal region. The southern part of Western Ghats covers the provinces of Kerala and Tamil Nadu. The annual rainfall ranges from 2,000 to 7,500 mm. Many streams and majors that originate in the Western Ghats and flow to east and west are Cauvery, Tambraparani, Vaigai, Chaliar, Bharatpuzha, Periyar, Pamba, etc. Since, the Western Ghats is one of the biodiversity hotspot in the world, it has variety of flora and fauna. In the southern part of Western Ghats, the present study was carried out in Nilgiri Biosphere Reserve.

The Nilgiri Biosphere Reserve (NBR) connects three provinces of Kerala, Tamil Nadu and Karnataka (latitude: 10°50'N - 12°16'N and longitude: 76°00'E to 77°15'E). In top of NBR, the world famous tourist spot, Ooty is found. On the way to Ooty, a pilot study was conducted from foot hills to top region during December 2019 and January 2020. In total nine streams were chosen for sampling (Fig. 1). In each sampling site, the stream puddles of bedrock and sand pools were noted for the presence of mosquito larvae. If mosquito larvae present in the puddle, sampling was done. The physical variables of latitude, longitude, elevation were noted by using GPS (Garmin, Germany), and chemical variables of pH, total dissolved solids, conductivity and salinity of puddle water were measured with the help of PCS tester 35 (Eutech instruments, India). The water temperature was noted by digital thermometer. The puddle depth and covering area were measured by using a measuring tape.

In the mosquito larvae containing stream puddles, puddles benthic substrates of small stones, woody debris and leaf litter were collected by using dip net. From the benthic substrates, the surface biofilms were scrapped using a hard tooth brush. The scrapped biofilm was collected in a small bowl with respective puddle water followed by the excess water was removed in the bowl and 1000 µl of sediments were taken in a 1.5 ml Eppendorf tube containing 500 µl of 70% ethanol. Then, it brought to the laboratory and preserved at -4 °C until analysis. Simultaneously, larvae and pupae were collected from puddle water using circular dip net for identification purpose. The collected larvae and pupae were placed in a plastic container half filled with respective puddle's water followed by all containers transported to the laboratory, reared the pupae till adult emergence and emerged adults stored in 80% ethanol.

### 2.2 Experimental design

The dominant riparian species nearer to the stream puddles were noted in the sampling sites. Of these, five riparian species were chosen for analysis: *Acacia caesia* (AC), *Cassia montana* (CM), *Pavetta indica* (PI), *Spondias pinnata* (SP) and *Tamarindus indica* (TI). The fresh fallen leaves were

collected under the respective riparian tree species. The collected leaves were taken in a polythene bag and it brought to the laboratory. Then all bags were preserved at 4 °C until analysis. The laboratory experiment was conducted during February 2020. Before experiment, 1 g of leaves were taken from the collected leaves for the following combination in three replicates as: AC, CM, PI, SP and TI-1 g of each (5 x 3 replicates = 15), AC+PI, CM+TI, CM+AC, CM+PI, SP+CM, SP+TI, SP+AC, SP+PI, TI+AC and TI+PI -0.5 g of each (10 x 3 replicates = 30), CM+TI+AC, CM+TI+PI, SP+CM+TI, SP+AC+PI and TI+AC+PI -0.33 g of each (5 x 3 replicates = 15), SP+CM+TI+AC+PI -0.2g of each (1 x 3 replicates = 3) and control - without leaves (1 x 3 replicates = 3).

A total 66 samples are taken in the individual leaf (15), two leaf species (30), three leaf species (15) and five leaf species combinations (3) with control (3). In each sample, 100 ml of distilled water and 1 ml of filtered stream puddle water were added. All containers were kept at 23 °C and 12 hours for day light and dark for 12 hours. This set up was maintained up to end of experimental period. A day before introduction of mosquito larvae (on 7<sup>th</sup> day), the eggs of *Aedes aegypti* hired from Centre for Research in Medical Entomology (CRME), Indian Council of Medical Research (ICMR), Madurai were positioned in a tray containing distilled water. Within 4-6 hours, the maximum number of eggs were hatched out and 50 larvae were transferred to each container using a visible glass Pasteur pipette to study the development of pupal period.

All containers were maintained at adjusted to stream puddle water (23 °C) up to 7 days or 90% pupae development. The developed pupae were killed using 70% ethanol. During the whole experimental study periods (leaves incubation and larval development periods), chemical characters of pH, conductivity, total dissolved solids, temperature and salinity of water in each container were measured. By the time, water with decaying leaves was shaken carefully to form sediments. After 3-5 minutes, 1000 µl of sediments were taken in a 1.5 ml Eppendorf tube with 500 µl of 70% ethanol. The sediments were collected throughout the experimental period. All sediments collected from both field and laboratory experiment was observed under stereo-binocular digital microscope (Optoscope, Germany).

### 2.3 Data analysis

To measure arithmetic mean and percentage for physical and chemical variables, microalgae population and immature mosquitoes, we used MS-Excel spread sheet. The Shannon, Simpson, Evenness and Margalef diversity indices for microalgae distribution in sampling sites were calculated. The statistical tests of student *t* test, *F* test, Mann-Whitney and Pearson correlation coefficient analysis were calculated for measuring relationship between microalgae and environmental variables among sampling sites. To determine the precise environmental factor for the distribution of microalgae, Principal Component Analysis was used. The graphical diagrams and other statistical measures were calculated by using a statistical software of PAST version 4.01 [17].

## 3. Results

### 3.1. Field analysis

#### 3.1.1. Physico-chemical parameters

The physico-chemical parameters and stream characters for sampling sites were measured. In the sampling sites, six

bedrock pools and three sand pools, to a total of nine were observed for the study. We did sampling over the elevation from 400 to 1600 meters. The mean water temperature was 25.5 °C. The average pool area was 40 cm in diameter in bedrock pools and 1.5 m in sand pools. The water depth was ranged from 5 to 20 cms. The mean pH of puddle water was 7.5. The mean total dissolved solids and salinity were 130 ppm and 90 ppt. The conductivity was ranged from 80 and 300 µSec. The riparian cover was between 30 and 90%. The surface floating vegetation or plant debris ranged from 10 and 20%.

### 3.1.2. Floral composition

A total of 2,260 individuals/0.1 ml of microalgae were observed under 13 species, 11 families and 5 classes (Tab. 1). The members of the class Bacillariophyceae were predominant (44%) in stream puddles. Next to Bacillariophyceae, Frailariophyceae occupied 38% of individuals. The family-wise analysis indicates that *Fragilaria* ceae had the higher percentage (37%) over other families. In the species-wise distribution, *Fragilaria* was dominated over (24%) the other species. The habitat-wise microalgal distribution showed that bedrock pool had twelve species in 12 families whereas sand pool had the three species in three genera. In the bedrock pools, *Fragilaria* was the dominant species, while *Fragilaria* and *Spirogyra* were equally occupied high percentage in sand pools. In the same stream puddles of sampling sites, six mosquito species in two genera (*Aedes* and *Anopheles*) were collected (Tab. 1). The members from the genus *Aedes* were predominant (58%) and *Ae. pseudotantaus* had the highest percentage.

### 3.1.3. Diversity indices

The diversity indices of Shannon, Simpson, Evenness and Margalef were calculated for microalgae distribution in stream puddles of nine sampling sites (Fig. 2). The stream puddles in bedrock pool had the higher number of taxa when compared to sand pools. The site 3 had the higher number of taxa (7). The Shannon diversity index showed that site 8 had the high value (1.582) and low diversity value in sand pool of site 5 (0). The site 3 had the high diversity value revealed by Simpson index and Margalef indices. Evenness index indicates that site 1 had the high value and low value observed in site 5. The diversity indices for mosquitoes in stream puddles revealed that site 5 had the higher number of taxa (2) and diversity values given by Shannon (0.693), Simpson (0.5), Evenness (1) and Margalef indices (0.315).

### 3.1.4. Relationship of microalgae

To test the relationship between microalgae diversity and environmental variables with mosquito larvae distribution, the student t test, F test, Mann-Whitney and Pearson correlation coefficient were calculated (Tab. 2). The results of statistical analyses indicate that longitude and elevation by t test and Mann-Whitney, elevation and pH by F test and riparian cover by correlation were significant factors for the distribution of microalgae in stream puddles, but the mosquito larval abundance was not related to the distribution of mosquito larvae revealed by correlation analysis.

The non-parametric analysis Principal Component Analysis (PCA) was used to understand the clear relationship between environmental variables including mosquito larval abundance and microalgae distribution in stream puddles. The results of

PCA showed that eigen and variance of axes were 1661.0 and 92.1% for PCA 1, and 1243.1 and 6.9% for PCA 2. The loadings of PCA showed that elevation and riparian cover were related with microalgae distribution in PCA1 and the factors of conductivity, total dissolved solids, salinity and mosquito larval abundance were significant factors in PCA 2 (Fig. 3). The scores of PCA indicate site 6 (Conoor B) in PCA 1 and site 2 (Mettupalayam stream) in PCA 2 were significantly related with the distribution of microalgae in stream puddles.

## 3.2. Laboratory experiment

The sampling of microalgae in stream puddles of nine sampling sites were compared with environmental variables and mosquito larval abundance, which showed the riparian cover, conductivity and total dissolved solids were the significant factors revealed by statistical analyses. Since microalga was negatively related with leaf litter density in stream puddles, riparian cover was positively related with microalgae distribution in mosquito abundance. Therefore, the laboratory experiment was conducted for determining the relationship between microalgae colonization during decaying leaves and the pupal development of mosquitoes. For this experiment, the mosquito larvae of *Ae. aegypti* were taken, because this species is common in the field survey.

### 3.2.1. Chemical characters of water

The chemical characters of pH, conductivity, total dissolved solids and salinity were measured during experiment. During the litter incubation period (first week), the range of pH was 7.1 to 8.2, 31 to 892 µSec for conductivity, 81 to 675 ppm for total dissolved solids and 22 to 435 ppt for salinity. In the mosquito larval development period, the high pH in AC, PI and TI-AC-PI (8.4) leaves container and maximum value of conductivity (1205 µSec), total dissolved solids (850 ppm) and salinity (600 ppt) were in TI-AC leaves container. The low chemical characters of pH, conductivity, total dissolved solids and salinity were found in control.

### 3.2.2. Colonization of microalgae

A total of 11,249 individuals/0.01 ml of microalgae were observed under six species during larval developmental periods (Initial (1<sup>st</sup> day), mid (3<sup>rd</sup> day) and end (6<sup>th</sup> day)). Of these, *Microcystis* had the highest percentage (95%) during all sampling. The colonization of microalgae (six species) during larval development period showed that: *Microcystis* was high in the water with combination of two leaves SP-AC at 1<sup>st</sup> day, CM-AC in 3<sup>rd</sup> day and control in 6<sup>th</sup> day, thus it was found higher in number at initial and decreased their numbers after successive days. The higher number of individuals of *Monoraphidium* was CM-TI at 1<sup>st</sup> day, TI-AC at 3<sup>rd</sup> day and SP-CM-TI at 6<sup>th</sup> day. The *Volvox* colonization was low during 1<sup>st</sup> day, while high during 3<sup>rd</sup> day (CM-PI) and decreases their density during 6<sup>th</sup> day. The individuals of *Spirogyra* were found only (AC, SP-TI and SP-CM-TI) during 1<sup>st</sup> day and they were absent in subsequent days. When compared to other four species, *Cladophora* and *Closterium* were found only during initial time (Fig. 4).

### 3.2.3. Microalgae and pupal development

The higher number of *Microcystis* was found in the water with CM-AC, SP-AC and TI. When compared to *Microcystis*, other algal species found in all containers were below 10%.

But *Monoraphidium* and *Volvox* were present in minimum numbers than the other species. Simultaneously, the development of pupae was found during 4<sup>th</sup> day in all containers except control and five mixed leaves. The higher number of pupae was observed in water of three leaves combination of SP-AC-PI (Tab. 3). To estimate the relationship between the abundance of microalgae found in different leaves combination container and pupal development of mosquito larvae, Pearson correlation co-efficient analysis was used (Tab. 3). The result of correlation analysis indicate that the abundance of algal species with three leaf combination water of SP-AC-PI's and two leaf combination water of AC-PI's had the high relationship.

#### 4. Discussion

Benthic algae are significant primary producers and determine a major role in lotic ecosystems at the interface of physico-chemical and biotic mechanisms of the food web [18]. Many reports to the connection between the properties of stream substrata and microalgae assemblages were conducted [19-21] and very few report concerning the relationship between microalgae and mosquitoes has received [22, 10]. But the attention on microalgal ecology in mosquito breeding habitat of puddles in lotic environment has not received. Hence, this is the first attempt made on microalgae community and their effect on the production of mosquitoes in stream puddles in the present study.

From the nine sampling sites, 13 species in 11 families of microalgae were collected in mosquito breeding stream puddles. This finding is comparably higher than our previous report that five species in three families of microalgae associated with leaf litter in riffle area observed from streams of Palani hills of Western Ghats [23]. The members in the class Bacillariophyceae was the predominant group of microalgae in stream puddles and *Fragilaria* dominated over the other species. This report is supported by the finding that the colonization of *Fragilaria* was high during decomposition of leaf litter [16]. Together, six mosquito species under two genera were collected in stream puddles of study area, of which, *Aedes* larvae had the highest percentage. In contrast, 16 mosquito species were observed in stream puddles of Agasthyamalai biosphere reserve [6].

The diversity analysis of microalgae in stream puddle indicates that the bedrock pool of site 3 had the high diversity value rather than other sites. It may be due to optimum environment and sufficient riparian cover and their input available in this site. The sand pool had the low diversity value observed in the study area. This finding may be the difference of colonization and water flow that if water flow suddenly decreases, the particular species of mosquitoes can lay their eggs and develop into pupa with the presence of minimum nutrients available in water, whereas in bedrock pool water may be retained for some week and it is possible to enhance the microalgae growth. For this reason, sand pools may have low diversity of microalgae.

The non-parametric test of Principal component analysis was used to find the relative environmental factor for the distribution of microalgae in mosquito breeding stream puddles. The result of PCA revealed that elevation and riparian cover were positively related with the distribution of

microalgae. The factor of elevation influences the diversity and distribution of periphytic microalgae [23]. But the riparian cover effect on microalgae in lotic environment received a little attention and the available literatures explain that benthic microalgae colonization in streams heavily influences with riparian cover and light availability [13]. The microalgae which live in the stream and could potentially colonize leaf detritus are generally high in omega-3 PUFAs (diatoms being especially rich in 20:5x3) and material with high algal content has a high ratio of the sum of omega-3 to omega-6 [24, 25].

During the experiment, the colonization of microalgae was well developed in all the containers with decaying leaves except control. Of which, *Microcystis* was occupied more than 90%. The addition of nutrient is possibly decline the competition among different bacterial species and stimulate an increase in population growth [26]. The release of dissolved organic matter by bloom-forming *Microcystis* is a pervasive in lentic freshwater ecosystem and their growth heavily influence the rapid depletion of dissolved oxygen in water [27, 28]. In the subsequent days, the population of *Microcystis* were reasonably decreased in all the containers except control. In control, the colonization of *Microcystis* was not found at initial time, but *Microcystis* was found in end of experimental period.

The cyanobacteria, *Microcystis* growth was consciously absent in container with SP, PI, SP-TI, CM-TI leaves. It may have inhibitory or anti-bacterial compounds present in these leaves, which can decrease or eliminate the cyanobacterial growth. More than, 50% of mosquito larval mortality was found in TI-AC and five leaves combination container. The pupal development of mosquitoes does not relate with cyanobacteria abundance and they were found irregular pattern that higher number of pupae produced container had the lower number of cyanobacteria as well as lower number of cyanobacteria container was produced higher number of pupae. It reflects that cyanobacterial population depends on the nutrition and bioactive compounds of the water and pupal development either relative with microalgae or dissolved nutrients in water.

In the present study, the pupal development of mosquitoes is positively correlated between SP-AC-PI and AC-PI containers and *Macroscystis* abundance. SP (*Spondias pinnata*) has excellent nutrient and growth factors, whereas both AC (*Acacia caesia*) and PI (*Pavetta indica*) are having anti-microbial compounds [29-31]. Some literatures explain that algae are considered as the main food component for mosquito larvae especially *Aedes* members [32]; diatomic algae do not get destructed in *Aedes* intestines and the role of algae for nutrition of mosquito larvae is still understudied [10].

Overall results of the present study highlight that riparian cover greatly influence the periphytic microalgae diversity and distribution in mosquito breeding stream puddles of sampling sites. Interestingly, diatoms were enormously present in the field, whereas few microalgal species was developed in laboratory experiment. Of these, the cyanobacterial species of *Microcystis* had the highest percentage existence during experimental period. Future study is needed to examine the relationship between microalgae abundance and pupal development of mosquitoes in the field as well as laboratory.



**Table 1:** Microalgae and mosquito distribution in stream puddles of sampling sites. ('+' -present, '-' -absent)

Class	Family	Genus	Sampling site								
			Kallar (1)	Mettu Palaayam (2)	Barliar A (3)	Barliar B (4)	Conoor A (5)	Conoor B (6)	Kunja Paarai (7)	Kotta Combai (8)	Aravenu (9)
Microalgae											
Bacillariophyceae	Amphipleuraceae	<i>Frustulia</i>	-	-	-	+	-	-	-	-	-
Bacillariophyceae	Aulacoseiraceae	<i>Aulacoseira</i>	-	-	-	+	-	-	+	-	-
Bacillariophyceae	Stauroneidaceae	<i>Craticula</i>	-	-	+	-	-	-	+	-	-
Bacillariophyceae	Eunotiaceae	<i>Eunotia</i>	-	-	-	+	-	+	+	+	-
Bacillariophyceae	Gomphonemataceae	<i>Gomphonema</i>	-	-	-	-	-	-	-	+	+
Bacillariophyceae	Pinnulariaceae	<i>Pinnularia</i>	-	-	-	+	-	-	-	-	-
Cyanophyceae	Nostocaceae	<i>Anabaena</i>	-	+	-	+	-	-	-	-	-
Fragilariophyceae	<i>Fragilaria</i> ceae	<i>Diatoma</i>	-	-	+	-	-	-	-	-	-
Fragilariophyceae	<i>Fragilaria</i> ceae	<i>Tabularia</i>	-	-	+	-	-	+	+	+	-
Fragilariophyceae	<i>Fragilaria</i> ceae	<i>Fragilaria</i>	-	+	+	-	-	+	+	+	+
Fragilariophyceae	Tabellariaceae	<i>Tabellaria</i>	-	-	+	-	-	-	-	-	-
Ulvophyceae	Cladophoraceae	<i>Rhizoclonium</i>	+	-	+	-	-	-	-	+	-
Zygnematophyceae	Zygnemataceae	<i>Spirogyra</i>	+	-	+	+	+	-	-	+	+
Mosquito larvae											
Insecta	Culicidae	<i>Aedes vittatus</i>	-	+	-	-	-	-	-	-	-
Insecta	Culicidae	<i>Aedes pseudothanasos</i>	+	-	+	-	+	+	-	-	-
Insecta	Culicidae	<i>Anopheles stephensi</i>	-	-	+	+	-	-	+	-	-
Insecta	Culicidae	<i>Anopheles aconitus</i>	-	-	-	-	-	-	-	+	-
Insecta	Culicidae	<i>Anopheles dravidicus</i>	-	-	-	-	-	+	-	-	-
Insecta	Culicidae	<i>Anopheles karwari</i>	-	-	-	-	+	-	-	-	+

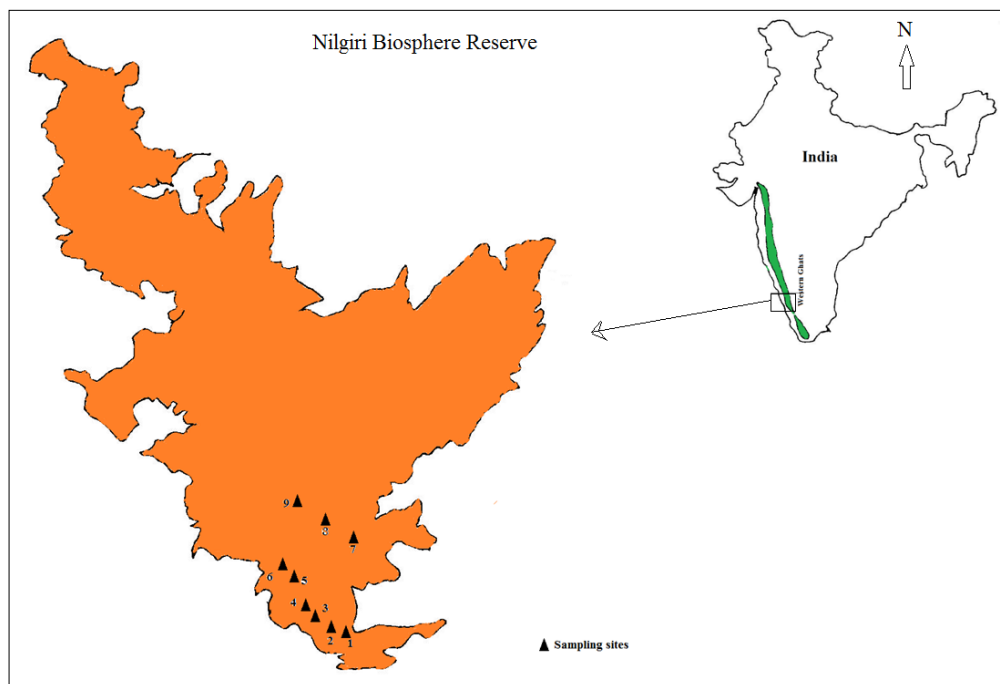
**Table 2:** Statistical analyses between microalgae and environmental variables with mosquito larval abundance in stream puddles of sampling sites.

	't' test (p= 0.05)	F test (p= 0.05)	Mann-Whitney (z) (p=0.001)	Pearson correlation(r)
Latitude	2.34	1.98	1.15	0.03
Longitude	8.65	4.10	3.53	-0.25
Elevation	7.20	518.3	3.53	0.30
Water temperature	0.64	73.38	0.61	0.11
Conductivity	5.96	20.87	3.53	0.14
TDS	5.45	10.54	3.44	0.15
pH	2.95	265.9	1.94	-0.01
Salinity	4.81	4.58	3.27	0.14
Pool diameter	0.60	1.11	0.48	0.03
Water depth	2.72	17.64	1.77	-0.17
Riparian cover	4.62	1.97	3.19	0.50
Leaf litter	0.84	3.52	0.89	-0.37
Mosquito larval abundance	2.80	9.29	2.03	-0.34

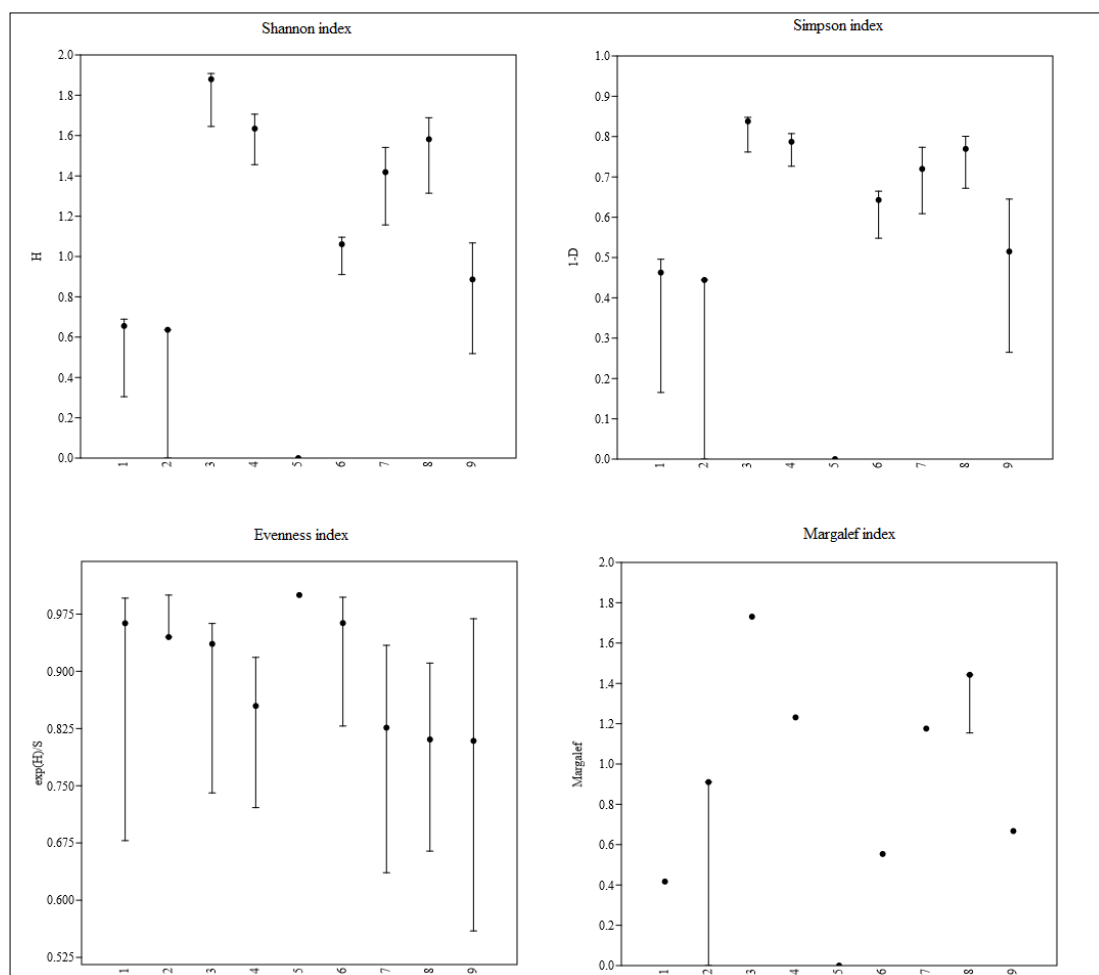
**Table 3:** Microalgae colonization (Abundance-No. of individuals/0.01 ml) and pupal development of *Aedes aegypti* in the water with different combination of leaves during experimental period. (Mi-Microcystis, Mo-Monoraphidium, Vo-Volvox, LA-Larvae (No.), LM-Larval mortality (%) and PU-Pupal development (No.)).

	1 <sup>st</sup> day			3 <sup>rd</sup> day			6 <sup>th</sup> day			1 <sup>st</sup> day			3 <sup>rd</sup> day			6 <sup>th</sup> day			Correlation (r)
	Mi	Mo	Vo	Mi	Mo	Vo	Mi	Mo	Vo	LA	LM	PU	LA	LM	PU	LA	LM	PU	
Control	0	0	0	40	0	0	804	0	0	50	0	0	50	0	0	20	0	30	0.086
SP	0	0	0	0	0	0	0	0	0	50	0	0	44	12	0	20	0	24	0
CM	0	3	0	0	0	0	200	0	86	50	0	0	30	40	0	8	0	22	-0.167
TI	720	0	0	186	0	0	115	0	0	50	0	0	26	48	0	14	0	12	0.325
AC	183	0	0	122	0	0	0	0	0	50	0	0	46	8	0	14	0	32	0.837
PI	0	0	0	0	0	0	0	0	0	50	0	0	46	8	0	10	0	36	0
SP-CM	156	15	0	165	0	15	46	0	0	50	0	0	36	28	0	28	0	8	0.574
SP-TI	0	0	0	0	0	0	0	0	0	50	0	0	44	12	0	14	0	30	0
SP-AC	831	0	0	140	0	0	371	0	14	50	0	0	30	40	0	10	0	20	0.331
SP-PI	91	0	0	0	0	0	0	0	0	50	0	0	36	28	0	12	0	24	0.498
CM-TI	0	54	0	0	13	0	0	0	0	50	0	0	30	40	0	18	0	12	-0.093
CM-AC	648	0	0	772	0	0	574	0	5	50	0	0	30	40	0	20	0	10	0.330
CM-PI	151	0	0	204	0	114	0	0	0	50	0	0	20	60	0	20	0	0	0.026
TI-AC	186	25	0	281	39	0	505	0	0	50	0	0	40	20	0	28	0	12	0.568
TI-PI	170	13	0	0	14	0	0	0	0	50	0	0	30	40	0	22	0	8	0.434
AC-PI	121	12	0	121	16	0	0	0	0	50	0	0	40	20	0	16	0	24	0.759
SP-CM-TI	190	0	0	79	0	0	105	49	3	50	0	0	36	28	0	22	0	14	0.519
SP-AC-PI	104	0	0	206	0	0	110	0	0	50	0	0	42	16	0	32	0	10	0.777
CM-TI-AC	148	0	0	95	0	0	158	0	0	50	0	0	32	36	0	22	0	10	0.391
CM-TI-PI	286	12	55	255	0	0	0	0	0	50	0	0	34	32	0	20	0	14	0.473

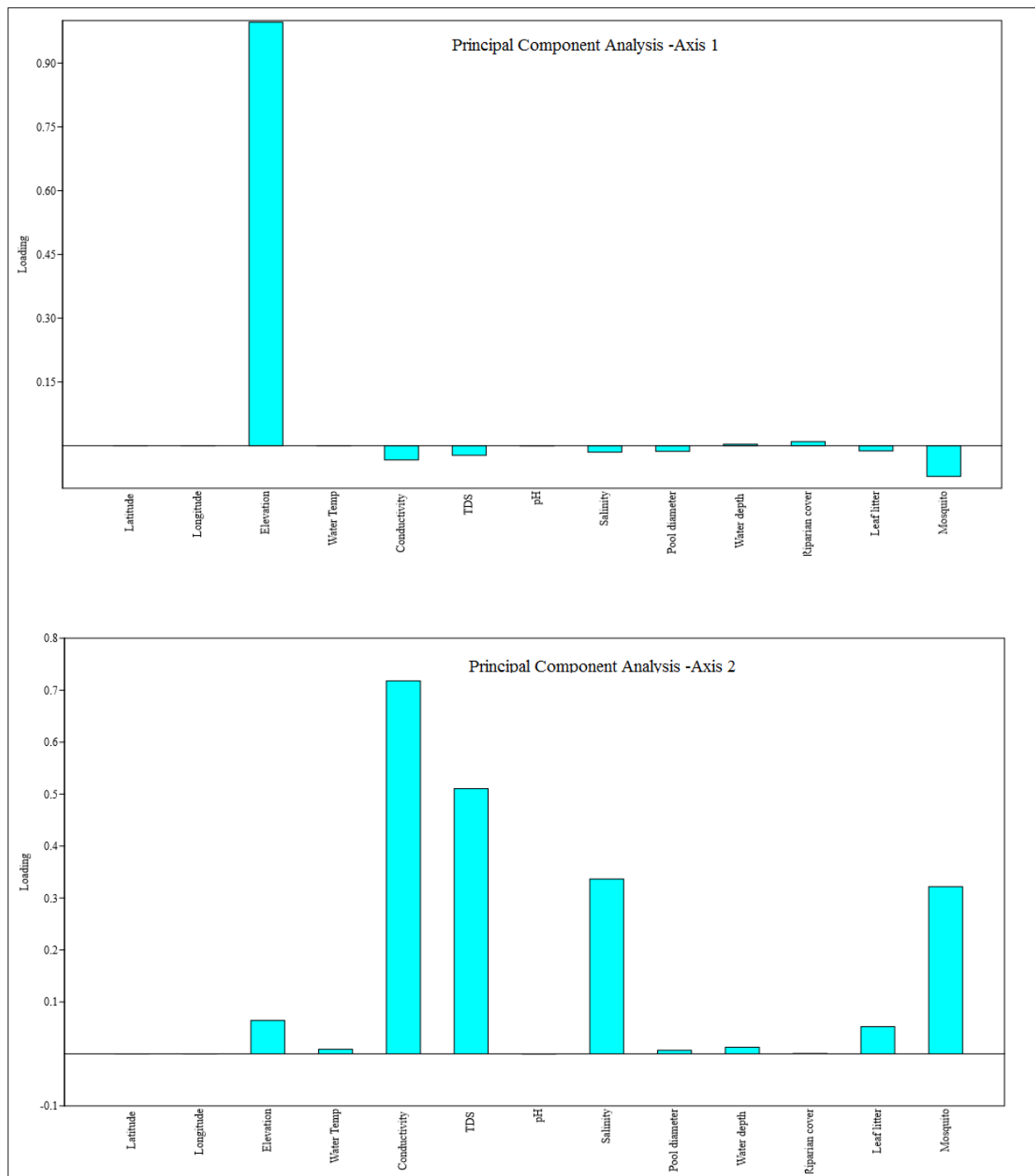
TI-AC-PI	174	8	0	0	0	0	178	0	0	50	0	0	40	20	0	28	0	12	0.539
SP-CM-TI-AC-PI	270	25	0	174	39	0	125	0	0	50	0	0	16	68	0	0	0	16	0.177



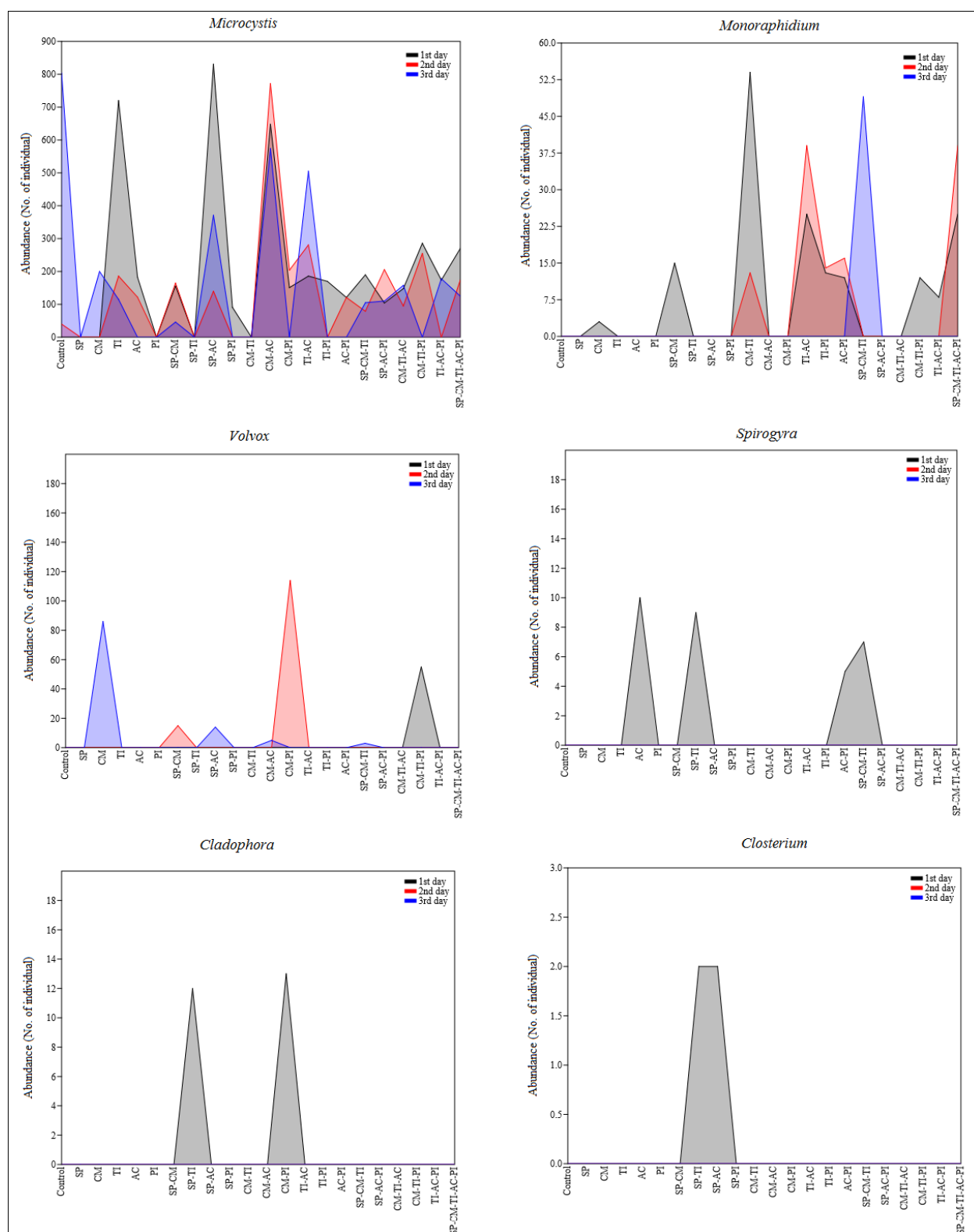
**Fig 1:** Sampling sites in Nilgiri Biosphere Reserve of Southern Western Ghats.



**Fig 2:** Diversity indices of microalgae associated with mosquito breeding habitat of stream puddles of nine sampling sites.



**Fig 3:** Loadings plots of Principal Component Analysis for microalgae and environmental variables in stream puddles.



**Fig 4:** Colonization of microalgae in water containing different combination of leaves during larval development period of *Aedes aegypti*.

#### Conflict of Interest statement

The authors have declared no conflict of interest.

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