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In vitro studies for development of periphyton and biofouling by aquatic insect larvae on substrates

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Abstract
The present study was designed to determine development of periphyton and biofouling by aquatic insect larvae on substrates like I) N1-Cotton stem II) N2-Sugarcane Baggasse III) N3-*Ipomea* stem IV) A1-Stones V) A2-Bisleri Bottle Strips VI) A3-Rubber Strips of Vehicle Tyre were assessed. Results showed that periphyton growth in case of Sedgewick Rafter counting and biomass on N1 substrate was 41666.67±577.3503 no.lit^{-1} and 33.79 gm.lit^{-1} respectively compare to remaining substrates, among periphytic community class chlorophyceae was diverse. N1 substrate was high in the form of aquatic insect larvae percentage due to nature and nutrient diffusing ability of substrates, all larval forms of insects *Culex* sp. was dominant 69.76%. The study revealed that N1 is most specific substrate for periphytic growth and ALI. Hence, N1 is used for improving primary productivity of fresh water ecosystem for aquaculture yields.

Keywords: Aquatic insect larvae; Biofouling; Cotton stems; *Ipomea sp*; Periphyton

1. Introduction
Periphyton is a complex aquatic biota and microorganisms attached on submerged substrates like stones, sticks and submerged surfaces including associated non-attached organisms and detritus [1, 3]. Periphyton is grazed by animals in aquatic ecosystem [17]. For the production of periphyton, variety of biodegradable and non-biodegradable substrates are used in aquculture and its concentration is higher on biodegradable substrates [10, 19].
Biofouling is the process in which organisms colonized on the different substrates inserted in water, species are microfoulers and macrofoulers that includes bacteria, viruses, protozoa, fungi, algae, large size organisms such as Mussels, Barnacels and aquatic insects larvae to cause biofouling in the systems [9, 11, 16, 23]. Biofouling development typically follows a regular sequence of events that mainly includes microfouling and macrofouling. Macrofouling is caused by settlement, attachment and growth of invertebrate larvae, translocating adults and drifting shells and exoskeleton [23]. Macrofoulers tend to be filter feeder, which means large quantities of nutrients and other materials are removed from water which is deposited on or in benthos. This deposition increases further fouling by micro and macrofoulers in that particular system and silts [9]. They also often have planktonic larvae which enhance dispersal capabilities and enable them to colonize areas that would otherwise be unavailable to adult life stages. The researcher reported that biofouling influenced by factors like water temperature, conductivity, pH, dissolved oxygen, organic content, nature of substrate, roughness of substrate surface, surface charge, hydrophobicity, hydrodynamic conditions, location and depth of water bodies, seasons etc [12]. The water quality is directly proportional to growth of mosquito larvae, salt and other dissolved organic substances acts as inhibitory factors on mosquito growth, dissolved organic matter such as effluent from sugar factories is inhibitory effect on breeding of *Anopheles sp.* presence or absence of plants in habitat also has an effect on mosquito larvae respiration [7, 13]. The hydrogen ion concentration does not affect directly on mosquito larvae but it affect flora and fauna in a habitat. The aim behind this study to determine specific substrate for the development of periphyton and biofouling by aquatic insect larvae.

2. Materials and Methods
2.1 Experimental design for growth of larvae in periphyton growing systems
Size of plastic trough was 38x23cm, 40x25.5cm and 15cm on base side, top side and depth

respectively. Plant materials were collected and sun dried. 1cm thick soil bed was put in base of all troughs for providing nutrients in the designed periphyton system and then added 125 gm garden wastes- fish manure in all troughs for fertilization in the systems. Substrates like N1-cotton sticks, N2-Sugarcane Baggasse and N3-*Ipomea* sticks, A1-Stones, A2-Bislari plastic bottle strips, and A3-Tyre Rubber strips were inserted in troughs. Size of biodegradable substrates were 14x2cm and Non-biodegradable substrates like A1-Stones size was random, A2-Bisleri bottles strips, bottles upper and lower surfaces were removed and was cut in to two halves 13x8cm in size, and A3-Tyre Rubber strips was 14x3cm in size and all substrates were equal (six) in number. Troughs were filled with one lit pond water and nine lit borewell water. Water level was maintained throughout experiment. 100 watts tungsten coil bulb was used as light source for photosynthesis activity in the systems and entire system was kept in experimental laboratory for 30 days incubation period [14, 22].

2.2 Collection of periphyton from substrates and fixation
Substrates removed from water and periphyton grown on surface were collected by using soft brush and rinsed with distilled water; final collection was done in tray. Large sized periphyton in the form of insect larvae was collected separately and other planktonic, algal filaments were collected separately and fixed by adding 1ml of 4% formalin for phytoplankton and zooplankton [1].

2.3 Staining of periphyton
Temporary slides were prepared by using ethanolic dehydration staining method. Acetocarmine stain was used and xylene as clearing agent and treatment different percentage grades of alcohol such as 30, 50, 70, 90, 100 for 5min respectively and mount in glycerin. It observed under compound microscope with 10X and 40X and photograph were taken by using TUCSEN made eyepiece attached camera [1].

2.4 Identification of periphyton
Periphyton was identified by using keys from the reference book fresh water biology [1, 21].

2.5 Quantitative analysis of periphyton
Periphyton was collected from substrates placed in experimental troughs and quantitatively measured by using Sedgwick Rafter Counting Cell method. The number of periphyton group as group/ml of sample was calculated. 1ml sample was used from 1lit sample made during periphyton scraping from each bundle. For each sample, number of periphyton was recorded and expressed numerically per ml of water. Periphyton density was calculated using the formula [2].

$N = (P \times C \times 100)/ml$

After it converted into /liter by multiply N X 1000.

Where, N: number of plankton cells

P: number of plankton counted in ten fields

C: volume of final concentrate of sample

ml: volume ml of water sample.

2.6 Determination of Biomass
Samples were centrifuged at 5000 RPM for 5min, supernatant was discarded and pellets were filtered through Whatman filter paper, air dried and weighed [1].

2.7 Preservation of Insect larvae
After four week incubation of the entire periphyton system larvae were collected in 4% formalin and later were preserved in 70% alcohol for long time preservation [14].

2.8 Larval count by direct method
Collected larvae were put in tray and then counted manually with the help of needle one by one [5].

2.9 Staining of larvae
Permanent slides of larvae were prepared by using ethanolic dehydration staining method. Acetocarmine stain, xylene and treatment of different percentage grade of ethanol such as 30, 50, 70, 90, 100 for 5 min respectively were done and Canada balsm used as mounting media. It observed under compound microscope with 10X and photograph were taken by using TUCSEN made eyepiece attached camera [1].

2.10 Identification of Larvae
The larvae was identified by using images on http://www.nhm.org/nature/blog/moth-flies-living-your-drain [4, 8].

3. Results
The results in table 1 showed that total periphytic growth and biomass of biodegradable and non-biodegradable substrates, total periphytic growth and biomass was recorded on the N1, N2, N3, A1, A2 and A3 substrates were 41666.67±577.3503 no. lit^{-1} and 33.79 gm. lit^{-1}, 34333.33±577.3503 no. lit^{-1} and 20.15 gm. lit^{-1}, 27000±1000 no. lit^{-1} and 28.85 gm. lit^{-1}, 23000±1000 no. lit^{-1} and 17.38 gm. lit^{-1}, 16666.67±1527.525 no. lit^{-1} and 7.9 gm. lit^{-1}, 21333.33±1527.525 no. lit^{-1} and 5.43 gm. lit^{-1}respectively. The highest periphytic growth and biomass recorded on the N1 substrates.

Table 1: Total periphyton no. lit^{-1} (Mean ± SD of three samples) and biomass gm. lit^{-1} on biodegradable and non-biodegradable substrates samples

Substrates		Total periphyton no. lit^{-1}	Biomass gm. lit^{-1}
Biodegradable	N1	41666.67±577.3503	33.79
	N2	34333.33±577.3503	20.15
	N3	27000±1000	28.85
Non-Biodegradable	A1	23000±1000	17.38
	A2	16666.67±1527.525	7.9
	A3	21333.33±1527.525	5.43

In table 2 data showed four periphytic groups present on the substrates has *Oedogonium sp., Zygnema sp.***,** *Closterium sp.*, *Chlamydomonas sp.* and *Cossmarium sp.*; these are members of chlorophyceae, *Diatom sp.;* member of Bacillariophyceae, *Daphnia sp.*; member of cladocera and *Cyclop sp.*; member of copepods. Chlorophyceae was diverse group in all periphytic community and highest count reported on N1 substrates.

Table 2: Periphytic diversity on biodegradable and non-Biodegradable substrates

Periphyton groups	Substrates					
	Biodegradable			Non-Biodegradable		
	N1	N2	N3	A1	A2	A3
Chlorophyceae						
Oedogonium sp.	8	5	2	3	2	2
Zygnema sp.	9	6	7	4	2	3
Closterium sp.	6	7	5	3	1	4
Chlamydomonas sp	2	3	4	1	2	3
.*Cossmarium sp.*	4	6	3	2	2	3
Bacillariophyceae						
Diatoms sp.	5	3	2	7	4	3
Cladocera						
Daphnia sp.	3	1	2	1	1	1
Copepod						
Cyclop sp.	4	3	2	2	3	1

In table 3 data showed that three different types of insects larvae; Mothfly, *Culex* and Chironimous were colonized on all substrates. % count of larvae were determined and their values like Mothfly number 44.32% high on the N3 substrate, larvae *Culex sp.* number 69.76% high on the N1 substrates and chironimous number 15% high on A2 substrates.

Table 3: Counting of larvae by direct counting method

Substrate	Biodegradable			Non-biodegradable		
Larvae	N1	N2	N3	A1	A2	A3
Mothfly (%)	28	37.87	44.32	28.30	20	25.92
Culex (%)	69.76	54	54.35	66.03	65	66.67
Chironimous (%)	1.6	7.5	3.78	5.66	15	7.40

4. Discussion

The present study revealed that biodegradable substrates showed highest growth of periphyton and larvae than non-biodegradable substrates due to physical appearance of substrates, perform great role in colonization of periphyton and larvae [3, 10]. In table 1 results showed SR-counting of periphyton has highest growth on cotton substrate as compared to other substrates [18]. In table 2 results showed *Chlorophyceae* was dominant; there were five members present on substrates. The *Bacillariophyceae*, *Cladocera* and *Copepods* having only one member present in periphyton colonization. *Diatom sp.* showed the diverse growth on stone substrate due to roughness of substrate along with calcium and phosphate content that increased growth of *Diatoms* [6]. In table 1 results revealed abundant biomass of periphyton on Cotton substrate as compared to other substrates and in table 3 results revealed larvae % count found Mothfly larvae % count high on N3 substrate, *Culex sp.* % count high on the N1 substrate and Chironimous % count high on the A2 substrate. The cotton stem has highest count of larvae due to nutrient diffusing ability of cotton stem from water, texture of substrates and hydrodynamic condition of substrates in four weeks time period that cause biofouling in periphytic based experimental systems [18]. The growth and development of larvae are dependent on nutrient availability, water flow rate, pH, temperature, total solids. The nutrient diffusing ability of biodegradable substrates and respiration capicity of aquatic insect larvae on plants showed high growth of larvae as compare to non-biodegradable substrates [7, 11]. The highest count (44.43%) of Mothfly on *Ipomoea sp.* was due to moist conditions and organic matter [20]. The *Culex* sp. was dominant on cotton stem. Dissolved organic matter present in the sugarcane baggasse hence it is inhibitory effect on breeding of mosquito larvae [7, 13].

5. Conclusion

It was concluded that there is an effect of substrate type natural or artificial on formation of periphyton and ALI. When biodegradable plant parts were used as substrates, the formation of periphyton and aquatic insect larvae was high whereas it was less on non-biodegradable substrates. The cotton stem waste has showed high periphyton and ALI formation amongst all biodegradable substrates. This study revealed that cotton is one of the most specific substrate for growth of periphyton and aquatic insect larvae. Hence, formation of periphyton and subsequent fouling on substrates which could have been used for improving primary productivity in the fresh water ecosystem for aquaculture yields.

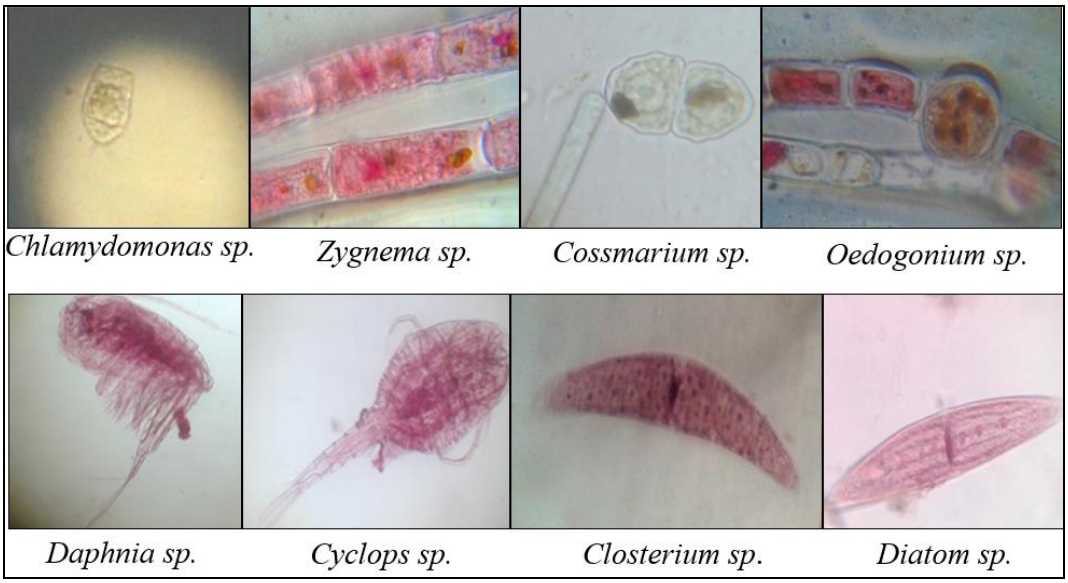

Plate 1: Periphytic diversity on the biodegradable and non-biodegradable substrates under microscope (40X

Mothfly *Culex sp.* *Chironimous sp.*

Plate 2: Larvae from periphyton systems

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