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Comparison of Periphyton Biomass on Coconut Coir and Bamboo Poles as Natural Substrates in Earthen Lined Pond

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Abstract

As a first step in assessing the viability of periphyton-based shrimp production in lined pond aquaculture systems, the effects of natural substrates (split bamboo poles and coconut coir) on development of periphyton were evaluated. Experiments were conducted in outdoor earthen lined pond to study periphyton biomass on two types of natural substrates such as split bamboo pole and coconut coir which was placed inside the earthen lined pond filled with seawater for duration of 45 days. The periphyton biomass was recorded at 0–40 cm depth, 40–80 cm depth and 80–120 cm for all the substrates. The periphyton biomass, in terms of dry matter (DM) from coconut coir was comparatively higher (3.4995±0.3120 mg cm^{-2}) than the split bamboo poles (3.2011±0.3044 mg cm^{-2}) during the experiment. 'T' test has affirmed that significant difference (p<0.05) was observed in the dry matter content of the periphyton among the substrates. 'T' test analysis of the data relating to periphyton biomass for split bamboo pole and coconut coir recorded at different depths indicated that upper 0–40 cm depth had higher values than the other depths. Furthermore, among the two substrates, coconut coir (3.4995±0.31201 mg cm^{-2}) was observed to be a better substrate than the split bamboo pole for periphyton biomass production. Coconut coir substrate can be utilised by fin and shellfishes as natural food.

Keywords: Natural substrates, periphyton, quantity, quality, natural food

1. Introduction

Aquaculture production in the world is depending on external resources like fertilizers and feed. These external inputs could have been used elsewhere and because of their cost, the poorer sectors of the community are excluded from participation (O'Riordan, 1992). To minimize costs, little attention was given to the periphyton-based food web. Periphyton has become a universally accepted expression for all organisms attached to a submerged substrate. Periphyton assemblages can reach high biomasses of upto 2350 mg m^{-2} of chlorophyll-*a* (Westlake et al., 1980). A minimum of 2 weeks was required for the development of peripyton before stocking of fishes (Azim et al., 2002). Fish production based on periphyton depends on artificial substrate type and preferred to use bamboo rather than PVC pipes or sugarcane bagasse bundles when culturing masheer (*Tor khudree*) fingerlings (Keshavanath et al., 2001). Some workers have compared the natural and artificial substrates for periphyton growth and found more periphytic growth on the natural substrates (Amisah

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et al., 2008; Keshavanath et al., 2012; Dutta et al., 2013). For the advantage of the substrates on shrimp growth, different authors carried out experiments from various aspects and obtained different results and provided different explanation including improvement of the water quality, addition of the natural food supplement, limitation of pathogenic bacteria reproduce, provide refuge for shrimp to escape any negative behavioural interactions and adding living space (Bratvold and Browdy, 2001; Kumlu et al., 2001; Burford et al., 2004; Preto et al., 2005; Arnold et al., 2006; Zarain-Herzberg et al., 2006; Ballester et al., 2007). Periphyton has been shown to be capable of improving the water quality by adsorpting and trapping heavy metals and organic molecules in water (Liu et. al., 2018; Chonova et al., 2018; Wang et al., 2019). Periphytic biofilms have become a common biological treatment form in water and/or wastewater disposal, and work efficiently in dealing with nutrient removal and several containments such as organic matters, nanoparticles and heavy metals (Liu et al, 2017; Shabbir et al., 2017; Tang et al., 2018). The substrate palm leaf and nylon net were compared for periphyton growth and found that the palm leaf had better periphyton growth than nylon net (Dutta et al., 2013). The substrates from organic origin attract more periphytic growth on them. Anand et al. (2014) used bamboo as a substrate in *Peneaus monodon* treatment pond and found periphyton biomass interms of dry matter over substrate was 5.9±0.7 mg cm^{-2}. The quantity and biomass of periphyton varied significantly with substrate type, fertilization level, environment conditions and taxonomic composition (Keshavanath et al., 2001; Makarevich et al., 1993).

Less work has been done on the periphyton growth on different types of natural substrate in seawater earthen line ponds. Hence, the present study was undertaken to quantify the periphyton biomass on different natural substrate such as split bamboo pole and coconut coir to find out the best suitable substrate for periphyton attachment in seawater earthen lined pond.

2. Materials and Methods

The outdoor experiments were carried out in 2 lined ponds, each having a water spread area of 30 m^2 during the period 2016-17 at Marine Research Farm Facility, Tharuvaikulam, Thoothukudi, Tamil Nadu, India. The area is located at 8°89′ N latitude and 78°17′ E longitude. The ponds were well exposed to sunlight and having water supply facilities. The water depth was maintained as 1.2 m over the study period. The experiment was carried out in duplicate with different natural materials for periphytic growth on them.

Two different types of natural materials (substrate) such as split bamboo pole and coconut coir were used for periphyton growth. These natural substrate materials were collected locally. Ponds were drained and renovated and all other undesirable aquatic weeds and organisms were removed. Both the substrates were driven vertically into the same pond to maintain the same environment. 5 Split bamboo poles (1.5 m total length / 1.20 m exposed length / 0.3 m free board) as substrate were used. These bamboo poles were tied in nylon ropes (5 mm) and vertically immersed in the experimental ponds. The space between each Split bamboo pole was 10 cm. Five coconut coir (1.3 m total length / 1.20 m exposed length / 0.1 m free board) as substrate were used. This coconut coir were tied in nylon ropes (5 mm) and vertically immersed in the experimental ponds. The space between each coconut coir given was 10 cm.

The mean diameter of the bamboo pole and coconut coir were 6.2 cm and 2.6 cm respectively. After the substrates were installed, the ponds were filled with sea water. The water depth in each pond was monitored daily at 1.20m and maintained by pumping sea water to replace losses at weekly intervals. Quicklime (CaO) was applied to the pond at the rate of 250 kg ha^{-1} (Azim et al., 2003). Three days after liming, ponds were fertilized with cow dung at the rate of 1,500 kg ha^{-1}. The application of cow dung was continued once in 10 days at a same dosage rate over the study period.

Periphyton samples were collected on 15th, 30th and 45th day between 10.00 to 11.00 hours. From each type of substrate, three places were selected by randomly and three composite samples of periphyton were taken at each of three depths (40, 80 and 120 cm below the water surface) $substrate^{-1}$ from 2×2 cm^2 area. These areas were carefully scraped with a scalpel blade to remove all periphyton visually without affecting the substrate. After sampling, the substrate was placed in their original positions and marked to exclude from subsequent sampling.

The composite samples of each depth were pooled. Pooled samples from three depth of each substrate were pre weighed and dried at 105 °C until constant weight and kept in a desiccator. The dry matter of the samples was determined by weight difference. The standard methods were used for the determination periphyton biomass interms of dry weight. (Anonymous, 1995). Another pooled composite sample were re-suspended in 50 ml distilled water and preserved in 5 % buffered formalin in sealed container. After vigorous shaking, sample was observed under binocular microscope.

Water samples were collected on biweekly basis between 10.00 and 11.00 am. Water quality parameters such as temperature by mercury thermometer, salinity by hand refractometer, transparency by secchi disc and pH by pH pen were measured directly from the ponds and dissolved oxygen was measured following Winkler method at laboratory. The water samples were filtered through GF / C Whatman glass fibre filter and the filtrate was analyzed for Nitrate-N (cadmium reduction), Nitrite-N and total ammonia nitrogen (TAN) (Phenol hypochlorite method). Non-filtered water column samples were analyzed for Chlorophyll 'a' by following standard methods. Biological oxygen demand (5 day BOD) of water samples was estimated following standard methods

(Anonymous, 1995).

Data collected from this study were analyzed statistically by 'T' test, One-way ANOVA, followed by Turkeys' test using SPSS 20.0.

3. Results and Discussion

There were 16 different phyto-periphytic microalgae grew on split bamboo pole and coconut coir immersed in seawater of the earthen lined pond (Table 1). During experiments, the group such as Bacillariophyceae (*Biddulphia, Chaetoceros, Coscinodiscus, Pleurosigma, Rhizosolenia, Triceratium, Nitzchia, Leptocylindrus, Climacosphenia, Climacosphenia*); Dinophyceae (*Ceratium, Peridinium, Thalassisothrix, Skeletonema*) and Cyanophyceae (*Tricodesmium, Oscillatoria*) were observed in both the natural substrates. Bacillariophyceae were found more on both the substrates, followed by Dinophyceae and Cyanophyceae. On split bamboo pole, the numbers of genera found were Bacillariophyceae (10), Dinophyceae (2), and Cyanophyceae (2) followed by Bacillariophyceae (10), Dinophyceae (2), and Cyanophyceae (2) in coconut coir. From the result obtained, it was found that on both natural substrates, the Bacillariophyceae grew more in numbers compared to other groups (Figure 1a, 1b).

Table 1: Types of Phyto-periphyton recorded from different natural substrates immersed in seawater earthen lined pond for different periods

No.	Phyto-eriphyton	15 Days		30 Days		45 Days	
		BP	CC	BP	CC	BP	CC
Bacillariophyceae							
1	*Biddulphia sp.*	+	+	+	+	+	+
2	*Chaetoceros sp.*	+	+	+	+	+	+
3	*Coscinodiscus sp.*	+	+	+	+	+	+
4	*Pleurosigma sp.*	+	+	+	+	+	+
5	*Rhizosolenia sp.*	+	+	+	+	+	+
6	*Triceratium sp.*	+	+	+	+	-	-
7	*Nitzchia sp.*	+	+	+	+	+	+
8	*Leptocylindrus sp.*	+	+	+	+	+	+
9	*Climacosphenia sp.*	+	+	-	-	+	-
10	*Gyrosigma sp.*	+	+	+	+	+	+
Dinophyceae							
11	*Ceratium sp.*	+	+	-	-	-	-
12	*Peridinium sp.*	+	+	+	+	+	-
13	*Thalassisothrix sp.*	+	+	+	+	+	+
14	*Skeletonema sp.*	+	+	+	+	+	+
Cyanophyceae							
15	*Tricodesmium sp.*	-	+	+	-	-	-
16	*Oscillatoria*	-	-	+	+	+	+

BP: Bamboo poles, CC: Coconut coir

The periphyton biomass from coconut coir was comparatively higher (3.4995±0.3120 mg cm^{-2}) than the split bamboo poles (3.2011±0.3044 mg cm^{-2}) during the experiment (Figure 2). 'T' test has affirmed that significant difference (p<0.05) was observed in the dry matter content of the periphyton among the substrates (Table 2).

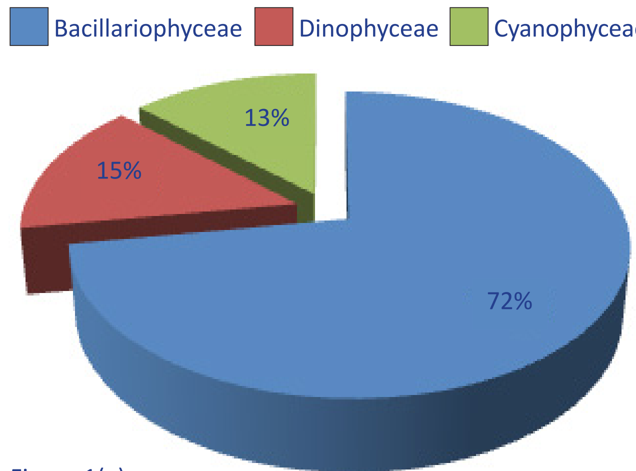

Figure 1(a)

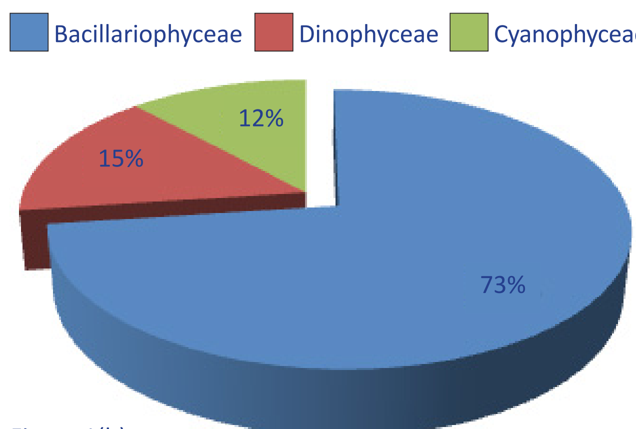

Figure 1(b)

Figure 1(a, b): Phyto-periphyton composition for different natural substrates. a) Phyto-Periphyton growth on split bamboo pole; b) Phyto-Periphyton growth on Coconut coir

Table 2: Periphyton biomass in terms of dry matter (mg cm^{-2}) of different substrates immersed in seawater earthen lined pond for different periods. Figures are means of samples from three depths, two ponds and three sampling dates (n = 18)

Substrate	N	Mean	Std. deviation	Std. error
Split bamboo poles	18	3.2011[a]	1.2913	0.3044
Coconut coir	18	3.4995[b]	1.3237	0.3120

Values with different superscripts in a mean column differ significantly at p<0.05. (n=18)

As per the 'T' test analysis of the data affirmed that high significance (p<0.05) was observed in periphyton biomass at depth 40 and 80 cm among all the treatments over the study period (Table 3) whereas in 120 cm there was no significant difference among the treatments studied. Periphyton biomass in coconut coir (4.1450) was more than the split bamboo pole (3.6717) at 40 cm depth. At 80 cm depth also, higher periphyton biomass was recorded in coconut coir (3.5325) than Split bamboo pole (3.2108). Same result was observed in 120 cm depth. Periphyton biomass in coconut coir (2.8210) was more than the Split bamboo pole (2.7208) at 120 cm depth (Figure 2).

Table 3: "T" test analysis of the data relating to periphyton biomass for different natural substrates recorded at different depths

Sl. No.	Parameters (Substrate)	Depth (cm)	SBP Mean±SE	CC Mean±SE	LS
1	SBP×CC	40 (surface)	3.6717± 0.5001	4.1450± 0.4319	S
2	SBP×CC	80 (Column)	3.2108± 0.5651	3.5325 ± 0.5059	S
3	SBP×CC	120 (bottom)	2.7208± 0.5340	2.8210 ± 0.6123	NS

SBP: Split bamboo poles; CC: Coconut coir; LS: Level of significance; NS: Non-significant; S: Significant

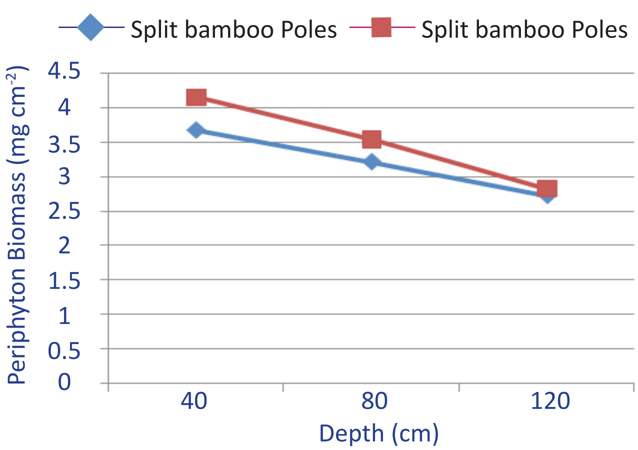

Figure 2: Periphyton Biomass at various depth for different natural substrates during the study

'T' test analysis of the data relating to periphyton biomass for split bamboo pole and coconut coir recorded at different depths indicated that upper 0–40 cm depth had higher values than the other depths. In the present study, the periphyton biomass from coconut coir was comparatively higher (3.4995±0.3120 mg cm^{-2}) than the split bamboo poles (3.2011±0.3044 mg cm^{-2}). So coconut coir was found to have a better surface structure for periphyton species for attachment. But, it is reported that bamboo pole might provide a better surface structure for periphyton species to attach to, or might leach nutrients beneficial to periphyton growth (Keshavanath et al., 2001). Bamboo was used as a substrate in *Peneaus monodon* treatment pond and found periphyton biomass interms of dry matter over substrate was 5.9±0.7 mg cm^{-1} (Westlake et al., 1980; Anand et al., 2014). The quantity of periphyton varied significantly with substrate type, fertilization level, environment conditions and taxonomic composition (Keshavanath et al., 2001; Makarevich et al., 1993). The Periphyton growth comparison study was made for four natural substrates such as bamboo mat, sugarcane bagasse, coconut leaf and palm leaf and reported that the periphyton growth was better on coconut leaf than other tested materials (Keshavanath et al., 2012). The substrate palm leaf and nylon net were compared for periphyton growth and found that the palm leaf had better periphyton growth than nylon net (Dutta et al., 2013).

Over all, periphyton biomass for split bamboo pole and coconut coir recorded at different depths indicated that upper 0–40 cm depth had higher values than the other depths (Figure 3). These results are in agreement with the findings of Konan- Brou and Guiral and Keshavanath, who reported maximum periphytic biomass levels coinciding with photosynthetic compensation depths (Konan-Brou and Guiral 1994; Keshavanath et al., 2001). Increasing the nutrient levels and using substrates that help to periphyton growth seem to be possible solution to enhance fish production in periphyton-based pond aquaculture system (Azim et al.,

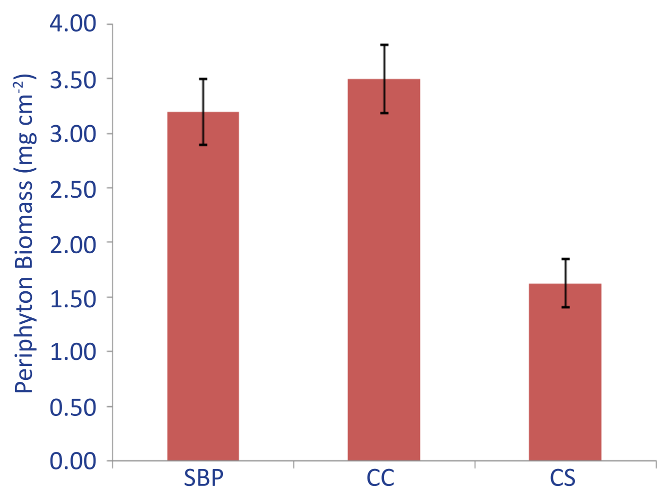

Figure 3: Periphyton Biomass for different natural substrates during the study

2004; Keshavanath et al., 2002). Microbial organisms and meio-macrofauna grown in the periphyton may also be utilized by cultured organism and therefore, the need for artificial feed can be substantially reduced by providing the substrates in aquaculture ponds (Zarain-Herzberg et al., 2006). Amisah et al. (2008) and Saikia and Das (2011) compared the biodegradable and non-biodegradable substances for periphyton based aquaculture to enhance fish production. They reported about 88 genera of periphytic microalgae composed of Chlorophyceae, Bacillariophyceae and

Cyanophyceae. Chlorophyceae and Cyanophyceae preferred rice stems, whereas, Bacillariophyceae preferred glass slide. In the present study, a total 16 genera were found and among them the Bacillariophyceae preferred natural substrates (split bamboo pole and coconut coir) for its growth and settlement in seawater medium in the earthen lined pond.

The mean and standard deviation of various physico-chemical water quality parameters recorded such as water transparency, water temperature, water salinity, water pH, dissolved oxygen, biological oxygen demand (BOD), total ammonia nitrogen (TAN), Nitrite-N, Nitrate-N and chlorophyll 'a' are given in Table 4. In the periphyton experimental pond, temperature, salinity, pH, nitrite and ammonia did not show significant difference (p>0.05) over the experimental period while secchi disk reading, BOD, dissolved oxygen, nitrate and chlorophyll 'a' were shown significant difference (p<0.05) over the experimental period (Table 3). The highest secchi disk reading was observed on 1st day (72.50±0.71 cm), while, it was lowest in the final day (47.00±.00 cm). The lowest and highest dissolved oxygen was recorded on 1st day and final day were 3.80±0.14 mg l^{-1} and 6.80±0.34 mg l^{-1} respectively. This level shows the good sign of dissolved oxygen in the pond and provide good environment for shrimp culture. The highest BOD reading was observed on final day (2.88±0.27 mg l^{-1}), while, it was lowest in the 1st day (1.75±0.07 mg l^{-1}). In periphyton experiment system, the water quality parameters recorded were under optimal condition throughout the study period[23]. Significantly higher value of Nitrate-N was observed on 45th day (0.41±0.02 µg.at.NO_3-N l^{-1}), while, it was lower on 1st day (0.31±0.01 µg.at.NO_3-N l^{-1}) of the experiment. With regard to chlorophyll 'a', significant higher values were observed in the final day (75.25±5.89 mg m^{-3}) and it was lower on 1st day (30.79±2.63 mg m^{-3}) of the experiment. On the contrary, the biological oxygen demand (BOD) showed variations among the sampling days and this would manifest the differential rates of consumption pattern of oxygen by the organisms over the experimental period. Several studies proved the reduction of BOD level in substrate installed tanks compared to non substrates tank (Keshavanath et al., 2001 and 2002; Ramesh et al., 1999; Umesh et al., 1999; Keshavanath et al., 2004; Dharmaraj et al., 2002; Mridula et al., 2003). In the present study also, the BOD values were low in the substrate added pond and this finding which fully concurs with the above workers findings. Results of the ANOVA showed that significant variations were observed in the Nitrate-N and chlorophyll 'a' concentrations over the experimental period. In ponds and lakes, phytoplankton productivity is positively correlated with nutrient concentrations (Boyd, 1990) and in periphyton based ponds, this relationship is interfered with by competition and interactions between periphyton and phytoplankton. Comparatively lower level of total ammonia nitrogen (TAN), NO_3-N, NO_2-N and PO_4-P were observed in substrate based system while no significance difference was noticed among the experimental ponds (Anand et al., 2015). Higher chlorophyll 'a'content in substrates treatment indicates the phytoplankton production, which is an indication of the positive effect on plankton nutritional quality (Azim et al., 2004).

Table 4: Physico-chemical parameters of seawater in earthen lined pond during experimental period

Water parameters	15 days	30 days	45days
Transparency (cm)	53.00±1.41^{a}	48.50±.70^{b}	47.00±.00^{b}
Water temperature (˚C)	32.00±.00^{a}	32.00±.00^{a}	33.00±.00^{a}
Salinity (ppt)	40.00±.00^{a}	42.00±.00^{a}	43.00±.00^{a}
Ph	7.85±.05^{a}	7.80±.00^{a}	7.85±.05^{a}
Dissolved Oxygen (mg l^{-1})	5.76±.22^{a}	6.00±.34^{ab}	6.80±.34^{b}
BOD (mg l^{-1})	2.32±.34^{a}	2.24±.27^{a}	2.88±.27^{a}
Nitrite (µg. at.NO_2-N l^{-1})	0.47±.08^{a}	0.45±.07^{a}	0.46±.04^{a}
Nitrate (µg. at.NO_3-N l^{-1})	0.33±.01^{b}	0.34±.01^{b}	0.41±.02^{a}
Ammonia (µg. at.NH_3-N l^{-1})	0.29±.02^{a}	0.30±.0^{a}	0.31±.01^{a}
Chlorophyll a (mg m^{-3})	40.50±9.98^{b}	61.21±8.64^{ab}	75.25±5.89^{a}

Values are expressed as (Mean±SD); Values with same superscripts in a row do not differ significantly at p>0.05. (n=2)

4. Conclusion

The present experiment for periphyton growth on natural substrates has shown that the coconut coir has the potential for better periphyton growth on them than the split bamboo pole in earthen lined pond seawater medium. The present work indicates that there is a good relationship between the substrates, periphyton and environment. Periphyton grown on the natural substrate is an important natural food source and the quantity of artificial feed can be reduced substantially for the fishes in the aquaculture system.

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