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Effect of Different Natural Substrates on Periphyton Production under Earthen Lined Seawater Pond for Periphyton based Aquaculture

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ABSTRACT

Background: Periphyton-based aquaculture improves water quality, provides a natural food supplement, limits harmful bacteria, provides a safe haven for shrimp to escape, and adds living space. The impacts of diverse natural substrates on periphyton formation were examined under earthen lined pond seawater systems as a first step in analysing the sustainability of periphyton-based shrimp production.

Methods: Natural substrates such as coconut coir, coconut shell and split bamboo pole were placed inside the earthen lined pond filled with seawater for duration of 45 days. Periphyton samples were obtained from each type of substrate on the 15th, 30th and 45th days of the experiment. For all substrates, periphyton biomass and chlorophyll 'a' pigment in the periphyton were measured at depths of 0 to 40 cm, 40 to 80 cm and 80 to 120 cm. Physico-chemical parameters of experiment pond water were recorded during periphyton samplings.

Result: During the experiment, coconut coir showed higher periphyton biomass in terms of dry matter (DM) (3.4995 0.31201 mg/cm^2) and chlorophyll 'a' pigment (3.2949 0. 27076 mg/cm^2). Duncan's Multiple Range Test and One Way ANOVA of diverse data reveal a significant difference (P 0.05) between the substrates in periphyton biomass and chlorophyll 'a' pigment. In all three substrates, a Student's 't' test analysis shows that the upper 0 to 40 cm depth yields more periphyton biomass and chlorophyll 'a' content than the other levels. Coconut coir was the best natural substrate for periphyton growth among the three.

Key words: Bamboo poles, Coconut coir, Coconut shell, Periphyton quantity.

INTRODUCTION

Aquaculture mainly depends upon external inputs for production such as fertilizer and feed. Because of the high cost of foreign inputs, poorer sectors were unable to participate in production (O'Riordan, 1992). As a result, natural substrate-based aquaculture is receiving more attention in order to reduce production costs. Periphyton refers to any organism that is connected to a submerged substrate. On a clean surface, the formation of a periphyton layer is usually initiated by the deposition of a coating of dissolved organic compounds to which bacteria are drawn by hydrophobic interactions (Cowling *et al*., 2000). Before stocking fish, let at least 2 weeks for periphyton to grow on the substrates (Azim *et al*. 2003). Periphyton attachment is higher in bamboo than in PVC pipes or sugarcane bagasse bundles, according to Keshavanath *et al*. (2001). Natural substrates support periphyton growth more than artificial substrates (Keshavanath *et al*., 2012; Dutta *et al*., 2013). Using three plant substrates, sugarcane bagasse, paddy straw, and dried Eichhornia, periphyton had a good influence on rohu development (Wahab *et al*., 1999). Several authors have undertaken various research on the effects of substrates on shrimp growth and discovered a variety of outcomes, including improved water quality, more natural food supplementation, bacterial growth inhibition, shrimp rescue, and the addition of living space (Zarain-Herzberg *et al*., 2006; Kumlu *et al*., 2001).

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Several studies have shown that bacterial and microalgal biofilms growing on natural or manufactured submerged substrates (periphyton) can successfully be used as the primary or sole food source for a variety of freshwater and brackish water fish species (Azim *et al*., 2002, 2004; Keshavanath *et al*., 2004). Promoting the growth of natural planktonic or benthic microbial (bioflocs) and microalgal communities (periphyton) in the pond environment is another option. Because they consume nutrients in autotrophic and heterotrophic activities, which speed up the removal of organic and inorganic wastes, water quality improves, and their biomass can be used as a food source by cultured species (Azim *et al*., 2002). The increased fish production was ascribed to the formation of bacterial biofilm on the substrate, which provides food for zooplankton and fish. Umesh *et al*. (2000) investigated the effects of microbial biofilm development on the growth of common carp, tilapia, and rohu fingerlings in biodegradable substrate such as sugarcane bagasse, and found a considerable increase in fish growth in ponds with substrate. The good impact of substrate-based biofilm or periphyton on overall pond ecology, combined with higher production and lower input costs, makes periphyton-based ponds a reliable production method.

Periphyton growth on various types of natural substrate in seawater earthen line systems or ponds has received little attention. In a seawater earthen lined pond, the current study was conducted to evaluate periphyton biomass and chlorophyll 'a' pigment on various natural materials such as split bamboo poles, coconut coir, and coconut shell.

MATERIALS AND METHODS

The experiments were carried out in two earthen lined ponds, each having water spread area of 30 m^2 during the period 2018-19 at Marine Research Farm Facility, Tharuvaikulam, Thoothukudi, Tamil Nadu, India. The area is located at 8°89′ N latitude and 78°17′ E longitude. Seawater was filled upto 1.2 m depth and water is maintained in this depth over the study period. The experiment was conducted in duplicate with natural substrate for periphyton attachment on them.

In this experiment, three different natural substrates such as split bamboo pole, coconut coir and coconut shell were used for periphyton growth and they were collected locally. All the substrates were introduced in one pond and in duplicate ponds. The five bamboo poles were tied in nylon ropes (5 mm) and vertically immersed in the experimental ponds leaving 10 cm space between each bamboo poles. Coconut coir were tied in nylon rope (5 mm) and vertically immersed in the experimental ponds leaving 10 cm space between each coconut coir. 1.3 m length nylon twine was taken and five coconut shells were arranged in each line and hanged vertically. These five nylon twines were tied in nylon ropes and immersed vertically in the experimental ponds (5 coconut shell × 5 nylon twines). The space between each nylon twine is 10 cm. The mean diameter of the coconut shell, coconut coir and bamboo pole were 11.4 cm, 2.6 cm and 6.2 cm respectively.

After the substrates were installed, the ponds were filled with sea water upto 1.20 m depth. Liming is done by applying Quicklime (CaO) at the rate of 250 kg/ha (Azim *et al*. 2003). After three days of liming, fertilization is done by applying cow dung at the rate of 1,500 kg/ha. The same dosage rate of cow dung application was continued once in 10 days over the study period.

Periphyton samples were collected in morning 10.00 to 11.00 am on 15th, 30th and 45th day of experiment. From each type of substrate periphyton samples were collected at each of three depths 40, 80 and 120 cm below the water surface from 2 × 2 cm^2 area per substrate. While sample collection the areas were scrapped carefully with a scalpel blade to remove all periphyton without damaging the substrate. After sampling, the substrates were marked and placed in their original positions 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. APHA (1995) standard methods were used for the determination.

The chlorophyll 'a' pigment in the periphyton was determined following standard method of APHA (1995). In addition to the samples taken from three depths, an extra 2 × 2 cm^2 composite periphyton sample was collected from each depth and pooled. After removal, the pooled sample was immediately transferred to a tube containing 10 ml 90% acetone, sealed and transferred to the refrigerator for storing overnight. Following the samples collected in the morning were homogenized for 30 seconds with a tissue grinder and centrifuged for 10 min at 3000 rpm. The supernatant was transferred to the 3.5 ml glass cuvettes and then acidified by the addition of three drops of 0.1 N HCL and absorption was measured at 663, 645 and 630 nm using a UV-VIS spectrophotometer. Chlorophyll 'a' pigment was calculated using the trichromatic equation given in APHA (1995).

$$\text{Chlorophyll 'a'} = 11.64E_{663} - 2.16E_{645} + 0.10E_{630}$$

Water quality parameters were also checked for every two weeks by taking water samples between 10.00 and 11.00 am. Water quality parameters were checked by using mercury thermometer for temperature, hand refractometer for salinity, secchi disk for transparency and PH pen for measuring PH. Dissolved oxygen was measured following Winkler method (APHA, 1995) at lab. The water samples were filtered through GF/C Whatman glass fibre filter and the filtrate was obtained. The filtrate was analysed for Nitrate-N, Nitrite-N and total ammonia nitrogen (TAN) by using the standard procedures given by APHA (1995). Chlorophyll a content is analysed from non-filtered water column samples by following standard methods as given by APHA (1995). The water sample was estimated for Biological oxygen demand (BOD) by following standard methods as given by APHA (1995).

Statistical analysis such as One-way ANOVA, Duncan's multiple range test using SPSS 20.0 and student's 't' test using Microsoft excel were used to analyse the data collected from this study.

RESULTS AND DISCUSSION

The periphyton biomass from coconut coir was comparatively higher (3.4995±0.31201 mg / cm^2) than the split bamboo poles (3.2011±0.30437 mg / cm^2) and coconut shell (1.6297±0.22078 mg/cm^2) during the experiment (Fig 1). The chlorophyll 'a' pigment recorded from coconut coir was comparatively higher (3.2949±0.27076 mg/cm^2) than the split bamboo poles (3.0062±0. 26531 mg / cm^2) and coconut shell (1.5569±0.19885 mg / cm^2) during the experiment (Fig 2).

One way ANOVA of the different data affirmed that significant difference ($P<0.05$) was observed in the dry matter content of the periphyton (Table 1) and chlorophyll 'a' pigment (Table 2) among the substrates. As per the Duncan's multiple range test, dry matter content of periphyton (Table 1) and chlorophyll 'a' pigment (Table 2) recorded for coconut coir and split bamboo poles were significantly higher ($P<0.05$) than coconut shell.

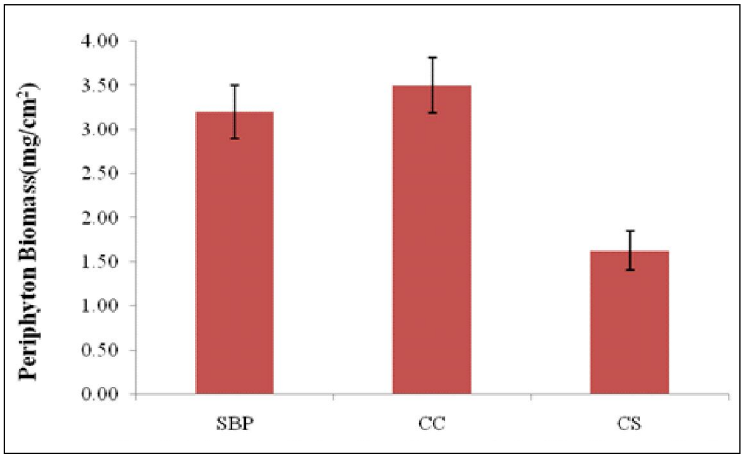

Fig 1: Periphyton Biomass for different natural substrates during the experimental period. SBL- split Bamboo poles, CC- Coconut Coir, CS- Coconut Shell.

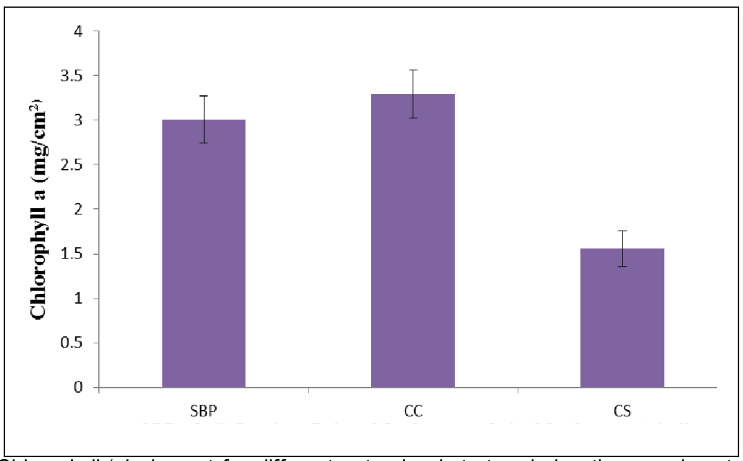

Fig 2: Chlorophyll 'a' pigment for different natural substrates during the experimental period. SBL- Spit Bamboo Poles, CC - Coconut coir, CS - Coconut shell.

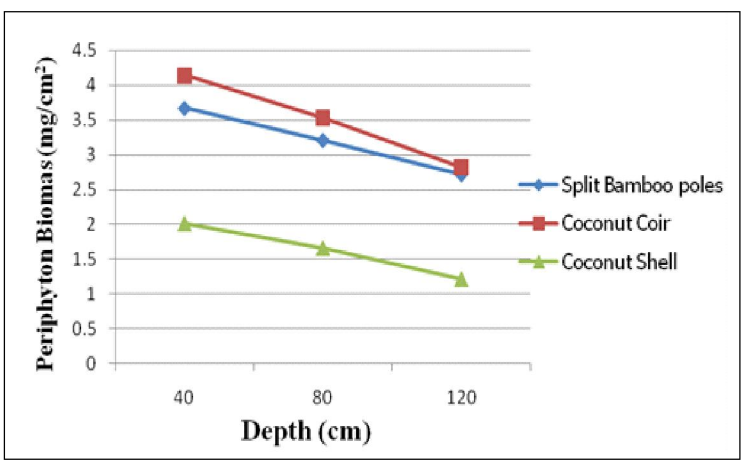

Fig 3: Periphyton Biomass at various depth for different natural substrates during the experimental period.

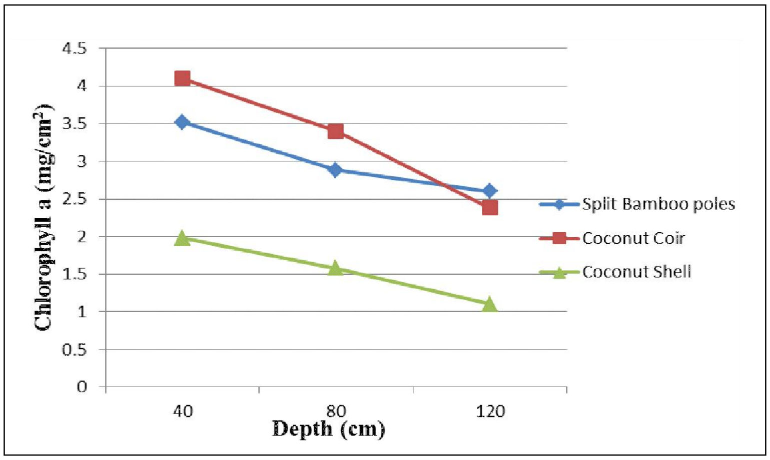

Fig 4: Chlorophyll ‘a’ pigment at various depth for different natural substrates during the experimental period.

As per the student’s ‘t’ test analysis of the data affirmed that highly significant was observed in periphyton biomass (Table 3) and chlorophyll ‘a’ pigment (Table 4) at various depth (40, 80 and 120 cm) among all the treatments over the study period.

The mean values of periphyton biomass (mg / cm^2) observed among all the treatments for 40, 80 and 120 cm depth over the study period is given in the following descending order:

40 cm depth

[Coconut Coir (4.145) > Spit bamboo pole (3.67166) > Coconut shell (2.01583)]

80 cm depth

[Coconut Coir (3.5325) > Spit bamboo pole (3.21083) > Coconut shell (1.66)]

120 cm depth

[Coconut Coir (2.821) > Spit bamboo pole (2.72083) > Coconut shell (1.21333)]

The mean values of chlorophyll ‘a’ pigment (mg / cm^2) observed among all the treatments for 40, 80 and 120 cm depth over the study period was given in the following descending order:

40 cm depth

[Coconut Coir (4.10) > Spit bamboo pole (3.52) > Coconut shell (1.979)]

80 cm depth

[Coconut Coir (3.398) > Spit bamboo pole (2.88) > Coconut shell (1.58)]

120 cm depth

[Spit bamboo pole (2.60) > Coconut Coir (2.386) > Coconut shell (1.105)]

Student’s ‘t’ test analysis of the data relating to periphyton biomass and chlorophyll ‘a’ pigment for split bamboo pole, coconut coir and coconut shell recorded at different depths indicated that upper 0 to 40 cm depth had higher values than the other depths such as 40 to 80 and 80 to 120 cm.

In the present study, coconut coir had better surface structure for periphyton species for attachment. But, Keshavanath *et al.* (2001) indicated that bamboo pole is having a better surface structure for attachment of periphyton and provide nutrients beneficial to the growth of periphyton. Several authors found that, based on the substrate type, rate of fertilization, different environment and species composition periphyton quantity is varied significantly (Makarevich *et al.*, 1993 and Keshavanath *et al.*, 2001). Keshavanath *et al.* (2012) made the periphyton growth comparison study and reported that the periphyton attachment was better on coconut leaf than bamboo mat,

Table 1: Periphyton biomass on dry matter basis (mg/cm^2) of various substratse.

Substrate	Split bamboo poles	Coconut coir	Coconut shell	Total
N	18	18	18	54
Mean	3.2011^a	3.4995^a	1.6297^b	2.7768
Std. deviation	1.29132	1.32374	.93667	1.4365
Std. error	0.30437	0.31201	0.22078	0.19549

Values with same superscripts in a mean row do not differ significantly at P>0.05. Samples from three depths, two ponds and three sampling dates (n = 18).

Table 2: Chlorophyll ‘a’ pigment (mg/cm^2) of various substrates.

Substrate	Split bamboo poles	Coconut coir	Coconut shell	Total
N	18	18	18	54
Mean	3.0062^a	3.2949^a	1.5569^b	2.6194
Std. Deviation	1.12564	1.14874	0.84363	1.28343
Std. Error	0.26531	0.27076	0.19885	0.17465

Values with same superscripts in a mean row do not differ significantly at P>0.05. Samples from three depths, two ponds and three sampling dates (n = 18).

sugarcane bagasse and palm leaf. Dutta *et al*. (2013) found that comparatively periphyton growth and attachment was better in palm leaf than nylon net. A positive effect of periphyton on growth of rohu was recorded using three plant substrates such as sugarcane bagasse, paddy straw and dried *Eichhornia* (Wahab *et al*., 1999). The higher production of fish was attributed to bacterial biofilm promoted on the substrate, which forms food for zooplankton and fish. Effects of microbial biofilm production on biodegradable substrate such as sugarcane bagasse on growth of common carp, tilapia and rohu fingerlings have been evaluated by Umesh *et al*. (2000) and recorded a significant increase in fish growth in pond with substrate. Shankar *et al*. (1998) indicated that, the increase in growth of fish in tanks with sugar bagasse, paddy straw were largely due to the increased zooplankton density in the water. This was influenced by a phenomenal increase in biofilm on the substrate on which both zooplankton and fish grazed.

Over all, higher values of periphyton biomass (Fig 3) and chlorophyll 'a' pigment (Fig 4) were recorded at upper 0 to 40 cm depth than the other depths (40 to 80 and 80 to 120 cm) in all the natural substrates. Similar results were found from the findings of Keshavanath *et al.* (2001), who highlighted the maximum periphytic biomass levels coinciding with photosynthetic compensation depths.

The physico-chemical water quality parameters were checked and the mean and standard deviation values are given in Table 5. In the periphyton experimental pond, there was no significant difference ($P>0.05$) observed in temperature, salinity, pH, BOD, nitrite and ammonia over the experimental period. But significant difference ($P<0.05$) was observed in secchi disk reading, dissolved oxygen, nitrate and chlorophyll 'a' over the experimental period (Table 5). The highest and lowest secchi disk reading was observed on 15th day (53.00±1.41cm) and final day (47.00±.00 cm). The lowest dissolved oxygen was recorded on 15th day (5.76 ±0.22 mg/l), while it was highest in the final day (6.80± 0.34 mg/l). This shows the good sign of dissolved oxygen and provide good environment in the pond for shrimp culture. The highest and lowest level of Nitrate-N value was observed on 45th day (0.41±0.02 µg.at.NO_3-N / l) and 15th day (0.33± 0.01 µg.at.NO_3-N / l) of the experiment. The highest and lowest level of chlorophyll 'a' was observed on final day (75.25±5.89 mg/m^3) and 15th day (40.50±9.98 mg / m^3) of the experiment. This result indicates the increment of periphyton production over the study period due to the

Table 3: Student's t-test analysis of the data relating to periphyton biomass for different natural substrates recorded at different depths.

Parameters (Substrate)	Depth (cm)	X_1	X_2	DF	Student's t value	P value	LS
SBP × CC	40 (surface)	3.67166	4.145	2	-2.84043	P<0.05	S
SBP × CC	80 (Colum)	3.21083	3.5325	2	-2.24191	P>0.07	NS
SBP × CC	120 (bottom)	2.72083	2.821	2	-0.48663	P>0.33	NS
CC × CS	40 (surface)	4.145	2.01583	2	12.8392	P<0.003	S
CC × CS	80 (Colum)	3.5325	1.66	2	7.925593	P<0.007	S
CC × CS	120 (bottom)	2.821	1.21333	2	4.165693	P<0.02	S
SBP × CS	40 (surface)	3.67166	2.01583	2	5.043586	P<0.01	S
SBP × CS	80 (Colum)	3.21083	1.66	2	6.00801	P<0.01	S
SBP × CS	120 (bottom)	2.72083	1.21333	2	5.964064	P<0.01	S

SBP-Split Bamboo Poles / CC-Coconut Coir / CS-Coconut Shell LS-Level of Significance / DF - Degrees of freedom / NS- Non Significant / S- Significant.

Table 4: Student's t-test analysis of the data relating to chlorophyll 'a' concentration for different natural substrates recorded at different depths.

Parameters (Substrate)	Depth (cm)	X_1	X_2	DF	Student's t value	P value	LS
SBP × CC	40 (surface)	3.52	4.10	2	-1.91515	P<0.09	NS
SBP × CC	80 (Colum)	2.88	3.398	2	-1.9682	P<0.09	NS
SBP × CC	120 (bottom)	2.60	2.386	2	3.0968	Pe"0.04	S
CC × CS	40 (surface)	4.10	1.979	2	23.4408	Pe"0.0009	S
CC × CS	80 (Colum)	3.398	1.58	2	15.0615	Pe"0.002	S
CC × CS	120 (bottom)	2.386	1.105	2	3.7505	Pe"0.03	S
SBP × CS	40 (surface)	3.52	1.979	2	6.7890	Pe"0.01	S
SBP × CS	80 (Colum)	2.88	1.58	2	6.6377	Pe"0.01	S
SBP × CS	120 (bottom)	2.60	1.105	2	4.8253	Pe"0.02	S

SBP- Split Bamboo Poles / CC- Coconut Coir / CS- Coconut Shell LS- Level of Significance / DF - Degrees of freedom / NS- Non Significant / S- Significant

Table 5: Physico-chemical parameters of seawater in earthen lined pond during the experimental period.

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

Values are expressed as (Mean±SD).

Values with same superscripts in a row do not differ significantly at P>0.05. (n=2).

natural substrate introduction. There is significant variations in biological oxygen demand (BOD) level among the sampling days and this manifest the differential rates of oxygen consumption over the experimental period by the organisms. Many authors proved that the BOD level was reduced in substrate installed pond compared to non substrates pond (control) (Keshavanath *et al*., 2001, 2002; Dharmaraj *et al*., 2002; Keshavanath *et al*., 2004). In the present study also, the BOD level was low in the substrate installed pond compared to non substrate pond and this coincides with the findings of above said reports. ANOVA results indicated that over the experimental period, significant variations were observed in the Nitrate - N and chlorophyll 'a' content. Phytoplankton productivity is positively correlated with nutrient concentrations in ponds and lakes (Boyd, 1990). In substrate treatments, higher chlorophyll 'a'content indicates the phytoplankton production, which is the positive effect indication on plankton nutritional quality (Azim *et al*. 2002).

CONCLUSION

In the study, the growth of periphyton is higher in coconut coir than split bamboo pole and coconut shell. Coconut coir is also enhancing as natural substrate for periphyton development in earthen lined seawater pond for aquaculture. Periphyton grown on this natural substrate serves as an important natural food source for the fishes and reduces the cost of production by reducing the artificial feed substantially in aquaculture ponds. By this way cost reduction can be done in feed in shrimp farming, hence increases the profit of culture. Further, research work is also required to determine the optimum density of natural substrates and economic viability of substrate based shrimp culture in earthen lined pond for adoption.

Conflict of interest: None.

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