The effect of Added Substrates on Growth Performance, Water Quality Dynamics, Proximate Composition of Whiteleg Shrimp, *Penaeus Vannamei* (Boone, 1931) in Outdoor Biofloc-Based Nursery Rearing System

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ABSTRACT

This study assessed the effect of different types of substrates integration in biofloc based nursery rearing system. The experiment was conducted comprising 100% of tank total surface area (0.86 m^2). Twenty four experimental units of 100 L capacity were stocked with *Penaeus vannamei* PL-10 (5.0 ± 0.00 mg) for 30 days. The experiment consisted of six treatments as biofloc(F), biofloc with bamboo mat (F+BM), biofloc with HDPL sheet (F+HDPL), biofloc with mosquito net screen (F+MNS), biofloc with shade net (F+SN) and a group without biofloc and without substrate as control (C) with four replicates. Among the treatments, significantly higher ($p<0.05$) final weight (1.1035±0.005g), survival rate (94.42±0.68%) and lower feed conversion ratio (1.1782±0.0048) were recorded in F+BM system followed by F+MNS and F+SN treatments. The substrates except HDPL sheet affected water quality since the concentrations of total ammonia, nitrate, nitrite and total suspended solids were significantly different ($p < 0.05$) from other treatments. The treatments had effect on the proximate composition of shrimp and biofloc; crude protein was significantly higher ($p < 0.05$) in tanks with bamboo mat as a substrates. Between different substrates the natural substrate as bamboo mat was the most suitable for nursery rearing of *P. vannamei* in the biofloc system followed by artificial substrate as mosquito net screen and shade net essentially because it could maintain levels of solids suspended in biofloc. Also, the microbial community associated with substrate was mainly regulating water quality variables and biofilm formation on substrate serve as natural feed which enhances the concentration of crude protein in shrimp and bioflocs.

Keywords: substrate, biofloc, nursery rearing, Penaeus vannamei, growth performance

1. INTRODUCTION

Globally 130.9 million metric tons of aquaculture products were produced, with a market value of USD 313 billion [1]. The most valuable class of fish traded worldwide is the crustacean, with a value share of 23% (USD 71.99 billion) and a production share of 8% (12.75 million metric tons) in the aquaculture industry. The most significant species of cultivated crustaceans is the Pacific white shrimp, *Penaeus vannamei* (Boone, 1931); its production accounted for 53.33% of all crustacean production [1]. This species dominates the Indian shrimp farming industry and offers a significant contribution accounting for

90.6% of the country's total shrimp aquaculture production [2]. However, the industry is currently dealing with a number of issues most notably the rise in the cost of shrimp feed and an emergence of new diseases, as a result of an over-reliance on a single species [3]. Considering this, shrimp farmers in India have begun to express interest in eco-friendly farming practices such as biofloc-based farming [4], which includes a variety of bacteria, microalgae, fungi, detritus, zooplankton, and other suspended organisms [5,6]. Culture of *P. vannamei* in biofloc systems has been shown to have several benefits, including increased growth and survival [7,8], decreased feed conversion ratio (FCR) [9] and disease prevention [6,7]. These bioflocs are of great importance for marine shrimp feeding because of their biological diversity [10].

Though the biofloc-based approach has many advantages over conventional farming, proper discharge of surplus suspended floc is the biggest challenge that produces more nitrogenous waste. This causes the water quality to deteriorate, which has a detrimental effect on the growth and health of cultured shrimp [11]. The incorporation of submerged substrates in the biofloc system assists in controlling the amount of high turbidity or flocculated particles generated by the biofloc system, which have been shown to reduce nitrogen metabolites [12,13]. The addition of substrates to the biofloc system expands the tank's surface area and offers shelter to cultured animals. It also provides an additional place for nitrifying bacteria for their growth and proliferation [14,12].

Nursery seed rearing of *L. vannamei* has witnessed remarkable advancements in recent years, particularly with the integration of biofloc technology and the introduction of substrate-based systems. In order to address issues that typically arise in the conventional prenursery system, such as biosecurity and toxic ammonia and nitrite [15], biofloc technology (BFT) has also been attempted to be used in the pre-nursery of *P. vannamei* PL [16,17]. There are many studies on biofloc-based culture systems, but there is a lack of information on the incorporation of various submerged substrata into the biofloc nursery system. To better understand how the integrated biofloc substrate system affects *P. vannamei* growth performance, water quality dynamics, proximate composition, its planktonic community and function in capturing flocculated particles during nursery rearing the current study was undertaken.

2. MATERIAL AND METHODS

2.1 Experiment location and Biological material:

The experiment was performed at the ADG Shrimp Farm, Nevare, Ratnagiri, ($17^0$6'55''N $73^0$17'16''E). Specific pathogen-free post-larvae (PL-7) of *P. vannamei* were obtained from a commercial Shrimp Hatchery Venture from Pudukuppam, Villupuram, Tamil Nadu. After 3 days OF acclimatization the post- larvae of PL-10 stage (Initial average length- 1.18 cm and Initial average weight 0.005 g) were transferred to the experimental units.

2.2 Experimental design and setup:

A 30-day outdoor experiment was conducted in Circular HDPE containers of 100 L capacity having surface area 0.86 m^2 and water depth 0.40 m. The experimental design consisted of six treatments comprising biofloc (F), biofloc with bamboo mat (F + B), biofloc with HDPL sheet (F + HDPL), biofloc with mosquito net screen (F + MNS), biofloc with shade net (F + SN) and a group without biofloc and without substrate as control (C) with four replicate. All the groups received 40% crude protein diet while floc received additional molasses as a carbon source for biofloc production with C: N ratio 6:1 [15].

The substrate was fixed on rigid metal wire used for attachment over the experimental tanks. The substrate area is calculated by considering both sides of substrate was distributed vertically to the water column to cover 100% of the tanks surface area. They were fastened vertically to the water column as 5cm below the surface and 5 cm above the tank floor. Two Sinkers are attached at the bottom of each substrate stripe to place it properly. During the experimental period, water was not exchanged but rather replaced with fresh water owing to evaporation to maintain salinity (28 psu). Throughout the experiment, suspended and settled particles were not removed from the water.

2.3 Water quality parameters:

Water quality parameters such as temperature, pH and salinity were recorded daily using mercury thermometer, refractometer and pH meter respectively while dissolved oxygen was recorded following standard method [18]. Other parameters such as the alkalinity, orthophosphate, total ammonia-N, nitrite-nitrogen (No_2-N), nitrate-nitrogen (NO_3-N), total suspended solids were recorded at three days interval. The concentration of chlorophyll-a in the water was recorded once in a five days as a measure of phytoplanktonic biomass. Estimation was carried out following standard methods [18,19].

2.4 Floc assessment:

The volume of biofloc was determined by sampling 1L of water at regular intervals of three consecutive days using the Imhoff cone (Borosil, India). Before quantifying the volume of flocculation, the water sample had been allowed 20 minutes to settle at the bottom of the cone [20]. On days 15 and 30 of the experiment, the floc particles in suspension (total suspended solids) and those that settled at the tank bottom (total bottom solid) were all collected and analysed [14]. In order to assess the total substrate solid from each treatment the substrate was delicately taken off. The solid particles that were adhered to the substrate were scrapped from 2.0 × 2.0 cm^2 area with sterile scalpel. In a pre-dried and pre-weighed container, the scrapped samples from five separate places of each substrate were combined and dried overnight at 105°C. The difference between the initial and final weights was represented as total substrate solid (mg/cm^2) [19].

2.5 Growth performance and survival:

Two biological variables such as average body length (ABL) and average body weight (ABW) were obtained weekly from approximately 10% sampling from each treatment replicate. Growth performance parameters based on these biological variables such as weight gain (WG), percentage weight gain (WG%), specific growth rate (SGR), feed conversion ratio (FCR) were also recorded on weekly basis. At the end of the trial survival% was calculated. These parameters were obtained using the following formulae [10].

i) Weight gain (WG)= $\text{Final average weight} - \text{Initial weight}$

ii) Specific growth rate (SGR % day^{-1}) $= \frac{\ln(\text{Final body weight}) - \ln(\text{Initial body weight})}{\text{Days of experiment}} \text{ X } 100$

iii) Feed Conversion Ratio (FCR)= $\frac{\text{Total dry weight of feed offered}}{\text{Total shrimp wet weight gained}}$

iv) Survival (%) $= \frac{\text{Final shrimp count}}{\text{Initial shrimp count}} \text{ X } 100$

2.6 Proximate composition of shrimp and biofloc:

Proximate composition of the shrimp and biofloc from all treatments were determined at the end of the experiment following the standard method [21]. Concentrated floc samples were taken from each experimental tank at the end of the trial using a 100 µm mesh net. These samples were then dried in an oven at 60°C and kept in a refrigerator till proximate analysis was done [22]. Shrimp samples were washed with deionized water to remove any adhering contaminant and drained using filter paper. The shrimp exoskeletons were peeled out and the meat was homogenized. The grounded samples were oven dried at 70°C and ground in

2.7 Statistical analysis

Water quality parameters, growth performance and proximate composition were analyzed by one-way ANOVA. To see the mean difference among the treatments Tukey's test [23] were further applied. The level of significance was made at 95% level. All analysis was performed using the statistical software package SPSS version 16.0 program.

3. RESULTS AND DISCUSSION

3.1 Water quality analysis:

The effect of different substrate addition in *p*. *vannamei* biofloc-based nursery rearing system on water quality parameters is presented in table 1.

Table 1: Water quality parameters measured for different treatments in outdoor biofloc-based nursery rearing system with added substrate of *L. vannamei* for 30 days

Parameters	C	F	F + BM	F + HDPL	F + MNS	F + SN
Temperature (^{0} C)	24.04 ± 0.04^{a}	24.02 ± 0.03^{a}	23.92 ± 0.07^{a}	24.00 ± 0.03^{a}	23.94 ± 0.02^{a}	24.06 ± 0.06^{a}
Salinity (psu)	29.40 ± 0.16^{a}	29.20 ± 0.16^{a}	29.56 ± 0.16^{a}	29.50 ± 0.16^{a}	29.55 ± 0.16^{a}	29.64 ± 0.16^{a}
pH	8.10 ± 0.007^{b}	8.04 ± 0.010^{a}	8.03 ± 0.005^{a}	8.03 ± 0.004^{a}	8.03 ± 0.004^{a}	8.03 ± 0.005^{a}
Alkalinity (mg L^{-1})	160.00 ± 1.47^{b}	153.43 ± 0.91^{a}	154.86 ± 0.60^{a}	154.07 ± 1.58^{a}	153.55 ± 0.56^{a}	153.73 ± 0.43^{a}
DO (mg L^{-1})	6.65 ± 0.03^{d}	5.91 ± 0.03^{c}	5.38 ± 0.02^{a}	5.67 ± 0.04^{b}	$5.57 \pm 0\ .02^{b}$	5.57 ± 0.03^{b}
Orthophosphate (mg L^{-1})	0.4132 ± 0.0016^{a}	0.5098 ± 0.0051^{b}	0.5390 ± 0.0044^{c}	0.5212 ± 0.0056^{bc}	0.5323 ± 0.0047^{c}	0.5268 ± 0.0019^{bc}
Total Ammonia Nitrogen (TAN) (mg L^{-1})	0.1068 ± 0.0018^{a}	0.5052 ± 0.0070^{d}	$0.3341 \pm 0\ .0007^{b}$	0.4848 ± 0.0111^{d}	0.4007 ± 0.0074^{c}	0.4202 ± 0.0033^{c}
Nitrite-N (mg L^{-1})	0.0140 ± 0.0006^{a}	0.2130 ± 0.0061^{d}	0.1590 ± 0.0046^{b}	0.1916 ± 0.0025^{c}	0.1652 ± 0.0020^{b}	0.1720 ± 0.0024^{b}
Nitrate-N (mg L^{-1})	0.52 ± 0.02^{a}	2.92 ± 0.06^{d}	1.87 ± 0.07^{b}	2.74 ± 0.04^{d}	2.30 ± 0.06^{c}	2.37 ± 0.07^{c}
Chlorophyll-a (µg L^{-1})	64.45 ± 0.75^{a}	113.70 ± 0.51^{b}	172.19 ± 1.98^{e}	115.41 ± 2.72^{bc}	133.36 ± 4.03^{d}	126.25 ± 3.07^{cd}
Biofloc Volume (ml L^{-1})	0.00^{a}	6.35 ± 0.06^{e}	3.48 ± 0.12^{b}	5.70 ± 0.06^{d}	4.32 ± 0.12^{c}	4.50 ± 0.08^{c}
Total suspended solids (mg L^{-1})	29.61 ± 0.65^{a}	124.50 ± 2.21^{d}	97.20 ± 2.41^{b}	110.09 ± 2.18^{c}	99.07 ± 1.94^{c}	108.34 ± 1.39^{d}

Note: n = 4, values are expressed as (Mean± SE). The means in the same row followed by different letters indicate significant difference between treatments by Tukey's test ($p < 0.05$)

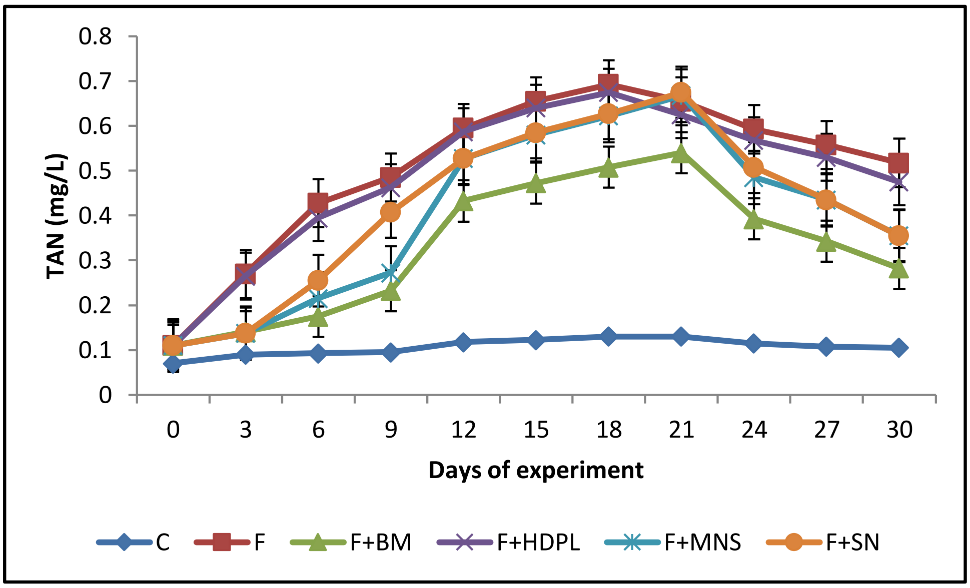
There were no significant differences among the treatments ($p > 0.05$) in terms of the mean values of temperature and salinity. The pH and alkalinity were significantly lower ($p \le 0.05$) in all biofloc treatment tanks compared with control. According to Boyd [24], the pH of water is directly correlated with its alkalinity and typically rises as alkalinity increases. However, throughout our investigation, we discovered that the pH began to decrease after a week of experimentation, and the similar tendency occurred with alkalinity. The similar conclusion had been drawn by Kim et al. [7], who reported that the biofloc treatment elevated the water's carbon dioxide concentration because of the respiration of heterotrophic organisms and notable nitrification.

The mean DO level in control was high from the start of the experiment while in all biofloc groups it started steadily declined after a week. The biofloc treatments resulted in lower DO levels compared to the control, due to increased demand from bacteria and microorganisms [25]. It was observed that the DO concentration in substrate based treatments significantly lower than only floc treatment. Also, the significantly lower amount of DO ($p \le 0.05$) in substrate added clear water rearing system tanks for *E. suratensis* was observed by Yadav et al. [26] as heterotrophic food synthesis consumes a significant amount of oxygen.

The orthophosphate concentrations were significantly higher in the treatments with substrate ($p < 0.05$) but not adequate to impact growth of experimental animals. Similarly, treatments with substrates exhibited significantly higher orthophosphate concentrations than treatments without substrates in *P. vannamei* culture was noted by Fleckenstein et al. [25]. Orthophosphate accumulation in biofloc systems occurred as a result of the steady feed supply, carbon source addition, and animal waste [27,28,12].

In the current investigation, TAN peaks were recorded on the 18th day (Fig. 1), exhibiting variability across the different treatments, which suggests that the incorporation of substrates constitutes an effective methodology for mitigating ammonia peaks, with its efficacy being contingent upon the specific types of artificial substrates introduced into the tanks. These results demonstrate a partial correspondence with the findings presented by Asaduzzaman et al. [29], who revealed that the addition of substrates that promote periphyton growth significantly lowered NO2–N levels and concurrently reduced TAN concentrations via the use of periphyton substrates.

Fig 1: Mean variation in water Total ammonia-N (TAN) for different treatments in outdoor biofloc-based nursery rearing system with added substrate of *L. vannamei* for 30 days



Throughout the experimental period for all treatments the mean nitrite concentrations were below 0.35 mg L^{-1}. Periphyton biofilms enhance nitrification processes [30], thereby sustaining low levels of NO2–N and TAN. The experiment focused on encouraging heterotrophic microorganisms and autotrophic algae communities to remove nitrogen metabolites from submerged substrates. However, increasing nitrite-N (Fig. 2) and nitrate-N (Fig. 3) levels from the 15th day onwards, with a peak on the 24th day, and concurrent reduction in the TAN level in all treatment groups may be attributed to the dominance of nitrifying bacteria. Lara et al.[12] discovered that increasing the amount of artificial substrates offered greater space for the attachment of a more diverse set of bacteria, resulting in more dynamic processing of nitrogen compounds.

Fig 2: Mean variation in water Nitrite-N for different treatments in outdoor biofloc-based nursery rearing system with added substrate of *L. vannamei* for 30 days

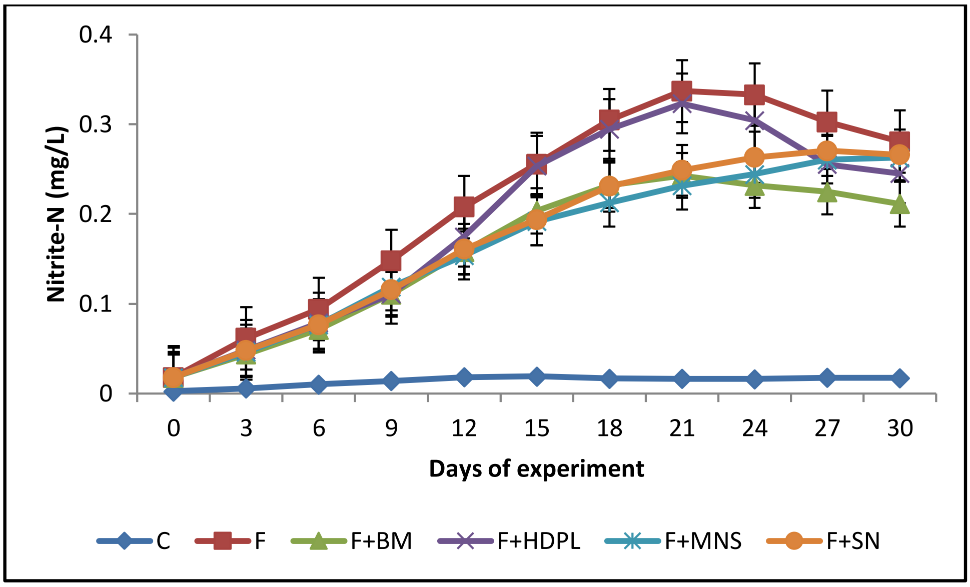
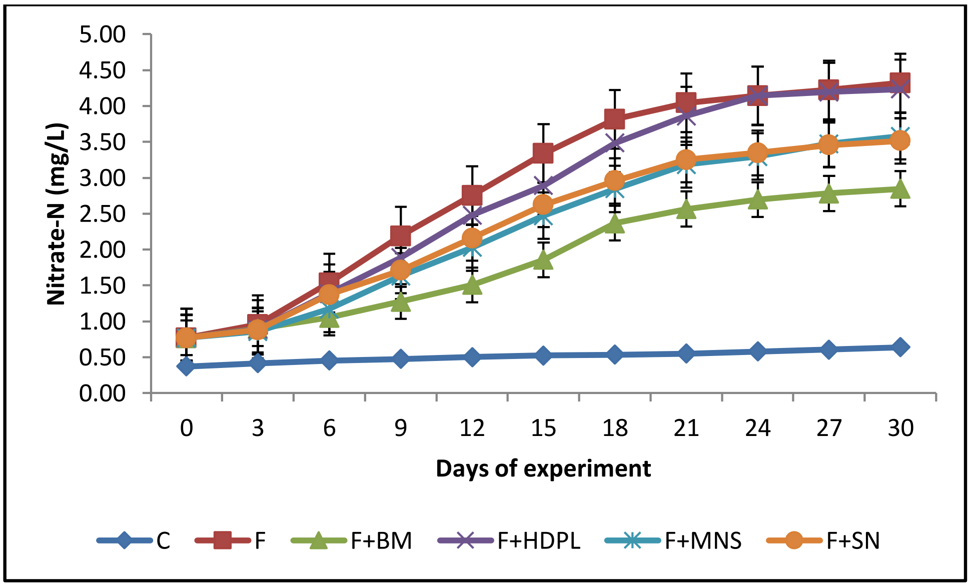


Fig 3: Mean variation in water Nitrate-N for different treatments in outdoor biofloc-based nursery rearing system with added substrate of *L. vannamei* for 30 days



The significantly higher chlorophyll-a (172.19 ± 1.98) was observed in F+BM treatment. Similarly, the higher periphyton chlorophyll-a in treatment with substrate was noted by Asaduzzaman et al. [29] was mainly because of higher rate of nutrient cycling within the periphyton biomass itself.

3.2 Floc assessment:

At mid and end of the experiment the three groups of the flocculated particles that were emerged in the experimental tanks as total suspended solid (TSS), total bottom solid (TBS) and total substrate solid (TSubS) were mentioned in Table 2.

Table 2: Solid particles of different treatments in outdoor biofloc-based nursery rearing system with added substrate of *L. vannamei*

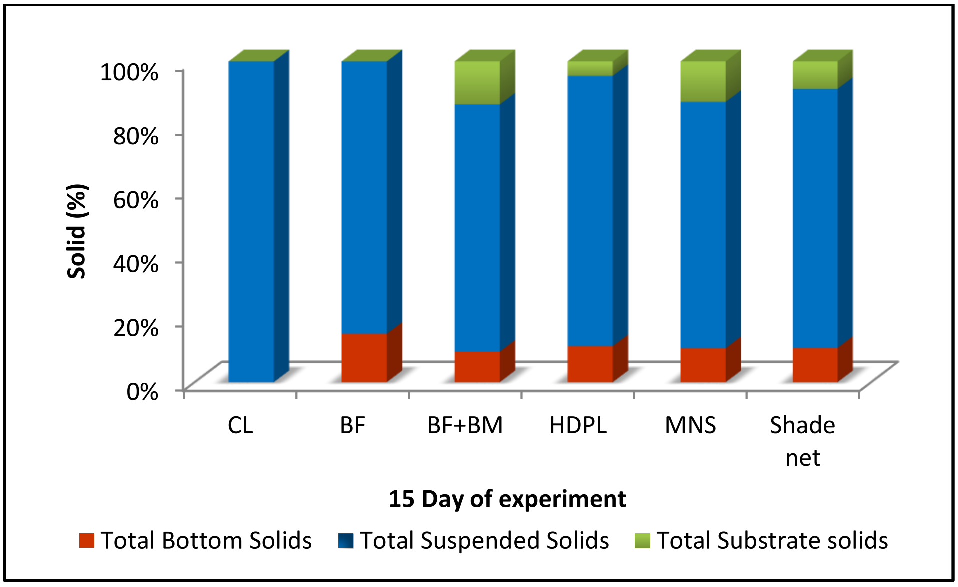
Parameters	C	F	F + BM	F + HDPL	F + MNS	F + SN
15th day reading:						
Total suspended solids (mg L^{-1})	26.75 ± 1.65^{a}	126.25 ± 4.02^{d}	99.00 ± 3.34 ^{b}	112.25 ± 3.09^{c}	104.75 ± 1.93^{bc}	105.50 ± 2.20^{bc}
Total bottom solids (mg L^{-1})	0.00	22.50 ± 0.28^{b}	12.25 ± 0.32^{a}	15.00 ± 0.20^{a}	14.50 ± 0.28^{a}	14.00 ± 0.40^{a}
Total substrate solids (mg L^{-1})	0.00	0.00	17.20 ± 0.00^{c}	6.02 ± 0.00^{a}	17.20 ± 0.00^{c}	11.18 ± 0.00^{b}
Total solids (mg L^{-1})	26.75 ± 1.65^{a}	148.75 ± 4.11^{c}	128.45 ± 3.05^{b}	133.27 ± 2.97^{b}	136.45 ± 2.17^{bc}	130.68 ± 2.06^{b}
30th day reading:						
Total suspended solids (mg L^{-1})	51.00 ± 1.08^{a}	212.50 ± 2.78^{d}	159.50 ± 2.87^{b}	189.75 ± 2.01 ^{c}	157.50 ± 2.39^{b}	184.75 ± 0.62^{c}
Total bottom solids (mg L^{-1})	00	86.50 ± 0.28^{d}	25.00 ± 0.40^{a}	60.25 ± 0.75^{c}	25.00 ± 0.40^{a}	40.25 ± 0.85^{b}
Total substrate solids (mg L^{-1})	00	00	34.40 ± 00^{d}	12.90 ± 00^{a}	30.96 ± 00^{c}	30.10 ± 00^{b}
Total solids (mg L^{-1})	51.00 ± 1.08^{a}	299.00 ± 2.73^{d}	218.90 ± 2.72^{b}	262.90 ± 2.73^{a}	213.46 ± 2.75^{b}	255.10 ± 0.40^{c}

Note: n = 4, values are expressed as (Mean± SE). The means in the same row followed by different letters indicate significant difference between treatments by Tukey's test ($p < 0.05$)

The biofloc system with incorporation of submerged substrate showed significantly lower level of TSS and TBS compared to only floc based treatment (Figure 4a,b). As compared to floc-based treatment (F), the inclusion of submerged substrates, bamboo mat (F + BM) and nylon mesh (F + MNS) and shade net (F + SN), resulted in up to 11.42%, 11.71% and 15.78% decrease in total bottom solid deposition levels respectively. Among the substrate the observation on development of biofilm as a substrate solid at 15th day and 30th day recorded that the growth of periphyton was more rapidly on bamboo mat (13.39 to 15.71%) which was significantly differ with mosquito net screen (12.61 to 14.50%), shade net (8.56 to 11.80%) and HDPL sheet (4.52 to 4.91%).

Fig 4: Solid particle percentage in different treatments in outdoor biofloc-based nursery rearing system of *L. vannamei* a) Observation on 15th day b) Observation on 30th day

a)



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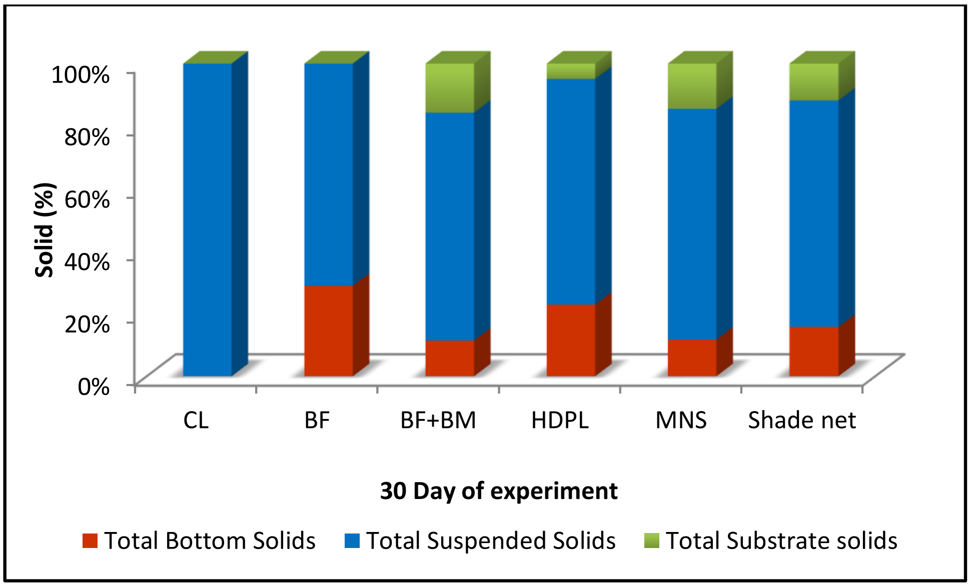

High suspended solids concentrations can impair shrimp growth and survival, as well as the population of some bacteria [31,5,32]. Furthermore, it was noticed that control of solids helps to maintain low nitrite concentrations in the system, owing to excess organic matter, implying a probable relationship between these two parameters [33]. In this experiment excess suspended particles in the biofloc system regulated utilizing different substrates. This finding can be explained by the probability that the placing of artificial substrates influenced water circulation in the tanks by decreasing turbulence. This could have improved sedimentation or particle aggregation, resulting in larger floc and mitigating the negative consequences of an excess of biofloc in the water. The incorporation of submerged substrates such as bamboo mat, mosquito net screen and shade net reduces the bottom deposition of excess solids. The bamboo mat was a natural substrate so periphyton development on it was so fast as compare to other; while HDPL sheet as a substrate had no any effect on bottom deposition and also on periphyton development may be due smooth surface of HDPL sheet was not allow the periphyton to attached on it and also its development. The periphyton biomass in terms total substrate solid (17.20-34.40 mg/L) increased steadily during the rearing period was significantly higher on bamboo mat (13.39 to 15.71%) but the rate of increase was slow. This might be because of higher grazing pressure on periphyton by the overall high biomass of PL. On the basis of Asaduzzaman et al. [29], Rezende et al. [27], Kumar et al. [14], and Lara et al. [12], the use of artificial substrates may help particles adhere to

the mats, filtering the water and lowering the amount of organic matter suspended in it and sludge production.

3.3 Growth performance and survival:

The effect of inclusion of different substrate on the growth performance parameters of *L. vannamei* in the biofloc-based nursery rearing system is presented in Table 3. In the present study, the substrate added treatment (except F+HDPL) performed better in body weight and related growth parameters as weight gain, SGR, and FCR. Several studies have revealed that growth performance of shrimps reared in substrate added systems is higher due to the increased availability of natural nourishment in the form of phytoplankton, zooplankton, heterotrophic beneficial bacteria, fungi, and other micro-macro organisms [9,34,4,35]. In contrary to artificial substrates like mosquito net screens, shade nets, or HDPL sheets, it has been observed that nutrient leaching happens when biodegradable natural substrates like bamboo are immersed in the water column, which accelerates the production of biofilms therefore when compared to other substrates, the growth performance was superior in bamboo mat (F+BM) [36,14].

Table 3: Growth performance (mean ± SE) of different treatments in outdoor biofloc-based nursery rearing system with added substrate of *L. vannamei* after 30 days

Parameters	C	F	F + BM	F + HDPL	F + MNS	F + SN
Initial ABW (g)	0.005 ± 0.001	0.005 ± 0.001	0.005 ± 0.001	0.005 ± 0.001	0.005 ± 0.001	0.005 ± 0.001
Initial ABL (cm)	1.18 ± 0.02	0.005 ± 0.001	0.005 ± 0.001	0.005 ± 0.001	0.005 ± 0.001	0.005 ± 0.001
Final ABW (g)	0.4275 ± 0.006^{a}	0.9100 ± 0.002^{b}	1.1035 ± 0.004^{e}	0.9350 ± 0.007^{bc}	1.0505 ± 0.0167^{d}	0.9575 ± 0.003^{c}
Final ABL (cm)	4.27 ± 0.025^{a}	4.90 ± 0.01^{b}	5.20 ± 0.010^{d}	4.90 ± 0.010^{b}	5.20 ± 0.010^{d}	5.00 ± 0.020^{c}
WG (g)	0.4225 ± 0.006^{a}	0.9050 ± 0.002^{b}	1.098 ± 0.004^{e}	0.9300 ± 0.007^{bc}	1.0455 ± 0.0167^{d}	0.9525 ± 0.003^{c}
SGR (% day^{-1})	15.88 ± 0.05^{a}	18.58 ± 0.01^{b}	19.27 ± 0.01^{e}	18.68 ± 0.02^{bc}	19.09 ± 0.05^{d}	18.76 ± 0.01^{c}
FCR	2.80 ± 0.042^{d}	1.64 ± 0.004^{c}	1.17 ± 0.004^{a}	1.60 ± 0.012^{c}	1.42 ± 0.022^{b}	1.46 ± 0.005^{b}
Survival (%)	67.75 ± 1.08^{a}	83.33 ± 1.17^{b}	94.41 ± 0.68^{d}	89.58 ± 1.02^{c}	88.00 ± 1.00^{c}	91.08 ± 0.59^{c}

Note: n = 4, values are expressed as (Mean± SE). The means in the same row followed by different letters indicate significant difference between treatments by Tukey's test ($p < 0.05$)

The survival of shrimp was considerably ($p < 0.05$) influenced by the presence of floc and substrate. At the completion of the experiment, the significantly highest survival rates (94.41%) were observed in bamboo mat as a substrate compared to all other substrate based treatment. As per references shrimp survival was typically higher in systems that integrate the biofloc and biofilm technologies [37,38,13]. Ferreira et al. [34] obtained survivals over 85.6% utilizing artificial substrates in a biofloc technology (BFT) system, whereas Schveitzer et al. [13] found significantly improved survival in tanks containing substrates (93.9 ± 2.4). Suggesting that added substrates had a favourable impact on shrimp PL and juvenile growth parameters [39,40].

3.4 Proximate composition of shrimp and biofloc:

The crude protein, crude lipid, crude fibre, NFE and GE content of the shrimp and biofloc was significantly higher in substrate added treatment except F +HDPL than control and only floc treatment (Table 4). The CP% in shrimp ranged between 57.43 to 65.27% was signicantly higher ($p < 0.05$) in F+BM, F+MNS and F+SN treatment while in a biofloc composition it was in between 37.01 to 41.71% which was signicantly higher only in F+BM (41.71 ± 0.19) treatment. The CL% in shrimp of F+BM (4.38 ± 0.05) and F+MNS (4.24 ± 0.07) treatment were maximum while in biofloc it is higher in F+BM (2.75 ± 0.09) treatment which differ significantly with other treatments. Shrimp in treatment F+BM (1.82 ± 0.03), F+MNS (1.71 ± 0.06) and F+SN (1.68 ± 0.02) content significantly higher CF% than other treatments. Also, in biofloc it was maximum in F+BM (14.48 ± 0.24) and F+MNS (13.75 ± 0.23) treatment. All treatments had equal percentage of moisture contents for both shrimp and biofloc.

Table 4: Proximate composition (mean ± SE) on dry weight basis of *Litopenaeus vannamei* and biofloc obtained at the end of experiment for different treatments

Treatment	CP (%)	CL (%)	CF (%)	Moisture (%)	Ash (%)	NFE (%)	GE (kcal l00g^{-1})
Shrimp							
C	57.43 ± 0.45^{a}	3.18 ± 0.03^{a}	0.88 ± 0.06^{a}	4.67 ± 0.10^{a}	11.15 ± 0.02^{a}	22.66 ± 0.53^{c}	514.75 ± 0.57^{a}
F	60.65 ± 0.29^{b}	3.61 ± 0.04^{b}	1.10 ± 0.05^{b}	4.77 ± 0.04^{a}	11.67 ± 0.04^{b}	18.19 ± 0.24^{b}	522.08 ± 0.26^{b}
F + BM	65.27 ± 0.49^{c}	4.38 ± 0.05^{d}	1.82 ± 0.03^{c}	4.85 ± 0.10^{a}	12.73 ± 0.05^{c}	10.92 ± 0.47^{a}	533.54 ± 0.85^{c}
F + HDPL	61.22 ± 0.79^{b}	3.66 ± 0.04^{b}	1.13 ± 0.03^{b}	4.87 ± 0.12^{a}	11.74 ± 0.02^{b}	17.35 ± 0.90^{b}	523.22 ± 1.03^{b}
F + MNS	64.87 ± 0.21^{c}	4.24 ± 0.07^{cd}	1.71 ± 0.06^{c}	4.77 ± 0.03^{a}	12.65 ± 0.06^{c}	11.74 ± 0.19^{a}	532.09 ± 0.65^{c}
F + SN	63.90 ± 0.58^{c}	4.17 ± 0.03^{c}	1.68 ± 0.02^{c}	4.75 ± 0.09^{a}	12.56 ± 0.03^{c}	12.92 ± 0.66^{a}	530.23 ± 1.02^{c}
Biofloc							
C	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F	37.01 ± 0.32^{a}	1.54 ± 0.06^{a}	12.42 ± 0.08^{a}	4.95 ± 0.13^{a}	12.55 ± 0.13^{a}	31.38 ± 0.45^{d}	474.50 ± 0.64^{a}
F + BM	41.71 ± 0.19^{d}	2.75 ± 0.09^{d}	14.48 ± 0.24^{b}	4.97 ± 0.24^{a}	16.25 ± 0.08^{c}	19.83 ± 0.44^{a}	488.63 ± 0.62^{d}
F + HDPL	37.46 ± 0.12^{ab}	1.63 ± 0.06^{ab}	12.57 ± 0.23^{a}	4.80 ± 0.09^{a}	13.37 ± 0.46^{a}	30.11 ± 0.41^{d}	475.68 ± 0.50^{ab}
F + MNS	39.61 ± 0.43^{c}	2.43 ± 0.05^{c}	13.75 ± 0.23^{b}	4.80 ± 0.15^{a}	14.80 ± 0.17^{b}	24.61 ± 0.44^{b}	483.58 ± 0.46^{c}
F + SN	38.18 ± 0.15^{b}	1.83 ± 0.06^{b}	12.62 ± 0.11^{a}	4.90 ± 0.13^{a}	14.77 ± 0.31^{b}	27.88 ± 0.55^{c}	477.94 ± 0.58^{b}

Note: The means in the same column followed by different letters indicate significant difference between treatments by Tukey's test ($p < 0.05$).CP, Crude protein; CL, Crude lipid; CF, Crude fiber; NFE, Nitrogen-free extract; GE, Gross energy

The composition of biofloc changed depending on the type of inputs used such as feed, feeding rate, fertilizers, carbon sources, C:N ratio, feed protein content, and due to various environmental factors [23,42]. The importance of substrate was demonstrated by the observation that the proximate composition of the biofloc, which was obtained from biofloc tanks with varying substrate, altered significantly between treatments in terms of all nutritional parameters. In comparison to other published values by Tacon et al. (35–38%) [10] and Azim and Little (38%) [22], the protein content of the biofloc in this investigation was moderate at 37.01% - 41.01%. According to Olier et al. [28], the use of a vertical substrate improved the performance of *P. vannamei* and conserved dietary protein.

The proximate value of the biofloc determines the biochemical content of shrimp [42,8]. It has been suggested that mixed biofloc, which is dominated by microalgae and autotroph bacteria, be used to cultivate juvenile *P. vannamei* since it reduced cost of production by using less oxygen and formulated feed while increasing production output [43]. During the experiment the biofloc has altered the biochemical composition of shrimp, resulting in higher levels of ash and fat in treatments with zero water exchange compared to the control groups. Higher concentrations of fatty acids (PUFA or HUFA), essential amino acids (methionine and lysine), and other nutrients in biofloc improve nutrient absorption [42,44]. The continuous supply of minerals and trace elements, particularly phosphorus, from the biofloc subsequently causes the overall quantity of ash in shrimp to increase. Several studies have reported varying levels of biofloc lipids content in the biofloc between 2.04 and 3.03 g/kg [45]. Low amounts of lipids were also discovered in bioflocs by Wasielesky et al. and Ju et al. (0.05 and 0.12–0.23 g/kg, respectively) [46, 47]. Differences in the bioflocs' microbial composition and culture conditions are more likely to be the cause of this variation.

4. CONCLUSION

In present experimental conditions, for nursery rearing of *P. vannamei* in a biofloc system, the inclusion of bamboo mat as a substrate enhanced the water quality parameters by maintaining levels of suspended solids by trapping the floc particles and allow to attach over solid surfaces which can easily be consumed by the shrimp resulted in better growth performance of and also contributed to the improved nutritional composition of floc and shrimp with the planktonic community associated with the substrate.

ETHICAL APPROVAL

The welfare and rearing of the experimental animal used to conduct this research adhered to the guidelines established by the committee for the purpose of control and supervision of experiments on animals, ministry of environment and forests (Animal Welfare Division), Government of India, regarding the care and treatment of animals in scientific research.

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