



Effects of different carbon sources on the growth of freshwater zooplankton *Moina micrura* (Kurz, 1875) in biofloc system

Chittaranjan Raul¹ • J. Praveenraj¹ • K. Saravanan² • R. Kiruba Sankar¹ • Udipta Roy³ • Himanshu Sekhar Swain⁴

¹ ICAR- Central Island Agricultural Research Institute, Port Blair, 744105, India


² ICAR-Central Institute of Brackishwater Aquaculture, Chennai, 600028, India

³ ICAR-Central Institute of Fisheries Education, Mumbai, 400061, India

⁴ ICAR-Central Institute of Freshwater Aquaculture, Bhubaneswar, 751002, India

Correspondence

Chittaranjan Raul; ICAR- Central Island Agriculture Research Institute, Port Blair, 744105, India

 raulchittaranjan339@gmail.com

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Abstract

A 30-day study was conducted to evaluate the population growth of *Moina micrura* in biofloc systems using different carbon sources: jaggery, rice bran, tapioca, and coconut oil cake. Coconut oilcake and rice bran based biofloc system produced significant higher populations densities (15042 ± 1018 and 13060 ± 1010 individuals L^{-1} , respectively) compared to jaggery and tapioca. The optimal production was achieved at a C:N ratio of 20:1 and a floc volume of $5 mL L^{-1}$, where peak population density was observed. *M. micrura* abundance showed a significant positive correlation ($p \leq 0.05$) with water quality parameters pH, alkalinity, TAN-N, nitrite-N, and nitrate-N. Enhanced growth and reproductive performance in complex carbon based biofloc systems were attributed to improved nutrient availability and microbial composition. The findings demonstrate that nutrient rich biofloc systems, particularly those based on rice bran and coconut oil cake under optimized conditions, can be effectively utilized for mass culture of *M. micrura*, supporting improved live feed production in aquaculture.

Keywords: biofloc; coconut oilcake; jaggery; *Moina micrura*; rice bran; tapioca

1 | INTRODUCTION

The freshwater aquaculture sector has witnessed remarkable growth through the diversification of candidate species and the intensification of culture systems (Thomas *et al.* 2021). However, the intensification of aquaculture systems generates nitrogen-rich effluent, and creates competition with agriculture for land resources (Piedrahi-ta *et al.* 2026). Recirculating Aquaculture System (RAS) systems have not fully addressed this issue due to their high investment and operational cost, whereas Biofloc Technology (BFT) has emerged as an environmentally

friendly and cost-effective aquaculture system (Gutierrez-Wing 2006). BFT is a recently adopted land-based aquaculture system that enables high density fish culture in limited areas while efficiently utilizing water resources (Raza *et al.* 2024). Biofloc is a complex of heterotrophic bacteria, organic matter, protozoa, ciliates, and flagellates that immobilizes total ammoniacal nitrogen and produces single cell protein in the form of floc (Avnimelech 2012; Khanjani *et al.* 2022). Bioflocs are rich in nutrients such as protein (25–50%), lipid (5–20%), vitamins, and minerals (Hargreaves 2013; Wang *et al.* 2016). The quantity and

quality of biofloc depend on the carbon to nitrogen (C:N) ratio maintained in the culture system through the addition of various carbon sources, such as simple carbon sources like molasses, jaggery, tapioca, and complex carbon sources of rice bran powder and oil cakes depending upon the cost-effective availability (Dauda *et al.* 2017; Bakhshi *et al.* 2018). Complex carbon sources are polysaccharide-based carbon sources, composed mainly of cellulose and hemicellulose, are metabolized more slowly by bacteria due to their complex structure, whereas simple sugars consist of sucrose are rapidly utilized as an immediate energy source. The floc consumed by fish helps enhance growth and reduce the feed conversion ratio (FCR) in the culture system (Avnimelech 2007; Zhao *et al.* 2012). In addition, there is evidence that floc consumption improves immunity and feed digestibility (Valle *et al.* 2015; Abreu *et al.* 2019). The nutritional quality of floc is strongly associated with the diversity of microorganisms (bacteria, phytoplankton, protozoa, and zooplankton) present in the biofloc (Emerenciano *et al.* 2011). Zooplankton diversity in biofloc systems is primarily governed by the interaction between carbon source type and C:N ratio, which regulate microbial community composition, while additional factors such as water quality, floc volume, stocking density, and environmental conditions further modulate trophic structure and species distribution (Avnimelech 2012; Crab *et al.* 2012). In biofloc system, commonly used external carbon sources such as jaggery, molasses, and tapioca are simple carbon, leading to proliferation of microbial communities dominated by protozoan ciliates and flagellates rather than large zooplankton groups like cladocerans and rotifers (Ebeling *et al.* 2006; Emerenciano *et al.* 2013; Hargreaves 2013).

Initially, tilapia and shrimp were cultured in biofloc systems, which were later standardised for various finfish and shellfish species (Browdy *et al.* 2012). In addition to grow-out culture, high density seed rearing of carp from the spawn to fingerlings can be practiced in a biofloc system to improve survivability (Swain *et al.* 2025). However, despite higher survivability of carp spawn, growth performance during spawn to fry stage in biofloc system is often limited, as spawn tend to consume more live feed than floc during nursery stage (Dey *et al.* 2022; Solanki *et al.* 2023). For nursery rearing of fishes, live feed plays a crucial role, and freshwater species generally prefer *Daphnia* sp. and *Moina* sp. as live feed supplement with formulated feed to enhance growth and survivability (Baiz 2018; Santhanam 2020; Ignatious *et al.* 2021). Enrichment of live feed is an important approach to enhancing the nutritional quality of live feed by supplementing limiting nutrients, thereby prompting the production of high-quality seed.

In biofloc based systems, enrichment of live feed such as *Moina* sp. can improve larval growth at a lower feed conversion ratio (FCR), ultimately reducing feed cost.

Moina sp. is a freshwater cladoceran characterized by an open brood pouch, distinguishing it from *Daphnia* sp. (Rottmann *et al.* 2003). It is a nutrient rich live feed containing 45–50 % protein and 20–27 % fat on a dry weight basis, with adult size ranging from 700–1000 μm (Islam *et al.* 2017). Nutritionally, *Moina* sp. is comparable with *Artemia* and *Rotifer*, being rich in essential amino acids (Mubarak *et al.* 2025). It commonly inhabits various freshwater habitats such as ponds, lakes, ditches, temporary water bodies, and swamps containing decaying organic matter (Petrušek 2002). *Moina* sp. can tolerate high temperatures and is often found in poor water quality environments, even those with zero dissolved oxygen concentration (He *et al.* 2001). *Moina* sp. grows under both autotrophic and heterotrophic conditions, and its population growth and survivability vary depending upon food availability (Sipaúba-Tavares and Rocha 2001). It can be mass cultured, with phytoplankton species such as *Chlorella* sp. and can thrive on various food sources including phytoplankton, yeast, bacteria, and detritus (Hena *et al.* 2025). Additionally, *Moina* sp. can utilise a wide range of food resources, including algae, heterotrophic ciliates, and detritus of plant and animal origin, for its growth and reproduction (Rasdi *et al.* 2020). It can also grow and reproduce in the absence of phytoplankton by consuming bacteria and protozoan ciliates, and flagellates (Wylie and Currie, 1991). Under normal condition, *Moina* sp. reproduce asexually, with the entire population consisting almost females (Martínez-Jerónimo *et al.* 2007). However, during adverse environmental conditions, such as food scarcity, sexual reproduction occurs, leading to the production of resting eggs (He *et al.* 2001). Most of the previous studies have focused on the use of zooplankton like artemia, rotifers, and copepods in biofloc for the growth of shrimp and fish (Kamrunnahar *et al.* 2019; De Andrade *et al.* 2021; Silva *et al.* 2021; Abbaszadeh *et al.* 2022). However, research on the culture of zooplankton by utilising biofloc as growth medium remains limited. *Moina* sp. can be cultured in batch and semi-continuous methods by supplementing the algal media. Therefore, to utilise nutrient rich biofloc water and improve the nutritional quality of floc, a study was conducted to evaluate the population growth and reproduction of *M. micrura* in the biofloc system with different carbon sources.

2 | METHODOLOGY

2.1 Experimental condition

An indoor trial was conducted for 30 days at the Marine-hill wet laboratory of ICAR-Central Inland Agricultural Research Institute (CIARI), Sri Vijay Puram, India. A completely randomized experimental design was followed for the culture of *M. micrura* with four treatment groups i.e., T1–JB (Jaggery based biofloc), T2–RB (Rice bran based biofloc), T3–TB (Tapioca based biofloc), and T4–CB (Coconut oil cake powder based biofloc). The best performing

carbon source treatment was selected for evaluating *M. micrura* population production at different C:N ratios and floc volume combinations. A 3×3 factorial experiment was conducted with three levels of C:N ratio (10:1, 15:1, and 20:1) and three levels of floc volume (5, 7.5, and 10 mL L⁻¹), resulting in nine treatment combinations. The experiment was laid out in a completely randomized design with (replications). Data were analysed using two-way ANOVA to assess main and interaction effects. Both experiments were conducted in 100 L capacity plastic tanks in triplicate. The water used in experiment was sand filtered, chlorinated with active chlorine of 15%, and subsequently dechlorinated through continuous aeration. No water exchange was performed during the experiment, and continuous aeration was maintained throughout.

2.2 Production and addition of *Moina micrura*

A pure culture inoculum of *M. micrura* maintained in the algal laboratory of ICAR-CIARI was used for this experiment. The species was cultured in a transparent glass fibre rectangular tank of 100 L capacity under a 12:12 hr photoperiod with a light intensity of 3000 lux provided by an LED bulb. The culture water was initially chlorinated with 15 ppm chlorine and maintained under constant aeration at a pH of 7.4. *M. micrura* were fed with yeast and harvested when the culture density reaches 50 to 60 individuals mL⁻¹, using a 50 µm mesh plankton net (with approximate body size of 700–900 µm adult). *M. micrura* @ 100 numbers L⁻¹ was supplemented to each treatment tank (Sipaúba-Tavares and Rocha 2001).

2.3 Fish waste collection and floc preparation

The initial fish solid waste was collected from an indoor Rohu (*Labeo rohita*) nursery tank. The Rohu seed was reared (from spawn to fry) in 10 numbers of rectangular FRP tanks, each with a capacity of 2000 L. The seed was fed with commercial powdered feed (CIFA starter) containing 10% moisture, 35% crude protein, 6% crude fat, and 5% crude fibre. The characteristics of rohu seed solid waste were analysed, showing TSS (64.4±9.3 g L⁻¹), COD (102.5±12.7 g L⁻¹), and total nitrogen (5.6±1.7 g L⁻¹). The total nitrogen content of solid waste was utilised for biofloc preparation, as the crude protein in the feed and fecal matter converted to ammonium by microbial degradation (Avnimelech 2007). Biofloc inoculum was prepared in a 10 L bucket using solid waste and selected carbon sources under continuous vigorous aeration. Jaggery, rice bran powder, tapioca, and coconut oil cake were used as carbon sources for different treatments, with known carbon percentages, maintaining a C:N ratio of 15–20:1 based on the calculation described by De Schryver *et al.* (2008). An external probiotic, CIBAFLOC+ (a mixed bacterial complex), was added to enhance biofloc production. The mixture was aerated vigorously for 48 hours to produce biofloc inoculum, which was then added to each

treatment tank. To ensure continuous floc production, the respective carbon sources and nitrogenous fish solid waste were added to each treatment at 5-day intervals. For the factorial experiment, Biofloc was produced using COC and RB at C:N ratios of 20:1, 15:1, and 10:1 and added to the tank according to the required floc volume.

2.4 Water quality parameters

Water samples were collected at 5-day intervals for the analysis of water quality parameters. The pH and temperature were measured using a digital probe (COM-360, HM Digital), and dissolved oxygen was measured by a digital probe (HACH). The Total Ammoniacal Nitrogen (TAN), Nitrite-N, and Nitrate-N were measured using a spectrophotometric method (APHA 2006). Biofloc volume was estimated by the Imhoff cone (Avnimelech 2012). Total alkalinity was measured by the titration method (APHA 2006), and microbial density was measured by the standard total plate count method (TPC) (Gilcreas 1966).

2.5 Proximate analysis

The proximate composition of *M. micrura*, and carbon source ingredients (jaggery, rice bran, tapioca and coconut oilcake) was analysed following the methods described by AOAC (2006). At the end of the experiment, floc samples were harvested using a 100 µm net and dried in a hot air oven at 60°C for proximate analysis. *M. micrura* samples were collected using a 50 µm plankton net and freeze-dried at -80°C for proximate analysis. Crude protein content was estimated using the Kjeldahl method using KELPLUS-CLASSIC DX (Pelican), while crude lipid content was estimated using the ether extraction method with a SCOS PLUS (Pelican) apparatus. Ash content was determined by incineration in a muffle furnace at 550°C for 6 hours, and moisture percentage was estimated using hot air oven at constant 105°C. The carbohydrate percentage content was calculated using the formula given by Wei *et al.* (2016).

2.6 Life table parameters and counting of *Moina micrura*

The life table demographics parameters, such as average longevity (L), Number of neonates produced per brooder (R), and time of first reproduction (T), were determined according to the formula given by Krebs (1985). Water samples were collected on the 5th day interval to estimate *M. micrura* density and identify floc associated organisms. Approximately 500 mL of water was collected from each experimental tank and subsequently filtered through different mesh size plankton nets of 200 µm, 150 µm, and 100 µm to reduce suspended solids. The filtrate was then passed through a 50 µm net for zooplankton retention and a 15 µm net for phytoplankton retention. Subsequently, a 25 mL of aliquot was fixed with 4% formalin and preserved for further analysis. A Stereo microscope (QUASMO, STAR-4) was used to identify phyto-

plankton and zooplankton associated with biofloc. Sedgwick rafter cell was used to count *M. micrura* and other zooplanktons. The number of *M. micrura* (Numbers L⁻¹) was calculated according to the formula by Byod and Lichtkopler (1979).

$$\text{Number of } M. \text{ micrura} / \text{L} = (T \times 1000) / (A \times N \times (\text{Volume of sample}))$$

Where T = total number of *M. micrura* counted, A = area of grid in mm²; N = number of grids counted; 1000 = area of counting chambers in mm²

2.7 Statistical analysis

All statistical analyses were performed using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). Data are presented as mean ± SE. Prior to analysis, all datasets were tested for normality using the Shapiro-Wilk test for homogeneity of variances using Levene's test. For the first experiment, differences among treatments were analysed using one-way ANOVA and means were compared using Tukey's HSD test at significant differences of $p < 0.05$. For the factorial experiment, involving different levels of C:N ratio and floc volume, data were analysed using two-way ANOVA to evaluate main effects and interaction effects of

each factor. The statistical model included C:N ratio and floc volume as fixed factors. Pearson's correlation analysis was performed to assess relationships between *M. micrura* population density and water quality parameters. Additionally, principal component analysis (PCA) was conducted to identify patterns and reduced dimensionality among the measured variables.

3 | RESULTS

3.1 Water quality parameters

The initial water quality parameters for each treatment are shown in Table 1. Throughout the experiment, the water temperature was 29.2±0.4°C. The mean values of pH, alkalinity, and dissolved oxygen were 7.8±0.5 ppm, 60±5 ppm, and 7.5±2 ppm, respectively. The initial hardness and total dissolved solids (TDS) values were 120±10 ppm and 150±12 ppm, respectively. Among the nitrogen compounds, nitrate-N (NO₃⁻) concentration was 3.2±0.8 ppm, followed by nitrite-N (0.05±0.01 ppm), and total ammoniacal nitrogen-N (0.01±0.00 ppm). The trends of water quality parameters such as pH, alkalinity, floc volume, TAN, nitrite-N, and nitrate-N was shown in Figure 1.

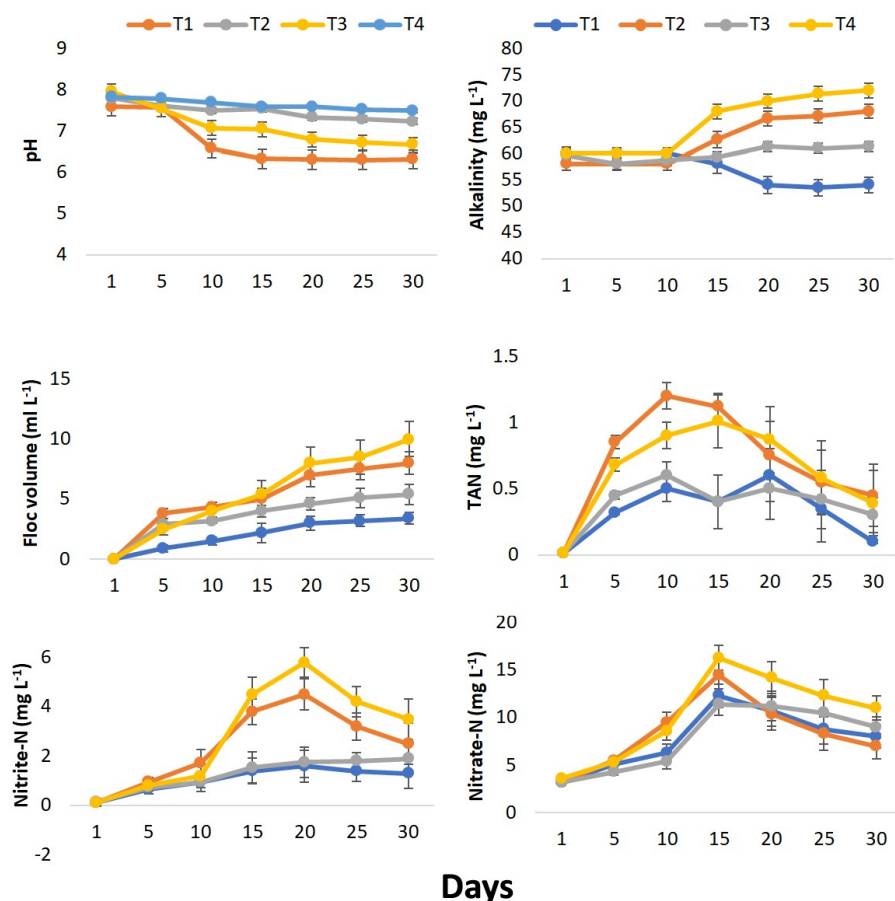


FIGURE 1 The trend of water quality parameters in different carbon source based biofloc treatments of the 30 days experiment. T1 = jaggery based biofloc; T2 = rice bran based biofloc; T3 = tapioca based biofloc; T4 = coconut oil cake powder based biofloc.

During the 30-day experiment, the floc volume increased significantly ($p \leq 0.05$) in the T2 and T4 treatments compared to T1 and T3. The final floc volumes were 8 and 10 ml L⁻¹ in T2 and T4 treatments, respectively. The TDS values ranged from 147±5 ppm to 172±8 ppm, with no significant difference ($p \geq 0.05$) among treatments. The Ph values of all treatments ranged from 7.6±0.25 to 7.9±0.3 at the beginning, but gradually decreased with time. By the end of the experiment, significantly lower Ph values ($p \leq 0.05$) were recorded in T1 (6.57±0.15) and T3 (6.31±0.23). The dissolved oxygen level showed no significant differences among treatments throughout the experimental period. A significant ($p \leq 0.05$) increase in TAN and nitrite-N concentrations was observed in T2 and T4 after the 15th day. The Nitrate-N concentration increased significantly up to 15th day, followed by a decreasing trend until the end of the experiment. Alkalinity showed a decreasing trend in T1, while it increased in T2 and T4 after the 15th day. The initial total plate count (TPC) value of water was 1±0.6×10³ CFUs ML⁻¹, which gradually increased, showing a significant difference ($p \leq 0.05$) in T1 (5.2±3×10⁷ CFUs ML⁻¹) and T3 (7.4±0.5×10⁷ CFUs ML⁻¹) treatments at the end of the experiment (Table 2).

3.2 Proximate composition of carbon source and *Moina micrura*

The proximate composition of carbon sources used for biofloc production, floc harvested from different biofloc treatments, and *M. micrura* were presented in Table 3,

Table 4, and Table 5, respectively. Among four carbon sources COC showed significantly higher ($p \leq 0.05$) crude protein and crude lipid than others, whereas crude fiber and ash were significantly higher ($p \leq 0.05$) in rice bran. There was a significant difference ($p \leq 0.05$) in the proximate composition of *M. micrura* with crude protein in T4, carbohydrate in T1, and ash content in T1. The proximate composition of *Moina*, except crude lipid, increased in rice bran and coconut oil cake based biofloc culture system than jaggery and tapioca. Floc produced by the addition of external carbon sources found significant variations in crude protein, crude lipid, and ash of T4 treatments than others. Crude protein of floc produced in COC based biofloc was significantly ($p \leq 0.05$) higher than other floc.

TABLE 1 Initial value of fresh water quality parameters (Mean ± SE, $n = 3$) used for the experiment of *Moina micrura* culture.

Water quality parameters	Value (mean)
Temperature (°C)	29.2±0.4
pH	7.8±0.4
Alkalinity (ppm)	60.4±5
DO (ppm)	7.5±0.6
Hardness (ppm)	120±12
TDS (ppm)	160.5± 15
TAN (ppm)	0.01±0
Nitrite-N (ppm)	0.05±0.01
Nitrate-N (ppm)	3.2±0.8

TABLE 2 Total plate count value in colony forming units (mean ± SE, $n = 3$) of microbes of water sample in different carbon sources based biofloc treatments results were analysed by performing one-way ANOVA ($p < 0.05$) and Tukey's test.

TPC (cfu/ml)	DAY-1	DAY-5	DAY-10	DAY-15	DAY-20	DAY-25	DAY-30
T1	^A 1±0.4×10 ³	^{AB} 4±1×10 ⁵	^C 4.4±1×10 ⁵	^A 5±1×10 ⁶	^B 8.4±0.3×10 ⁶	^B 6.4±0.3×10 ⁶	^A 5.2±0.3×10 ⁷
T2	^A 1±0.8×10 ³	^A 3±0.6×10 ⁴	^B 3.8±0.6×10 ⁴	^A 4.5±1.2×10 ⁵	^A 1.5±0.4×10 ⁶	^A 3.5±0.4×10 ⁶	^{AB} 6.5±0.7×10 ⁶
T3	^A 1±0.6×10 ³	^C 5±1.2×10 ⁵	^C 5.9±1.2×10 ⁵	^C 7±0.9×10 ⁶	^B 9±1.5×10 ⁶	^B 8.1±1.5×10 ⁶	^{AB} 7.4±0.5×10 ⁷
T4	^A 1±0.7×10 ³	^A 3±0.8×10 ⁴	^B 4.3±0.8×10 ⁴	^A 5±1.3×10 ⁵	^A 2±1×10 ⁶	^A 3.8±1×10 ⁶	^A 5.4±1×10 ⁶

T1 = jaggery based biofloc; T2 = rice bran based biofloc; T3 = tapioca based biofloc; T4 = coconut oil cake powder based biofloc. Different superscript letters along each column indicate significant differences.

TABLE 3 Proximate composition of carbon sources (jaggery, rice bran, tapioca and coconut oil cake) used for floc production in different treatments. The values are expressed in mean ± standard error ($n = 3$).

Ingredients	Moisture (%)	Crude protein (%)	Crude lipid (%)	Crude fiber (%)	Total ash (%)	Nitrogen free extract (%)
Jaggery	7.8±1.7 ^a	1.1±0.21 ^a	0.2±0.03 ^a	0.15±0.02 ^a	3.2±0.61 ^b	87.5±3.6 ^b
Ricebran	9.8±1.2 ^{ab}	11.8±2.5 ^b	7.3±1.6 ^c	15.5±3.9 ^c	18.7±3.7 ^c	36.8±4.6 ^a
Tapioca	10.8±1.9 ^b	2.1±0.32 ^a	1.9±0.21 ^b	1.5±0.35 ^b	1.2±0.34 ^a	82.5±5.9 ^b
Coconut oil cake	10.2±1.8 ^b	22.1±2.6 ^c	11.4±2.1 ^d	15.7±3.1 ^c	4.2±0.92 ^b	36.4±4.3 ^a

Different superscript letters along each column indicate significant differences.

TABLE 4 Proximate composition of *Moina micrura* (% dry weight) collected from control and biofloc treatments after 30 days. Results were analysed by performing one-way ANOVA and the Tukey's test. The values are expressed as mean±SE (n = 3).

Treatments	T1	T2	T3	T4	p-value
Moisture (%)	87.3±4.3 ^a	86.4±4.9 ^a	87.5±5.1 ^a	85.8±4.3 ^a	0.810
Crude Protein (%)	22.5±2.6 ^a	27.3±2.8 ^b	23.2±3.1 ^a	32.3±3.4 ^c	0.030
Crude Lipid (%)	1.01±0.3 ^a	2.7±0.97 ^b	1.11±0.6 ^a	3.4±1.1 ^c	0.020
Ash (%)	7.8±1.4 ^a	12.1±2.2 ^b	8.2±1.8 ^{ab}	18.3±2.5 ^c	0.020

T1 = jaggery based biofloc; T2 = rice bran based biofloc; T3 = tapioca based biofloc; T4 = coconut oil cake powder based biofloc. Different superscript letters along each column indicate significant differences.

TABLE 5 Proximate composition of floc collected from different treatments. Results were analysed by performing one-way ANOVA and the Tukey's test. The values are expressed as mean±SE (n = 3).

Treatments	T1	T2	T3	T4	p-value
Moisture (%)	87.3±4.3 ^a	86.4±4.9 ^a	87.5±5.1 ^a	85.8±4.3 ^a	0.810
Crude Protein (%)	22.5±2.6 ^a	27.3±2.8 ^b	23.2±3.1 ^a	32.3±3.4 ^c	0.030
Crude Lipid (%)	1.01±0.3 ^a	2.7±0.97 ^b	1.11±0.6 ^a	3.4±1.1 ^c	0.020
Ash (%)	7.8±1.4 ^a	12.1±2.2 ^b	8.2±1.8 ^{ab}	18.3±2.5 ^c	0.020

T1 = jaggery based biofloc; T2 = rice bran based biofloc; T3 = tapioca based biofloc; T4 = coconut oil cake powder based biofloc. Different superscript letters along each column indicate significant differences.

3.3 Life table parameters and population growth of *Moina micrura*

Life table parameters like longevity (L), number of neonates produced per brooder (R), and time at first reproduction (T) of *M. micrura* were shown in Figure 2. The longevity of *M. micrura* varied between 7 to 15 days in all biofloc treatments in which, coconut oil cake based biofloc treatment showed significantly ($p \leq 0.05$) higher longevity than other treatments. The lowest longevity was

found in jaggery based biofloc treatment, and there was no significant ($p \geq 0.05$) difference between RB and tapioca based biofloc treatments. The number of neonates produced between 3 to 11 per brooder in all treatments. The RB and COC based biofloc treatments showed significantly ($p \leq 0.05$) higher numbers of neonates than other treatments. The time of first reproduction in all treatments did not vary significantly ($p \geq 0.05$), and ranges from 4 to 6 days.

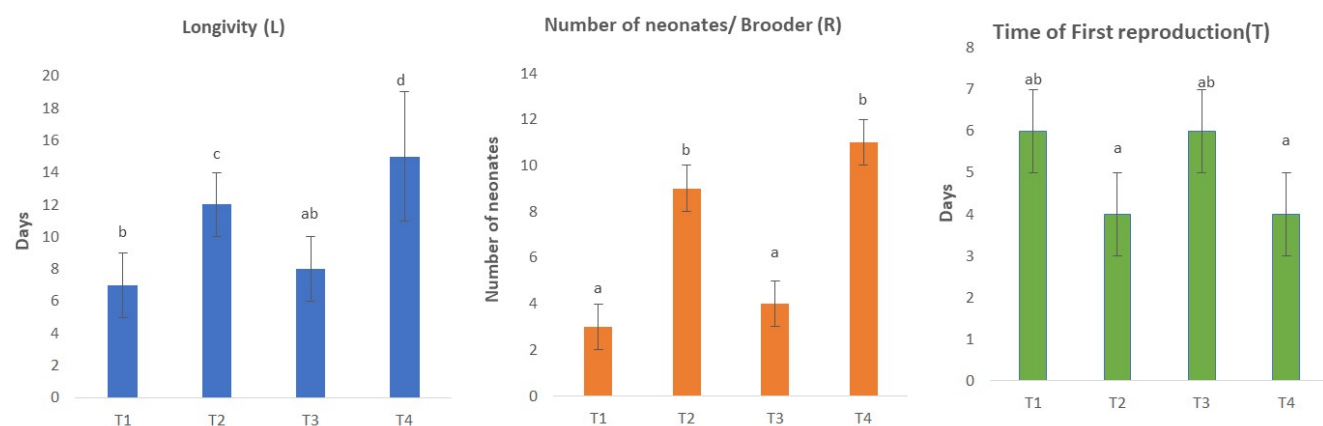


FIGURE 2 Longevity (L), Production of neonates/ Brooder (B) and Time of first reproduction (C) of *Moina micrura* in different biofloc treatments. The data correspond to the mean ± SD. Results were analysed by performing one-way ANOVA and the Tukey's test. Mean values in the same colour column with different superscripts differ significantly. T1 = jaggery based biofloc; T2 = rice bran based biofloc; T3 = tapioca based biofloc; T4 = coconut oil cake powder based biofloc.

The population growth curve of *M. micrura* in different carbon source based biofloc systems showed (Figure 3) a significant ($p \leq 0.05$) increase in the number with an increase in culture period in T2 and T4 treatments compared to other treatments. *M. micrura* reached peak density in both treatments on the 20th day, and the pairwise

comparison using the post hoc test showed that there was no significant ($p \geq 0.05$) difference between T2 (13060 ± 1010 individuals L^{-1}) and T4 (15042 ± 1018 individuals L^{-1}) treatments. The T1 and T3 treatments showed peak population of 4655 ± 272 and 5187 ± 310 individuals L^{-1} on the 15th day, having no significant ($p \geq 0.05$) differ-

ence between them. The abundance of *M. micrura* across all biofloc treatments showed significant difference ($p = 0.030$, one-way ANOVA). The Pearson correlation coefficient analysis in the Figure 4 identified a significant increase ($p \leq 0.05$) in *Moina* population growth with respect to several factors like floc volume ($p = 0.028$), pH ($p = 0.007$), alkalinity ($p = 0.004$), TAN ($p = 0.001$), nitrite-N ($p = 0.002$) and nitrate-N ($p = 0.030$) and a negative non-significant ($p \geq 0.05$) relationship with TPC.

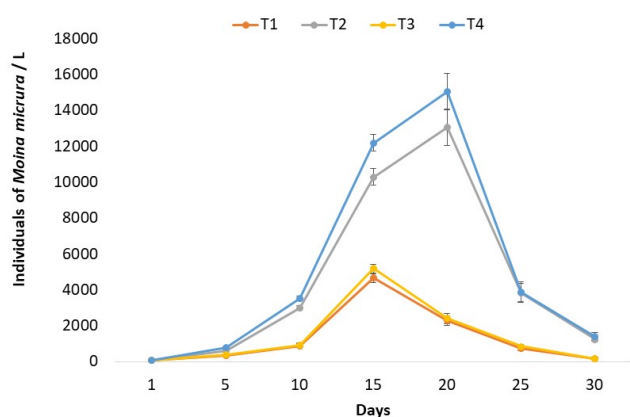


FIGURE 3 Population growth of *Moina micrura* cultured in different carbon source based biofloc system. Data are expressed in (mean \pm SE; $n = 3$). T1 = jaggery based biofloc; T2 = rice bran based biofloc; T3 = tapioca based biofloc; T4 = coconut oil cake powder based biofloc.

3.4 Effect of C:N ratio and floc volume on *Moina micrura* production

From the factorial experiment, it was observed that in both the COC and RB based biofloc systems, the production of *M. micrura* was significantly higher ($p \leq 0.05$) at C:N ratio of 20:1 and a floc volume of 5 mL L⁻¹ combined factorial treatment (Table 6). Among the three C:N ratio and floc volume, higher individual C:N ratio and lowest floc volume showed the highest *Moina* population growth. The peak population density of *M. micrura* was 14012 \pm 1082 individuals L⁻¹ and, 13010 \pm 1077 individuals L⁻¹ for the COC and RB biofloc systems, respectively. The lowest population density of *M. micrura* was recorded at a C:N ratio of 10:1 with a floc volume 10 mL yielding, 7090 \pm 566 individuals L⁻¹ in the COC based biofloc system and 7063 \pm 549 individuals L⁻¹ in the rice bran based biofloc system. The pareto chart of standardized effect (Figure 5) showed that there was an individual and combined significant effect ($\alpha = 0.05$) of both factors (C:N ratio and floc volume) on the production of *M. micrura*. The diversity and density of phytoplankton and zooplankton encountered in different treatments of biofloc were mentioned in Table 7 and Table 8. The types of phytoplankton observed were *Rhizosolenia* sp., *Scenedesmus* sp., *Chlorococcus* sp., *Closterium* sp., *Oscillatoria* and *Chlamydomonas* sp. (Figure 6). Types of zooplankton found were under ciliates and flagellates groups like *Colpidium* sp., *Sporozo-*

an sp., *Anisonema* sp., *Paramecium* sp., *Lepadella* sp., *Halteria* sp., *Phelodina* sp., and *Vorticella* sp. (Figure 7). The density of zooplankton observed in COC based biofloc treatment showed significantly ($p \leq 0.05$) higher numbers (213 \pm 79 individuals mL⁻¹), and the lowest was observed in the jaggery based biofloc system. There was no significant difference in phytoplankton numbers in all treatments.

TABLE 6 Peak density of *Moina micrura* in 3 \times 3 factorial combination of C:N ratio and floc volume. The factorial combination of C:N = 15:1 and floc volume = 7.5 mL was taken as the midpoint of the factorial combination treatments. The factorial combination treatments conducted in triplicate manner and results expressed in mean \pm SE. Results were analysed by performing two-way ANOVA and Tukey's test.

C:N ratio	Floc Volume (mL/L)	Peak Density of <i>Moina micrura</i> (Nos/L)	
		COC	RB
20:1	5	14012 \pm 1082 ^f	13010 \pm 1077 ^f
20:1	7.5	12033 \pm 1065 ^e	11058 \pm 1081 ^e
20:1	10	9808 \pm 981 ^c	9030 \pm 954 ^c
15:1	5	10185 \pm 1072.9 ^e	10136 \pm 1088 ^e
15:1	7.5	10111 \pm 1052 ^d	10055 \pm 978 ^d
15:1	10	9008 \pm 855 ^b	8068 \pm 856 ^{bc}
10:1	5	8035 \pm 758 ^a	9010 \pm 764 ^c
10:1	7.5	8021 \pm 651 ^a	8016 \pm 653 ^{ab}
10:1	10	7090 \pm 566 ^a	7063 \pm 549 ^a

COC = coconut oil cake; RB = rice bran

TABLE 7 Total numbers of phytoplankton in water sample of different treatments of biofloc system. Results were analysed by performing one-way ANOVA and Tukey's multiple range test. The values are expressed as mean \pm SE ($n = 3$).

Phytoplankton (individuals/mL)	Treatments			
	T1	T2	T3	T4
<i>Rhizosolenia</i> sp.	20 \pm 4	40 \pm 9	30 \pm 7	60 \pm 11
<i>Scenedesmus</i> sp.	10 \pm 4	30 \pm 9	20 \pm 7	20 \pm 13
<i>Chlorococcus</i> sp.	20 \pm 5	25 \pm 8	34 \pm 8	25 \pm 11
<i>Closterium</i> sp.	28 \pm 7	40 \pm 7	20 \pm 7	25 \pm 12
<i>Oscillatoria</i> sp.	30 \pm 6	52 \pm 7	55 \pm 9	60 \pm 12
<i>Chlamydomonas</i> sp.	98 \pm 11	80 \pm 18	120 \pm 12	90 \pm 7
Total	206 \pm 37 ^A	262 \pm 58 ^A	279 \pm 48 ^A	280 \pm 66 ^A

T1 = jaggery based biofloc; T2 = rice bran based biofloc; T3 = tapioca based biofloc; T4 = coconut oil cake powder based biofloc.

4 | DISCUSSION

4.1 Water quality parameters of biofloc based system

The higher TAN concentration observed in coconut oil cake and rice bran based biofloc systems compared to jaggery and tapioca treatments may be attributed to dif-

ference in nitrogen mineralization and microbial dynamics. Faster nitrification process and greater heterotrophic bacterial assimilation observed in simple carbon biofloc systems compared to complex carbon systems (Browdy et al. 2012; Ferreira et al. 2016; Bakhshi et al. 2018). Despite elevated TAN levels, no adverse effects on *M. micrura* were observed, likely due to the low concentration of toxic unionized ammonia. The maximum recorded TAN (1.2 ppm in COC) corresponded to a calculated unionised ammonia level of only 0.033 ppm, which is well below the

reported toxic threshold (> 2.5 ppm) (Arauzo 2003). The unionized ammonia concentration was calculated according to the method of Emerson et al. (1975). Since ammonia toxicity is strongly influenced by pH and temperature, the relatively stable temperature and pH below 7 in the present study limited the formation of unionized ammonia. These conditions suggest that the biofloc system maintained a favourable environment for *M. micrura* despite variation in TAN levels.

Correlation		<i>Moina</i> density	Floc volume	TDS	pH	DO	Alkalinity	TAN	NO2-N	NO3-N	TPC
Correlation	<i>Moina</i> density	1.000	.438	.143	.519	-.132	.646	.615	.669	.433	-.324
	Floc volume		1.000	.142	-.109	-.368	.703	.647	.864	.745	.466
	TDS			1.000	.357	-.065	.371	-.108	.028	-.066	-.070
	pH				1.000	.279	.387	-.025	.006	-.258	-.526
	DO					1.000	-.159	-.244	-.413	-.587	-.336
	Alkalinity						1.000	.323	.725	.441	.047
	TAN							1.000	.730	.765	-.010
	NO2-N								1.000	.854	.198
	NO3-N									1.000	.203
	TPC										1.000

FIGURE 4 Correlation coefficient of *Moina micrura* numbers with different water quality parameters. KMO Measure of sampling adequacy: 0.663; Lowest value: RED, Highest value: Blue, Mid value: Yellow; Extraction method: PCA.

Pareto Chart of the Standardized Effects

(response is *Moina* density, $\alpha = 0.05$)

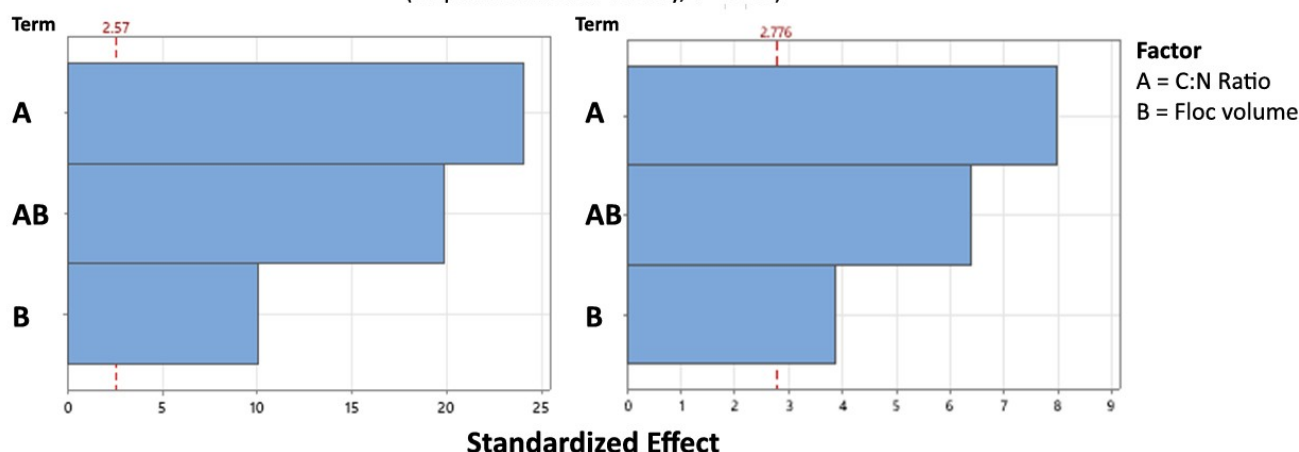


FIGURE 5 Pareto chart of the standardized effects of factors and their combined on production of *Moina micrura* in coconut oilcake (left) and rice bran (right) based biofloc system.

TABLE 8 Total numbers of ciliates and flagellates in water sample of different treatments of biofloc system. Results were analysed by performing one-way ANOVA and the Tukey's multiple range test. The values are expressed as mean±SE ($n = 3$).

Ciliates & flagellates (individual/ mL)	Treatments			
	T1	T2	T3	T4
<i>Clopidium</i> sp.	8±2	18±7	6±1	22±11
<i>Sporozoon</i> sp.	8±2	33±8	8±3	43±15
<i>Anisonema</i> sp.	0	14±7	0	18±7
<i>Paramecium</i> sp.	0	10±7	6±3	11±6
<i>Phelodina</i> sp.	12±3	22±6	10±4	36±11
<i>Halteria</i> sp.	8±3	10±5	6±3	10±6
<i>Lepadella</i> sp.	14±6	26±7	8±3	33±12
<i>Vorticella</i> sp.	16±6	28±5	12±2	38±11
Total numbers	66±22 ^A	161±51 ^B	56±18 ^A	213±79 ^C

Floc volume increased in all treatments with the periodic addition of carbon sources. These complex carbon-

based flocs may have supported protozoan ciliates and flagellates, which served as an important food source for *M. micrura*. The total plate count (TPC) value of bacteria in the RB and COC biofloc systems was comparatively lower, likely due to the grazing activity of ciliates and flagellates, which feed on bacteria and dissolved organic matter (Van Wichelen *et al.* 2016; Fedonenko *et al.* 2017). Khanjani *et al.* (2022) reported that simple carbon sources such as starch and molasses support higher densities of heterotrophic bacteria than complex sources like barley, flour, corn. In the present study, the pH and alkalinity decreased more in jaggery and tapioca based biofloc systems than in those using complex carbon sources such as rice bran and COC. This can be attributed to nitrification process (Ebeling *et al.* 2006). Alkalinity is the pH buffering capacity; as the pH decreases, the H⁺ ion increases to neutralize the HCO₃⁻ and CO₃²⁻ utilized, causing a decrease in alkalinity.

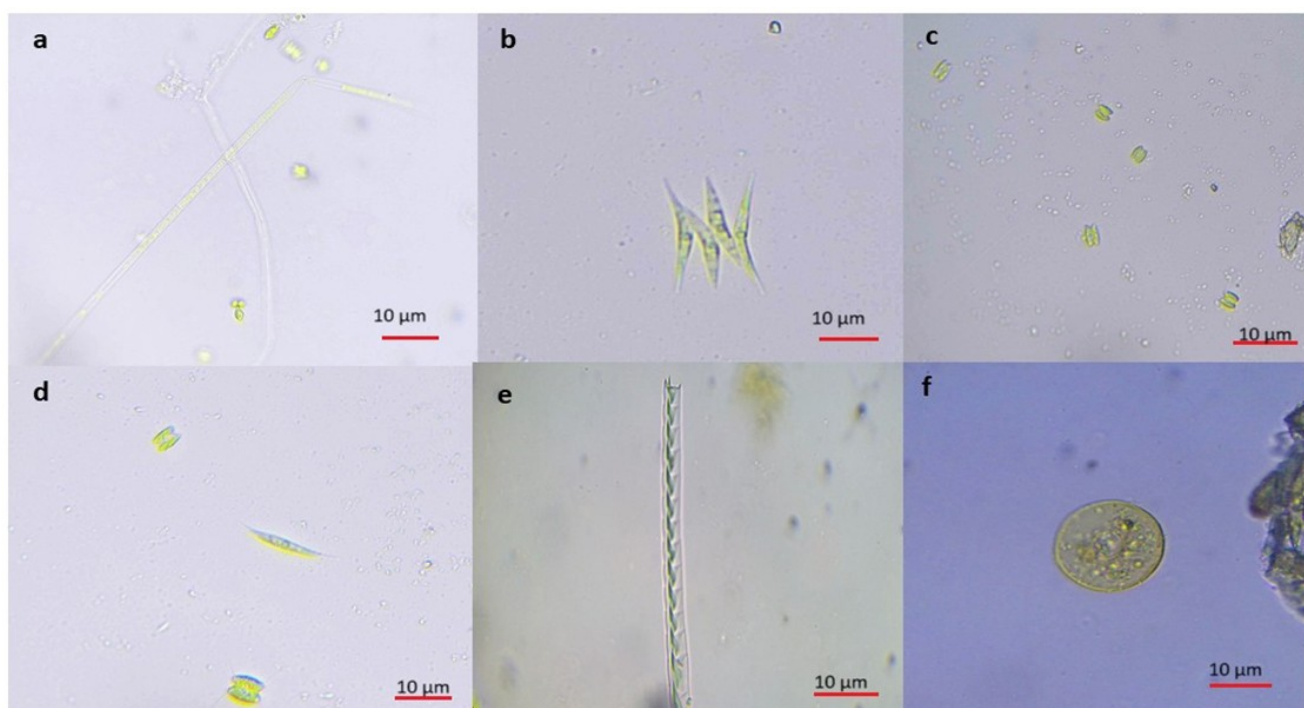


FIGURE 6 Microscopic images taken at 40x magnification of phytoplankton diversity associated with biofloc of different treatments. a. *Rhizosolenia* sp., b. *Scendesmus* sp., c. *Chlorococcus* sp., d. *Cosmarium* sp., e. *Oscillatoria* sp., f. *Chlamydomonas* sp.

4.2 Proximate composition of carbon source and biofloc
Moina micrura's proximate composition varies according to the carbon source utilized in the production of biofloc. The higher crude protein content of COC and RB compared to jaggery and tapioca results in the increased protein level of *M. micrura* cultured under these treatments. Similarly, the higher crude lipid content of COC may contribute to the increased lipid percentage of *M. micrura* compared to other biofloc treatments. The higher carbo-

hydrate content in jaggery based biofloc, on the other hand, increases the carbohydrate proportion in *Moina* cultured in biofloc system. Shidik *et al.* (2021) reported that *M. macroopa* produced in the biofloc system contained 4.62% crude protein and 0.25 % crude fat on a wet weight basis, which is comparable to values obtained in a chlorella-based culture system. The crude protein content of *M. micrura* produced in a biofloc system varies 30–45% (dry weight basis) meeting the protein requirements of

most commercial fish feed, which ranged from 30 to 43% (Li *et al.* 2002; Southgate 2003). *Moina* is widely used as live feed for the larval and nursery rearing of Indian major crabs (*Catla catla*, *Labeo rohita*, and *Cirrhinus mrigala*), tilapia, air breathing fishes (magur, singhi), as well as ornamental fishes including livebearers, *Betta splendens*, and gold fish (*Carassius auratus*) (Fermin 1991; Rottman *et al.* 2003). The dietary protein and lipid requirements of these fish larvae typically range from 35–55% and 6–15%, respectively (Jobling 2012; De Silva 1994). In this context, *M. micrura* produced using COC based biofloc system, with an approximate composition of 45% protein and 23% lipid, provides a nutritionally rich live feed capable of meeting the essential nutrient demands of these fish larvae during developmental stage. The crude lipid content in commercial fish feed generally ranges from 2 to 10%, whereas *M. micrura* produced a

biofloc system contain much higher levels, ranging from 20 to 24 % crude lipid (Southgate 2003). The higher crude lipid content in biofloc reared *M. micrura* may be attributed to their feeding on bacteria associated with floc, as the heterotrophic bacteria are known to store up to 20% fat in their cells (Yousuf *et al.* 2010). Therefore, biofloc produced *M. micrura* contains a sufficient amount of crude protein and lipid to be used as quality live feed. The elevated carbohydrate content (22–28%) in *M. micrura* cultured in a biofloc system makes it a suitable live feed for herbivorous and omnivorous fishes. In addition to providing protein sparing effect, carbohydrates enhance the availability of digestive enzymes, which are particularly essential for herbivorous and omnivorous fishes than carnivorous (Hepher 1988; Hertrampf and Piedad-pascual 2012).

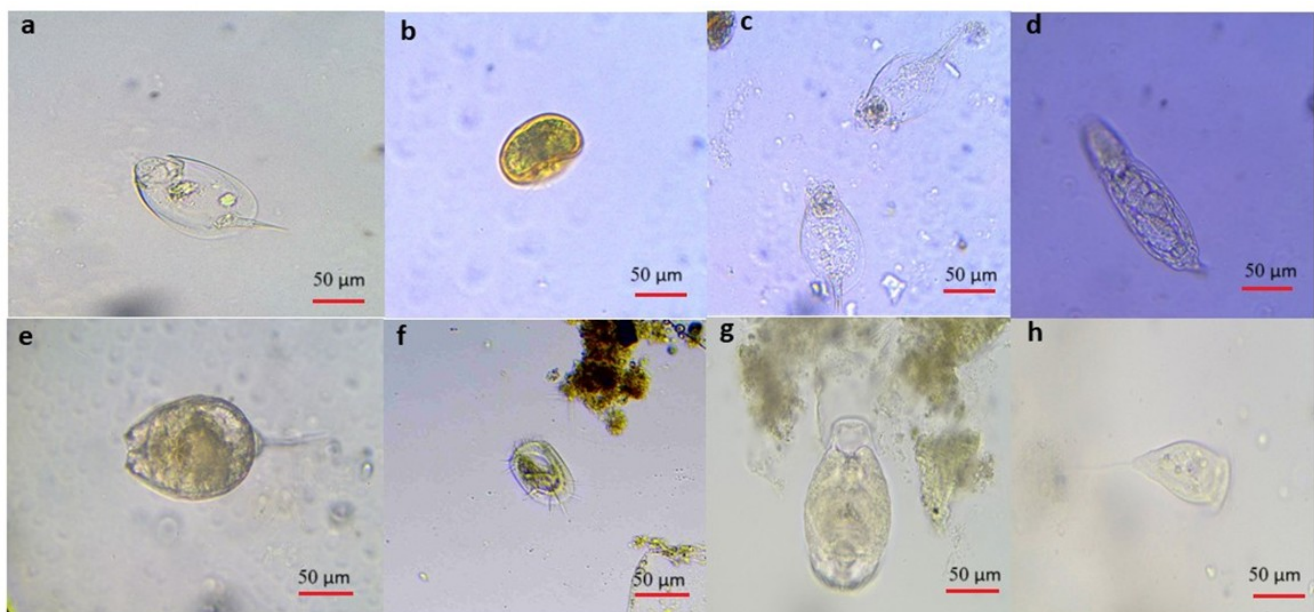


FIGURE 7 Microscopic images taken at 20x magnification of ciliates and flagellates diversity associated with biofloc of different treatments. a. *Clopidium*, b. *Sporozoon*, c. *Anisonema*, d. *Paramesium*, e. *Lepadella*, f. *Halteria*, g. *Phelleodina*, h. *Vorticella*.

4.3 Population growth dynamics of *Moina micrura* in biofloc system

This study revealed that the population growth of *M. micrura* was higher in the coconut oil cake and rice bran based biofloc system compared to those based on jaggery and tapioca. During the early neonate stage, the survivability of *M. micrura* improved due to the presence of phytoplankton such as *Scenedesmus* and *Chlamydomonas* in all biofloc treatments (Boersma and Vijverberg 1996), as the smaller sized neonates were unable to filter feed on larger ciliates and flagellates. During the unavailability of a phytoplankton diet, *Moina* utilized protozoan ciliates and flagellates of size less than 40 µm (De Andrade *et al.* 2021). The ontogenic shift in the food preference of adult

M. micrura toward floc associated protozoan ciliates and flagellates positively influenced its survivability and reproduction in the biofloc system (Kumar and Hwang 2008). The high abundance of ciliates and flagellates in the coconut oil cake based biofloc served as an additional food source, supporting a higher multiplication rate during the adult stage. Biofloc produced using complex carbon sources produces diverse floc composition, enriched with protozoan ciliates and flagellates, compared to those formed in simple carbon sources (Ferreira Marinho *et al.* 2014; Manan *et al.* 2017; De Andrade *et al.* 2021). Other than ciliates and flagellates, *Moina* sp. fed on bacteria as a single cell protein source (Miah *et al.* 2013; Castro *et al.* 2017). As the population of *M. micrura* increased in the

COC based biofloc system, the TPC value of biofloc associated bacteria decreased, as clearly indicated by the correlation co-efficient in this study. The production of particulate organic matter was higher in RB and COC based biofloc system, which provided an additional food source that supported greater population growth of *M. micrura* compared to a simple carbohydrate-based system (Rottmann *et al.* 2003). In this study, the time of first reproduction and longevity remained similar across all treatments; however, the number of neonates produced per brooder was reduced by half in jaggery and tapioca based biofloc treatments due to the lower availability of food source compared to RB and COC based biofloc treatments. The highest population growth of *M. micrura* in this study was in the range reported for *Chlorella* sp. based mass culture system of *Moina* sp. (Kaeoprakan *et al.* 2025), and it exceeded the growth achieved in other organic waste media such as chicken manure, pig manure, cow dung, and food waste (Kabery *et al.* 2019). Mass culture of *Moina* sp. was successfully achieved using both *Chlorella vulgaris* and yeast (*Saccharomyces cerevisiae*) as feed sources. Supplementation with chlorella inoculum resulted in peak population densities of approximately 5000–10000 individuals L⁻¹ under optimized conditions, including high algal density and suitable temperature (Lavens and Sorgeloos 1996; Sipaúba-Tavares and Rocha 2001). In contrast, the yeast-based culture system supported higher *Moina* densities, reaching 10000–15000 individuals L⁻¹ (Hagiwara *et al.* 2001; Rottman *et al.* 2003). The higher production in yeast-based system attributed due to the higher protein content and greater digestibility compared to chlorella facilitating improved population growth of *Moina* (Islam *et al.* 2015). Many studies have reported that freshwater cladoceran such as *Moina* sp. and *Daphnia* sp. can grow in biofloc system, depending on the carbon source or type of media uses (Miah *et al.* 2013; Castro *et al.* 2017; Shidik *et al.* 2021). They feed on dissolved organic particles, algae, protozoa, and bacteria associated with the flocs (Perera *et al.* 2022).

The population growth of *M. micrura* in the COC and RB based biofloc system increased with a higher C:N ratio, but decreased as floc volume rose. A higher C:N ratio enhances the mineralization of complex carbon sources like rice bran, and coconut oil cake into simpler organic particles (Avnimelech 2012; Bowszys *et al.* 2020), which served as food for *M. micrura* and other microorganisms such as protozoan ciliates and flagellates. This mineralization also releases essential nutrients like nitrogen and phosphorus, supporting primary productivity. However, when floc volume increases, suspended particles cause higher turbidity, which reduces phytoplankton concentration in the biofloc system (Boyd 2020). This floc associated turbidity may influence in the reduction of the initial food availability to the neonates of *M. micrura*. Therefore, the level of *Moina* productivity reduced at higher levels of floc vol-

ume. The result of *M. micrura* culture obtained from this study under controlled indoor biofloc conditions may not be directly extrapolated to outdoor pond systems due to environmental variability such as temperature, light and predation pressure, while strain specific difference among *M. micrura* and other cladoceran zooplankton may further influence reproducibility. Therefore, future study needs to be evaluated long-term stability and seasonal performance under outdoor condition. Additionally, co-culture trials with fish larvae under field conditions are essential to validate the practical applicability and scalability of the biofloc based culture system.

5 | CONCLUSIONS

The present study demonstrated that the growth, reproduction and nutritional quality of *M. micrura* was significantly influenced by the type of carbon source in the biofloc system. Among all treatments, coconut oilcake and rice bran based biofloc systems supported superior population density, enhanced longevity, and higher neonate production compared to jiggery and tapioca based biofloc system. The highest productivity was achieved at a C:N ratio of 20:1 combined with a lower floc volume (5 mL L⁻¹), indicating that optimized nutrient mineralization and moderate turbidity are critical for maximizing *Moina* production. Improved proximate composition, proximate composition, particularly crude protein and lipid content, further highlights the stability of complex carbon sources for enriching live feed quality. The positive relationship between *M. micrura* abundance and key water quality parameters confirms the ecological compatibility of biofloc based culture systems. From a practical prospective, the use of low cost and locally available carbon sources such as rice bran and coconut oilcake enhances economic feasibility. This approach shows strong potential for scalable mass production of high-quality live feed and can be effectively integrated into hatchery and nursery system, reducing resilience on conventional live feeds. Further field scale validation recommended to support commercial application.

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ETHICAL APPROVAL

Ethical approval was taken for this study.

CONFLICT OF INTEREST

No conflict of interest declared by author.

AUTHORS' CONTRIBUTION

The conceptualization of the research, methodology and original draft preparation were carried out by Chittarajan Raul, R Kiruba Sankar and K Sravanan. The proximate composition analysis and microscopic observations done by J Praveenraj, Himanshu Sekhar Swain and Udipto Roy. Statistical analysis of raw data and correction of manuscript done by Chittarajan Raul and all other others.

DATA AVAILABILITY STATEMENT

All data related to this research will be available on request basis.

REFERENCES

- Abbaszadeh A, Mozanzadeh MT, Qasemi A, Oujifard A, Bahabadi MN (2022) [Effects of the addition of *Calanopia elliptica*, *Artemia franciscana*, and *Brachionus rotundiformis* in a nursery biofloc system on water quality, growth, gut morphology, health indices, and transcriptional response of immune and antioxidant-related genes in *Penaeus vannamei*](#). *Aquaculture International* 30: 653–676.
- Abreu JL, Brito LO, de Lima PCM, Silva SMBCD, Severi W, Gálvez AO (2019) [Effects of addition of *Navicula* sp. \(diatom\) in different densities to postlarvae of shrimp *Litopenaeus vannamei* reared in a BFT system: growth, survival, productivity and fatty acid profile](#). *Aquaculture Research* 50(8): 2231–2239.
- AOAC (2006) Official Methods of Analysis. 18th Edition, Association of Official Analytical Chemists, Gaithersburgs, MD.
- APHA (2006) Standard methods for the examination of water and wastewater, 21st edition. American Public Health Association, Washington DC.
- Arauzo M (2003) [Harmful effects of un-ionised ammonia on the zooplankton community in a deep waste treatment pond](#). *Water Research* 37(5): 1048–1054.
- Avnimelech Y (2007) [Feeding with microbial flocs by tilapia in minimal discharge bio-flocs technology ponds](#). *Aquaculture* 264(1-4): 140–147.
- Avnimelech Y (2012) *Biofloc technology: a practical guide book*, 2nd ed. The World Aquaculture Society, Baton Rouge, LA, USA, pp. 73–91.
- Baiz AI, Paruğ Ş, Abdulrahman N (2018) Utilization of *Chlorella* and *Daphnia* as natural food sources and their combination as a feed supplement compared to commercial feed for common carp (*Cyprinus carpio*). University of Sulaimani.
- Bakhshi F, Najdegerami EH, Manaffar R, Tukmechi A, Farah KR (2018) [Use of different carbon sources for the biofloc system during the grow-out culture of common carp \(*Cyprinus carpio* L.\) fingerlings](#). *Aquaculture* 484: 59–267.
- Boersma M, Vijverberg J (1996) [Food effects on life history traits and seasonal dynamics of *Ceriodaphnia pulchella*](#). *Freshwater Biology* 35: 25–34.
- Bowszys M, Jaworska B, Kruk M, Gozdziejewska A (2020) [Zooplankton response to organic carbon content in a shallow lake covered by macrophytes](#). *Chemical and Ecology* 36(4): 309–326.
- Boyd CE (2020) [Suspended solids, color, turbidity, and light](#). In: *Water Quality* (pp. 119–133). Springer International Publishing.
- Browdy CL, Ray AJ, Leffler JW, Avnimelech Y (2012) [Biofloc-based aquaculture systems](#). In: JH Tidwell (Eds) *Aquaculture Production Systems* (1st ed., pp. 278–307). Wiley.
- Castro JM, Castro GM, Dávila FS, Castro AEC (2017) Density comparison of *Moina macrocopa* (Straus, 1820) cultured at different temperature conditions (19, 23 and 25 °C) fed with bacteria obtained from Biofloc system. *Journal of Entomology and Zoology Studies* 5(6): 2433–2437.
- Crab R, Defoirdt T, Bossier P, Verstraete W (2012) [Biofloc technology in aquaculture: beneficial effects and future challenges](#). *Aquaculture* 356–357: 351–356.
- Dauda AB, Romano N, Ebrahimi M, Karim M, Ikhsan NFM, ... Ekasari J (2017) [Different carbon sources affects biofloc volume, water quality, and the survival and physiology of African catfish *Clarias gariepinus* fingerlings reared in an intensive biofloc technology system](#). *Fisheries Science* 83(6): 1037–1048.
- De Andrade RJV, Dos Santos EP, De Almeida Costa GK, Campos C, da Silva SMBC, ... Brito LO (2021) [Effect of different frequencies of the addition of *Brachionus plicatilis* on the performance of *Litopenaeus vannamei* in a nursery biofloc system with rice bran \(anaerobic and aerobic\) as an organic carbon source](#). *Aquaculture* 540: 736669.
- De Schryver P, Crab R, Defoirdt T, Boon N, Verstraete W (2008) [The basics of bio-flocs technology: The added value for aquaculture](#). *Aquaculture* 277(3–4): 125–137.
- Dey SS, Tibile RM, Pawase AS, Meshram SJ, Pathan DI, Nayak A (2022) [Evaluation of different stocking densities and carbon: nitrogen ratios on growth, body composition and production performance of *Labeo rohita* \(Hamilton, 1822\) spawn in biofloc based-nursery system](#). *Aquaculture Research* 53(14): 4989–5005.
- Ebeling JM, Timmons MB, Bisogni JJ (2006) [Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia–nitrogen in aquaculture systems](#). *Aquaculture* 257: 346–358.
- Emerenciano M, Ballester EL, Cavalli RO, Wasielesky W (2011) [Effect of biofloc technology \(BFT\) on the early postlarval stage of pink shrimp *Farfantepenaeus paulensis*: growth performance, floc composition and salinity stress tolerance](#). *Aquaculture International*

- tional 19(5): 891–901.
- Emerenciano M, Cuzon G, Paredes A, Gaxiola G (2013) [Evaluation of biofloc technology in pink shrimp *Farfantepenaeus duorarum* culture: growth performance, water quality, microorganisms profile and proximate analysis of biofloc](#). *Aquaculture International* 21(6): 1381–1394.
- Fedonenko O, Marenkov O, Sharamok T, Kolesnik N, Grygorenko T, Symon M (2017) *Basics of aquaculture and hydrobiotechnology*. World Scientific News 88(1): 1–57.
- Fermin AC (1991) [Freshwater cladoceran *Moina macrocopa* \(Strauss\) as an alternative live food for rearing sea bass *Lates calcarifer* \(Bloch\) fry](#). *Journal of Applied Ichthyology* 7(1): 8–14.
- Ferreira LMH, Lara G, Wasielesky W, Abreu PC (2016) [Biofilm versus biofloc: are artificial substrates for biofilm production necessary in the BFT system?](#) *Aquaculture International* 24(4): 921–930.
- Ferreira Marinho Y, Brito LO, Figueiredo Da Silva CV, Santos IG, Galvez AO (2014) [Effect of addition of *Navicula* sp. on plankton composition and postlarvae growth of *Litopenaeus vannamei* reared in culture tanks with zero water exchange](#). *Latin American Journal of Aquatic Research* 42(3): 427–437.
- Gilcreas FW (1966) [Standard methods for the examination of water and wastewater](#). *American Journal of Public Health and the Nation's Health* 56(3): 387–388.
- Gutierrez-Wing MT, Malone RF (2006) [Biological filters in aquaculture: trends and research directions for freshwater and marine applications](#). *Aquacultural Engineering* 34(3): 163–171.
- Hagiwara A, Gallardo WG, Assavaaree M, Kotani T, De Araujo AB (2001) [Live food production in Japan: recent progress and future aspects](#). *Aquaculture* 200(1–2): 111–127.
- Hargreaves JA (2013) *Biofloc production systems for aquaculture*. SRAC Publication No. 4503. Southern Regional Aquaculture Center, USA.
- He ZH, Qin JG, Wang Y, Jiang H, Wen Z (2001) [Biology of *Moina mongolica* \(Moinidae, Cladocera\) and its perspective as live food for marine fish larvae: review](#). *Hydrobiologia* 457: 25–37.
- Hena SM, Akanda AM, Ritu JR, Sumon MAI, Haque MM, Khan S (2025) [Enhancing the efficiency of the cultivation of cladocerans \(*Moina* sp. and *Diaphanosoma* sp.\) through dietary diversification strategies](#). *Helicon* 11: e41608.
- Hepher B (1988) *Nutrition of pond fishes*. Cambridge University Press.
- Hertrampf JW, Piedad-Pascual F (2012) *Handbook on ingredients for aquaculture feeds*. Springer Science & Business Media.
- Ignatious B, Asha A, Anil MK (2021) *Package of aquaculture practices*.
- Islam M, Hassan M, Begum M, Punom N, Begum M, ... Rahman M (2017) [Effects of feeding zooplankton, *Moina macrocopa* \(Strauss, 1820\) on the growth of Nile tilapia *Oreochromis niloticus* L](#). *Bangladesh Journal of Scientific and Industrial Research* 52(2): 81–88.
- Jobling M (2012) [National Research Council \(NRC\): Nutrient requirements of fish and shrimp](#). *Aquaculture International* 20: 601–602.
- Kabery K, Anisuzzaman M, Jeong U, Kang SJ (2019) [Culture of *Moina macrocopa* using different types of organic wastes](#). *Asian Journal of Fisheries and Aquatic Research* 4(1): 1–13.
- Kamrunnahar K, Md A, Jeong UC, Kang SJ (2019) [Mass culture of *Moina macrocopa* using organic waste and its feeding effects on the performance of *Pagrus major* larvae](#). *Egyptian Journal of Aquatic Research* 45(1): 75–80.
- Khanjani MH, Mohammadi A, Emerenciano MGC (2022) [Microorganisms in biofloc aquaculture system](#). *Aquaculture Reports* 26: 101300.
- Krebs CJ (1985) *Ecology. The experimental analysis of distribution and abundance*. Pearson Education, London.
- Kumar R, Hwang JS (2008) [Ontogenetic shifts in the ability of the cladoceran, *Moina macrocopa* Straus and *Ceriodaphnia cornuta* Sars to utilize ciliated protists as food source](#). *International Review of Hydrobiology* 93: 284–296.
- Lavens P, Sorgeloos P (1996) *Manual on the production and use of live food for aquaculture*. FAO Fisheries Technical Paper No. 361, Rome.
- Li MH, Manning BB, Robinson EH (2002) [Comparison of various animal protein sources for growth, feed efficiency, and body composition of juvenile channel catfish *Ictalurus punctatus*](#). *Journal of the World Aquaculture Society* 33(4): 489–493.
- Manan H, Moh JHZ, Kasan NA, Suratman S, Ikhwanuddin M (2017) [Identification of biofloc microscopic composition as the natural bioremediation in zero water exchange of Pacific white shrimp, *Penaeus vannamei*, culture in closed hatchery system](#). *Applied Water Science* 7(5): 2437–2446.
- Martínez-Jerónimo F, Rodríguez-Estrada J, Villaseñor-Córdova R (2007) [Effect of culture density and volume on *Moina micrura* \(Kurz, 1874\) reproduction, and sex ratio in the progeny](#). *Hydrobiologia* 594: 69–73.
- Miah MF, Roy S, Jinnat E, Khan ZK (2013) [Assessment of *Daphnia*, *Moina* and *Cyclops* in freshwater ecosystems and the evaluation of mixed culture in laboratory](#). *American International Journal of Research in Formal, Applied & Natural Sciences* 4(1): 1–7.
- Mubarak AS, Sulmartiwi L, Mosyafwa MSS (2025) [The](#)

- Influence of different concentrations of isolated soy protein (ISP) powder in rice bran suspension as feed on the production of *Artemia salina* cysts. *Journal of Aquaculture Science* 10(1): 1–8.
- Perera HMRP, Walpita CN, Patabandi SK, Kumara CJ (2022) Morphological identification and comparative growth of tubificid worms in culture media supplemented with different feed types. *AgInsight* 2022: 238–240.
- Petrusek A (2002) *Moina* (Crustacea: Anomopoda, Moinidae) in the Czech Republic: a review. *Zoologica Bohemica* 66: 213–220.
- Piedrahita-Rodríguez S, Corredor-Muñoz LS, Colmenares-Quintero JC, Coz A, Colmenares-Quintero RF (2026) Hydrogen-centred process framework for the integrated valorisation of livestock and fisheries residues with biochar-based soil regeneration in coastal regions. *Processes* 14(4): 693.
- Rasdi NW, Arshad A, Ikhwanuddin M, Hagiwara A, Yusoff FM, Azani A (2020) A review on the improvement of cladocera (*Moina*) nutrition as live food for aquaculture using valuable plankton fisheries resources. *Journal of Environmental Biology* 41: 1239–1248.
- Raza B, Zheng Z, Yang W (2024) A review on biofloc system technology, history, types, and future economic perceptions in aquaculture. *Animals* 14(10): 1489.
- Rottmann RW, Graves JS, Watson C, Yanong R (2003) Culture techniques of *Moina*: the ideal *Daphnia* for feeding freshwater fish fry. *EDIS* 2003(16): 1–5.
- Santhanam P, Ananth S, Kumar SD, Piliyan R, Moorthy K, ... Perumal P (2020) Culture of different live fish food organisms. In *Fish Nutrition and Its Relevance to Human Health* (pp. 333–365). CRC Press.
- Shidik TSAN, Ekasari J, Jusadi D, Setiawati M (2021) Productivity and quality of *Moina* sp. cultivated with various culture medium. *Jurnal Akuakultur Indonesia* 20(2): 148–162.
- Silva DA, Lima PCM, Silva AEM, de Oliveira FPRC, da Silva SMBC, ... Brito LO (2021) Effects of adding rotifers on the water quality, plankton composition and growth of Pacific white shrimp, *Litopenaeus vannamei* juvenile, when cultured with biofloc technology. *Aquaculture Research* 52: 4380–4393.
- Sipaúba-Tavares LH, Rocha O (2001) Production of zooplankton (*Moina* spp.) in different culture systems. *Brazilian Journal of Biology* 61(3): 1–10 (in Portuguese).
- Solanki S, Meshram SJ, Dhamagaye HB, Naik SD, Shingare PE, Yadav BM (2023) Effect of C/N ratio levels and stocking density of catla spawn (*Gibelion catla*) on water quality, growth performance, and biofloc nutritional composition in an indoor biofloc system. *Aquaculture Research* 2023(1): 2501653.
- Southgate PC (2003) Feeds and feed production. In: *Aquaculture: Farming aquatic animals and plants* (pp. 172–198). Fishing News Books.
- Swain HS, Banu H, Velmani V, Das PC (2025) Microbe-driven farming system enhances production, water consumption efficiency, and economic returns in a sustainable high-density nursery rearing of *Labeo rohita* (Hamilton, 1822). *Aquaculture International* 33(5): 317.
- Thomas M, Pasquet A, Aubin J, Nahon S, Lecocq T (2021) When more is more: taking advantage of species diversity to move towards sustainable aquaculture. *Biological Reviews* 96: 767–784.
- Valle BCS, Dantas Jr EM, Silva JFX, Bezerra RS, Correia ES, ... Soares RB (2015) Replacement of fishmeal by fish protein hydrolysate and biofloc in the diets of *Litopenaeus vannamei* postlarvae. *Aquaculture Nutrition* 21(1): 105–112.
- Van Wichelen J, Vanormelingen P, Codd GA, Vyverman W (2016) The common bloom-forming cyanobacterium *Microcystis* is prone to a wide array of microbial antagonists. *Harmful Algae* 55: 97–111.
- Wei Y, Liao SA, Wang AL (2016) The effect of different carbon sources on the nutritional composition, microbial community and structure of bioflocs. *Aquaculture* 465: 88–93.
- Yousuf A, Sannino F, Addorisio V, Pirozzi D (2010) Microbial conversion of olive oil mill wastewaters into lipids suitable for biodiesel production. *Journal of Agricultural and Food Chemistry* 58(15): 8630–8635.
- Zhao P, Huang J, Wang XH, Song XL, Yang CH, ... Wang GC (2012) The application of bioflocs technology in high-intensive, zero exchange farming systems of *Marsupenaeus japonicus*. *Aquaculture* 354: 97–106.



- C Raul**  <http://orcid.org/0009-0003-6122-2264>
- J Praveenraj**  <http://orcid.org/0000-0002-5471-6931>
- K Saravanan**  <http://orcid.org/0000-0002-9695-5172>
- R Kiruba Sankar**  <http://orcid.org/0000-0003-2154-2573>
- U Roy**  <http://orcid.org/0000-0002-5497-2118>
- HS Swain**  <http://orcid.org/0000-0001-8143-5150>