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Production performance of tilapia and bell pepper in FLOC ponics, a bio-integrated system of Biofloc technology with aquaponics

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ABSTRACT

An experimental trial for 120 days was conducted to optimize the stocking density of the fish in a recirculatory bio floc- integrated nutrient film technique (BFT-NFT) aquaponic system using GIFT (*Oreochromis niloticus*) and Bell pepper (*Capsicum annum*). The experiment followed Completely Randomized Design (CRD) with three treatments and a control in triplicates. The treatments were BFT-NFT aquaponics with different stocking densities of fish, such as 150 no/m^3 (T1), 250 no/m^3 (T2) and 350 no/m^3 (T3), and control (C) was conventional NFT aquaponics without bio floc with a stocking density 150 no/m^3. All the treatments, as well as the control, were maintained with the same plant density of 12 nos/m^2. Fish with an average body weight of 1.44±0.12 g and bell pepper with plant height of 10.16±0.17 cm were used for the study. The result demonstrated that the treatment group with bio floc integrated NFT performed better ($p<0.05$) in terms of production of both plant and fish compared to control. The control (C) showed significantly higher FCR^{plant} (0.49±0.04) and lower Plant-fish revenue ratio (PFRR) (0.03±0.010) compared to the treatment groups ($p<0.05$). Among the different treatment groups, better fish mean body weight (80.55 g), specific growth rate (3.44% day^{-1}), and feed conversion ratio, (1.1) were observed in T1. The result of the present study suggests that the integration of Biofloc technology with aquaponics has the potential to enhance plant and fish growth, thereby utilizing aquaculture discharge for agricultural crop production. A stocking density of fish at the rate of 150/m^3 and plant density of 12 no. /m^2 is ideal for an integrated Biofloc NFT aquaponics system.

Key words: *Biofloc, Bell pepper, GIFT, Nutrient film technique, Stocking density*

Introduction

Sustainable intensive aquaculture techniques are required to enhance aquaculture production to meet the growing demand for fish (Boyd *et al.*, 2020). The technologies that can produce maximum yield by using minimum resources with minimum impact on the environment need to be standardized and commercialized to achieve the goal. Recirculatory aquaculture system (RAS) is one such advanced aquaculture technology that is sustainable and can support intensive culture conditions (Masser *et al.*, 1999). But in a high- density culture system, filtration technology and the lack of a cost-effective method of removing excess nitrogenous waste becomes a major problem in maintaining the system

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(Lee *et al.,* 2000**).** Technologies such as aquaponics and biofloc technology use cost-effective filtration systems to remove the nitrogenous waste in an environmentally friendly manner and also provide better yield by doing the same.

Aquaponics is the integration of aquaculture and hydroponics to effectively reuse aquaculture system waste as plant fertiliser (Thakur *et al.,* 2023). The plant component utilises the nitrogenous waste from aquaculture and helps in recycling the waste water. Similarly, biofloc technology (BFT) is considered an efficient recirculatory aquaculture system that does not use traditional biofilters, whereas it relies upon the manipulation of carbon: nitrogen ratio to support the flocculation of heterotrophic bacteria, which in turn assimilate the nitrogenous metabolites. In this system, there is a constant mixing of suspended particles known as the flocs in the water column, and the flocs act as the additional food source for fish species, which has the filter-feeding abilities (De Schryver *et al.*, 2008; Avnimelech, 2006).

Later on, it was found that the biofloc system can be combined with other agricultural units, where the excess dissolved nutrients from the biofloc system can be assimilated by plants into plant biomass **(**Pickens, 2015). FLOCponics is a type of aquaponics system that combines biofloc-based aquaculture with hydroponics. In FLOCponics, nutrients from a biofloc-based fish culture are used to fertilize soilless plants. The water and nutrients are reused to irrigate plants in a hydroponics subsystem (Pinho, 2022). FLOC ponics is a new research field, and little is known about its sustainability, resource use efficiency, and fish and plant productivity. The Nutrient Film Technique (NFT) and Deep-Water Culture (DWC) are the most used hydroponics subsystems in FLOC ponics. However, the success of any aquaculture technology relies upon the optimization of culture conditions such as stocking density, water quality parameters, feed composition, feeding rate etc.

Fish stocking density is a vital factor in any culture system as it determines the growth, production, survival, size variation, etc (Hussain *et al*., 2014). In an aquaponics system, the quantity of feed given to the fish determines the proportion of nutrients reaching to the plants. Hence, the stocking density and the feeding rate become the major factors for the fish as well as for the plant growth (Yildiz and Bekcan, 2017). Research have been done on determining the optimum fish-to-plant stocking density in the conventional aquaponic systems (Kamal, 2006; Hussain *et al.*, 2014; Yýldýz and Bekcan, 2017).Promising result in the production of fish and plants in biofloc-integrated aquaponics was observed by Pinho et al. (2017), Da Rocha *et al.* (2017), Pinho *et al.* (2020), and Pinheiro *et al*. (2020). However, optimising the stocking density of fish in biofloc integrated aquaponic systems is limited. Hence, this study was carried out to estimate the production performance of GIFT and bell pepper in in NFT with and without biofloc as well as to optimise the stocking density of GIFT.

Materials and Methods

Experiment site and set up

The experiment was conducted to study the effect of biofloc on fish growth and plant production in BFT integrated aquaponics system. The experiment was carried out for a period of 120 days at ICAR-Central Institute of Fisheries Education, Mumbai, India, from April 2018 to August 2018.

The experimental setup was with three treatments having different stocking densities of fish ie, 150 no/m^3(T1), 250 no/m^3(T2) and 350 no/m^3(T3) in BFT-AQP system and a control (C- conventional aquaponics) with stocking density of 150 no/m^3 in which all the treatments and control was in triplicates. BFT-NFT aquaponics system was designed in such a way that the water from the BFT culture unit(250 L Fibre reinforced tank) was allowed to pass through a vertically placed geotextile cloth filter placed inside the tank. The filter consists of a 7-inch diameter perforated pipe wrapped using a geotextile membrane, which is a nonwoven fabric material having a mesh size of 0.15mm with needle-punched pores throughout the fabric material that allows water to pass through. The filter was maintained within the biofloc fish culture tanks so that the flocs are retained within the fish tank and the filtered water passes from the filter to the plant component via a submersible pump of 0.02hp fitted inside the filter (Fig.1). The plant component of NFT(NFT channel) consists of 5-inch diameter pipe with holes large enough (about 8.3cm diameter) for a plastic cup of around 3-inch size to fit into it. The plastic cup having perforations of 2.5 cm length and 1.5cm breadth was used and was filled with gravel of 0.7-inch size. The gravels act as media for supporting the plants, helping them anchor and and stabilise in the NFT channel. The water from the biofloc fish culture tank was allowed to pass through the NFT channel as a thin film, touching the root fibres and irrigating them by supplying nutrient-laden water to them, which is the mechanism of the nutrient film technique. The roots of the plant can absorb nitrogenous nutrients dissolved in the irrigating water from the fish tank. From the NFT channel, water flows down to the fish tank by force of gravity (Fig.1). The water flow rate was controlled with the help of a ball valve and was adjusted at 8.3 L/min. Any loss of water from the fish tank due to handling and transpiration was compensated by adding fresh water. The water pumps were maintained at a timing of 20 min on and 40 min off during every single hour using a cyclic timer (Crouzet TMR 48 L).

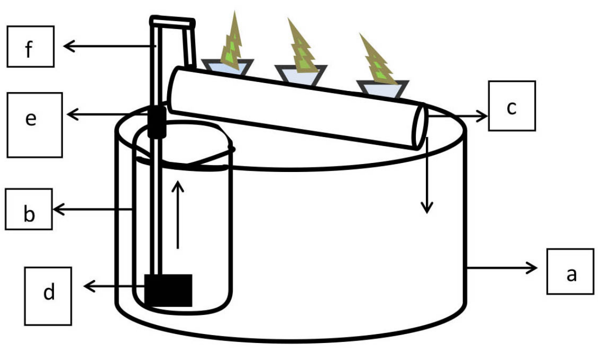

Fig. 1. A single unit of NFT used in the experiment a. Fish rearing tank. b. Filtration unit c. NFT channel d. Pump e. Ball valve f. Inlet pipe

Development of Biofloc, stocking of fish and plant in the system

The GIFT fingerlings were obtained from the Rajiv Gandhi Centre for Aquaculture and were acclimatised for 25 days in a 1000 L FRP tank ata feeding rate of 5% prior to the commencement of the experiment. Biofloc was prepared by following the methods of Hargreaves (2013). The inoculum was prepared by adding 20 g pond soil, 10 mg Ammonium sulfate, and 200 mg wheat flour per litre of water. The mixture was then kept overnight for incubation with proper aeration. After 24 hours, the inoculum was transferred to the culture tanks at the rate of 1 l per 100 l. In order to maintain the flocs in suspension, continuous aeration was provided to the tanks from blowers with an aeration capacity of 120 l/min and 4 aeration lines of 5 l/min capacity. The carbon: nitrogen ratio(C:N ratio) was maintained at 15:1(Hargreaves, 2013 and Avnimelech, 1999) and the carbon source used was commercially available wheat flour which was added on a daily basis until the floc was developed on 15 days (De Schryver *et al*., 2008). The carbon source-to-feed ratio was 1.08. That is every 10g feed required around 10.8 g of carbon source. When the floc volume reached to 5 ml/l, fishes of average size 1.44±0.12 g (weight) were stocked in different tanks of BFT-NFT aquaponics system depending on the stocking density with T1 (150 no/m^3), T2 (250 no/m^3) and T3(350 no/m^3) following completely randomised design (CRD) in triplicates. The control unit in triplicates was a conventional NFT aquaponic system, where the fish tank does not have bio floc and the water from the tank was directed to the NFT channel, and the stocking density of fish in the unit was maintained at 150 no/m^3.

The plant density was kept constant in both the control and treatment groups at the rate of 12 plants/m^2 (Kamal, 2006). Initially, the plants were grown in soil media for 20 days. When the plants developed roots with 3-5 true leaves, it was carefully transferred to the plastic cups containing gravel and fitted to the pipes of the NFT, with the roots touching the film of water running through the pipe. The plants were harvested at the end of the experiment (120days), and their height, chlorophyll-a and fruit yield content were measured.

Feed and feeding of fish

Fishes were fed with commercial feed containing 30 % crude protein at the rate of 2% of their biomass. The total feed requirement for a day was divided equally and was given in the morning at 10.00 and in the morning at 10.00 and in the evening at 17.00 hours. Sampling of fish (n=15) was carried out every 15 days interval to measure growth and biomass estimation and feeding was adjusted as per new biomass attained.

Fish growth and water quality parameters

The parameters like mean bodyweight (g), mean length (cm) was measured. Other important parameters representing growth and feed utilisation were evaluated with the following equations.

Specific growth rate (SGR) (% day^{-1})= (ln (W2) -ln (W1) / no. of days) x 100,

Feed conversion ratio (FCR) = feed given (dry weight in g)/body weight gain (wet weight in g), Protein efficiency ratio(PER): net weight gain (wet weight ing)/protein fed (g),

Percentage weight gain (PWG) (%) = ((final weight-initial weight)/initial weight) x 100,

Water quality parameters like temperature, pH, dissolved oxygen, ammonia, nitrate, floc volume, total alkalinity and phosphate were measured in every 10 days interval according to the standard methods of APHA, (2018). The quantity of potassium in the culture water was measured at the end of the experiment by flame atomic emission spectrometry (FAES) using a flame photometer (Elico CL 378, Hyderabad, India). The floc volume was measured using an Imhoff cone by allowing the floc to settle for 20-30 minutes and was expressed in ml/l.

Plant growth and yield

The plant growth parameters, such as plant height (cm) and yield (g/plant), were measured. In addition, the following indices were calculated (Colt, J. and Semmens, K., 2022).

FCR^{plant} = Feed Fed/ ΔW^{plant}

Plant-fish mass ratio at the time of harvest h ($PFRM_h$) $=W_t^{plant}/W_t^{fish}$

Plant-fish revenue ratio (PFRR) = $PFRM_h$ ($Farmgate^{plant}/Farmgate^{fish}$),

Where,

ΔW^{plant} = final weight of plant – Initial weight of plant (kg)

W_t^{plant} is the weight of the plant at the time t

W_t^{fish} is the weight of fish at the time t

$PFRM_h$ is Plant-fish mass ratio at harvest time h,

$Farmgate^{plant}$ = Farmgate price for plants ($/kg)

$Farmgate^{fish}$= Farmgate price for fish ($/kg

Sampling and Chlorophyll a estimation of plants

For estimating chlorophyll content, a leaf extract from the plants of the aquaponic unit was prepared by using 80% acetone (v/v). Fully expanded leaves were cut into pieces and weighed to 1g and then ground for 3-5 minutes in a mortar and pestle by adding acetone following Kamble *et al.*(2015). About 7 ml of chilled aqueous 80% acetone was added to the extract, centrifuged for 5 minutes at 3000 rpm and then the absorbance was determined at 630, 645 and 665 nm spectrophotometrically. Chlorophyll-a was determined using the equation: Chl a = (11.6x665nm)-(0.14x630nm)-(1.31x645nm)

Statistical analysis

Statistical analysis of growth, water quality and immune parameters was performed by one-way ANOVA (Analysis of variance) using SPSS software. In addition, the data were checked for normal distribution and then the outliers were removed and analysed for variance. The one-way ANOVA was coined out for the separation of means at a significance level of $p<0.05$, and the significant differences among treatments were determined by the Duncan multiple range test (DMRT) as DMRT involves the computation of numerical boundaries that allow for the classification of the difference between any 2 treatments means as significant or non-significant. This requires the calculation of a series of values, each corresponding to a specific set of pair comparisons. The dependent variables were measured in a continuous scale.

Results and Discussion

Fish growth in biofloc system

The highest mean body weight (80.55±0.43g), SGR (3.44±0.09% day^{-1}), PER (2.63±0.03) and PWG (6085±217.00%) of fish was observed in T1 (150 no/

Table 1. Growth performance of fish during the experiment period of 120 days

Parameter	Treatments T1	T2	T3	Control	P value
Mean Body weight (g)	$80.55±0.43^{a}$	$61.50±0.26^{c}$	$43.37±0.49^{d}$	$65.30±0.38^{b}$	0.000
Specific Growth Rate (% day^{-1})	$3.44± 0.09^{a}$	$3.08± 0.16^{bc}$	$2.82± 0.03^{c}$	$3.18± 0.01^{ab}$	0.014
Feed Conversion Ratio	$1.10±0.05^{c}$	$1.22±0.04^{bc}$	$1.46±0.03^{a}$	$1.27±0.03^{b}$	0.003
Feed Conversion Efficiency	$0.9088±0.04^{a}$	$0.8212±0.03^{ab}$	$0.6851±0.01^{c}$	$0.7836±0.01^{bc}$	0.006
Protein Efficiency Ratio	$2.63±0.03^{a}$	$2.00±0.06^{b}$	$1.39±0.02^{c}$	$2.14±0.10^{b}$	0.000
Percentage weight gain (%)	$6085.8±217.00^{ab}$	$4128.0±279.64^{a}$	$2871.4±123.27^{a}$	$4742.9±240.55^{ab}$	0.030
Initial length (cm)	$3.66±0.29^{a}$	$3.93±0.29^{a}$	$3.56±0.29^{a}$	$3.23±0.0.29^{a}$	0.355
Final length (cm)	$15.43±0.23^{a}$	$15.8±0.45^{a}$	$15.4±0.15^{a}$	$13.33±0.61^{b}$	0.000

Values in the same row with different superscripts differ significantly ($P < 0.05$) for each parameter. One-way ANOVA was used following Duncan multiple range test in SPSS-16.0.

Control: 150 no./m^3, T1: 150 no./m^3, T2: 250 no./m^3, T3: 350 no./m^3

m^3) with BFT-NFT. A significant difference ($p<0.05$) was observed for these parameters between the treatments and conventional NFT control (Table 1). The initial length did not vary significantly among the treatments and control, whereas the final length showed a significant difference ($p<0.05$) between treatments and control. However, there was no significant difference between the final lengths of fish across different treatments. FCR was found to be lowest in T1 (1.10±0.05), whereas the highest value was observed in T3 (1.46±0.03), the treatment with the highest fish density.

Tilapia has the ability to make use of the organisms associated with periphyton (zooplankton, phytoplankton, heterotrophic bacteria) as their food source (Martýnez-Cordova *et al.,* 2014). The present study also showed better growth performance of GIFT in treatment groups, that is, biofloc integrated NFT system compared to control, conventional NFT system without biofloc. Haque *et al.* (2016) observed that there was a significantly higher individual harvesting weight, individual weight gains and specific growth rate in the GIFT tilapia reared in a biofloc system compared to the red tilapia, attributing to the higher grazing of periphyton by GIFT in a biofloc system. A similar observation of a higher growth rate of fish in biofloc systems was reported by various authors (Luo *et al.,* 2014 and Xu and Pan, 2012). Availability of the in-situ food can be the major reason for the enhanced fish growth in the biofloc system in our study compared to the conventional NFT aquaponics. Similarly, Azim and Little (2008) also reported 45% higher net fish production in biofloc-based culture compared to the conventional clear water systems. These results indicate that the integration of biofloc in aquaponics can yield better fish production compared to conventional aquaponics systems.

Ekasari, J. and Maryam, S. (2012) indicated that the stocking density influences the growth and survival of Tilapia in biofloc technology. Likewise, Haridas *et al.* (2017) also reported increased growth performance of GIFT in biofloc treatment groups with the lowest stocking density. Similarly in the present study, the highest mean body weight, SGR, PER, PWG, and lower FCR were observed in the treatment with the lowest stocking density (T1) compared to higher stocking densities. The crowding stress, competition etc. induced by the higher stocking density might have negatively impacted the growth performance of GIFT in T3 and T2 compared to T1. Similarly, Mair (2002a, b) and Martin *et al.* (2005) also reported that the interactions and competition for food result in the varying growth of fish at different stocking densities. Hence, the lower stocking density of 150 no/m^2 can be used for the rearing of GIFT in bio floc integrated NFT to get optimum growth of fish.

Plant growth parameters

There was a significant difference ($p<0.05$) in the fruit yield of bell peppers between the control and treatments. There was no significant difference in the fruit yield among the treatments. However, slightly higher fruit yield was observed in T1 (155.10±3.76gm/plant). The height of the bell pepper plant showed significant difference within the treatment groups as well as the NFT control (Table 2). The maximum average plant height was observed in T3 (44.56±1.98 cm) followed by T1 (40.6±1.67cm). Similarly, the chlorophyll-a content in the plant was highest in treatments, T1(12.93 ± 0.36μgL^{-1}) followed by T3, T2 and the lowest was found in NFT control.

Plant growth parameter is an important criterion for evaluating the suitability and efficiency of the aquaponics system. Also, it can be considered as the crucial parameter for deciding the type of aquaponics technique to be adopted for the growth of the plant. Plants and vegetables like herbs and

Table 2. Growth response of bell pepper in NFT at the end of experiment days

Parameter	Treatments				P value
	T1	T2	T3	Control	
Plant height (cm)	40.6±1.67^{ab}	39.43±0.28^{b}	44.56±1.98^{a}	32.7±1.20^{c}	0.003
Yield (gm/plant)	155.10±3.76^{a}	138.87±5.18^{a}	118.07±4.83^{a}	47.4±6.30^{b}	0.001
Chlorophyll-a(ìgl^{-1})	12.93 ± 0.36^{a}	10.15±0.14^{c}	11.28±0.27^{b}	8.96±0.09^{d}	0.000

Values in the same row with different superscripts differ significantly ($P<0.05$) for each parameter. One-way ANOVA was used following Duncan multiple range test in SPSS-16.0
Control: 150 no./m^3,T1: 150 no./m^3, T2: 250 no./m^3, T3: 350 no./m^3

lettuces, which require low to medium nutrients, perform well in aquaponics systems (Diver, 2006). In the present study, bell pepper was grown in the nutrient film technique of aquaponics integrated with bio floc (BFT-NFT) in order to evaluate the effect of bio floc effluent on the growth of the plant. It was found that the plants grown in the BFT-NFT system showed the highest yield (T1) than those in the conventional NFT aquaponics. A study conducted by Pinho *et al.* (2017) also observed a better growth of lettuce in biofloc integrated aquaponics system thus supporting the present observations. Pickens, (2015) conducted a similar study on the biofloc system for tilapia production with greenhouse cherry tomatoes and observed that the soilless growing technique can be successfully integrated with aquaculture effluent. The lower production in the control may be due to the availability of lower amount of nutrients in the conventional system that leads to lower growth and yield compared to the biofloc integrated aquaponics (BFT-NFT). Castillo-Castellanos *et al*. (2016) also reported a similar observation of lower fruit yield in conventional aquaponics compared to hydroponics. In the present study, higher fruit yield, and plant height were observed in treatments compared to control. This might be due to the higher nutritional demand for the bell pepper and comparatively lower availability of nutrients in conventional aquaponics systems compared to the bio floc integrated treatments. Diver, (2006) also reported a higher nutrient demand in fruiting vegetables like tomatoes, bell peppers, and cucumber. However, there was no significant difference in fruit yield among the treatment groups. However, the fruit yield showed a decreasing trend with the increase in stocking density, whereas the plant height showed an increasing trend with an increase in stocking density. This can be due to the fact that in the higher stocking densities (T2 and T3) a gradual accumulation of NO_3-N was observed that led to enhanced vegetative growth, suppressing the fruiting ability of plants. Similar observations were earlier made by Rakocy *et al.* (2006), Lennard and Leonard (2006) and Palm *et al.* (2014), thereby substantiating the observation made in the present study. The result of this study indicates that fruit-yielding plants like bell pepper can grow well in nutrient-rich Biofloc water compared to conventional aquaponics. The lower stocking density can yield better fish and plant produce compared to higher stocking densities.

Yield and resource use efficiency

In the present study, the farm gate price of fish was assumed as INR 250 and Capsicum as INR100 per kg and hence the FCR^{plant} in the treatment showed significantly lower values compared to that of control (0.49±0.04). There was no significant difference among the treatments. However, a slightly lower value was observed in T3 (0.28±0.02) followed by T2 (0.31±0.01), T1 (0.35±0.02) and control. A significant difference was observed in the PFRMh, with the highest in T1 (0.19±0.015) and lowest in control (0.07±0.025), as shown in Table 3.

Similarly, the PFRR of the present study also showed significantly higher value T1 (0.08±0.006), and lowest in control (0.03±0.010).

Feed Conversion Ratio, is a metric commonly

Table 3. Water Quality parameter observed during the experiment period of 120 days

Parameter	Treatments				P value
	T1	T2	T3	Control	
Temperature (^{0}C)	29.80±0.17^a	29.66±0.23^a	29.36±0.11^a	29.64±0.24^a	0.943
pH	7.69±0.01^a	7.70±0.04^a	7.88±0.13^a	7.4±0.03^b	0.009
Dissolved Oxygen (mgl^{-1})	4.58±0.02^b	4.39±0.05^b	4.61±0.04^b	6.11±0.27^a	0.000
Ammonia (mgl^{-1})	0.27±0.01^a	0.17±0.01^b	0.15±0.02^b	0.23±0.03^{ab}	0.041
Nitrate (mgl^{-1})	2.38±0.12^b	3.35±0.12^a	3.10±0.05^a	2.11±0.10^b	0.000
Floc volume (ml^{-1})	25.72±0.38^b	28.84±0.71^a	29.66±1.45^a	0.00±0.00^c	0.000
Sludge Volume index	0.08±0.00^b	0.10±0.00^a	0.09±0.00^a	0.00±0.00^c	0.000
Total Alkalinity (ppm)	147.00±6.08^b	144.67±14.84^b	137.67±1.20^b	170.67±2.18^a	0.002
Phosphate (mgl^{-1})	5.44±0.17^b	6.08±0.07^a	6.16±0.14^a	5.16±0.14^b	0.004
Potassium (mgl^{-1})	36.43±3.35^b	46.93±3.27^a	54.06±3.00^a	26.60±3.19^b	0.001

Values in the same row with different superscripts differ significantly (P<0.05) for each parameter. One -way ANOVA was used following Duncan multiple range in SPSS -16.0
Control: 150 no./m^3,T1: 150 no./m^3, T2: 250 no./m^3, T3: 350 no./m^3

used in aquaculture and aquaponics to measure the efficiency of converting feed into biomass. In an aquaponics system, FCR can be calculated for both the plant and fish components to assess the overall efficiency of the system (Colt and Semmens, 2022). In the present study, FCR^{Plant} was significantly lower in treatments compared to control. However, values fell within the theoretical range of 0.064–0.564 kg/kg (Colt and Semmens, 2022), which indicates the optimum performance of the plants in aquaponics as well as bio floc integrated aquaponics systems. The plant-fish mass ratio in aquaponics refers to the proportion of plant biomass to fish biomass in an aquaponics system, and the plant-fish revenue ratio -PFRR indicates the revenue ratio (Colt, J. and Semmens, 2022). Both $PFMR_h$ as well as PFRR were significantly higher in T1 followed by T2, T3 and the lowest values were observed in control. The poor plant growth response in the control, even after the absorption of the phosphorus, can be due to the fact that, apart from phosphorus, other parameters like the amount of nitrate, microbial floc and its interaction also might have influenced the enhanced plant growth in the integrated system. The results indicate the better performance of the bio floc integrated aquaponics system compared to the Clearwater system, especially in the lower stocking densities.

Water quality parameters

The mean water temperature during the study period was 29°C± SE with no marked variation between the treatments and control (Table 4). The pH value observed was within a range of 7.3 to 8.1, with a significant difference ($p<0.05$) between the treatment and control. The highest value was in T3, followed by T2 and T1. The mean dissolved oxygen concentration in all the treatments and control varied significantly ($p<0.05$), with the highest value in NFT control (6.11±0.27 mgl^{-1}) followed by treatments T3, T1 and T2.

The pH was in the range of 7.4 to 7.8 for the control and treatments. However, the pH value was significantly higher in treatments compared to the control in the present study. A similar value of pH was previously obtained by Boyd, 1990, and Ekasari e*t al*., 2015 in aquaponics systems. The dissolved oxygen level in the treatment was significantly lower than that in the control. It is reported that nitrification requires approximately 4 mg of oxygen and 8 mg of HCO_3^- for oxidizing 1 mg of TAN (Sharmaand Alhart, 1977). The reduced DO level in the treatment can be due to the consumption of oxygen by the bacteria in the floc (Hargreaves, 2013). However, in the present experiment continuous aeration maintained dissolved oxygen level above 4ppm in all the treatments.

The level of ammonia was approximately within the range of 0.18-0.27 mgl^{-1}, showing a significant difference ($p<0.05$) between treatments and control. The highest value was observed in T1 followed by NFT control and the lowest was in T3. The highest nitrate level was observed in T2 (3.35± 0.12 mgl^{-1}) and the nitrate range varied between 2.11± 0.10 to 3.35± 0.12 mgl^{-1} with a significant difference between the treatments and control.

The level of ammonia in the present study was safe for the growth of the fish. There was a gradual accumulation of ammonia in the first month, which may be due to the poor activity of the ammonia-oxidising bacteria, and then the value began to decrease, which indicates the establishment of an active nitrification process (Fig. 2). The Nitrate-N is the preferred form of nitrogen for growing higher plants (Rakocy *et al.,* 2006). In the present study, nitrate level showed reduced values towards the end of the experiment due to its uptake by the plants (Fig. 3). The floc volume showed a significant difference between treatments, with the highest volume in T3 (29.66±1.45 ml/l) followed by T2 (28.84±0.71 ml/l) and T1 (25.72±0.38 ml/l). The floc level was sufficient throughout the experiment. The floc volume showed fluctuation in the treatments during the culture period. After 30 days of culture, the highest floc volume was observed in T1 followed by T2 and T3. Whereas T2 and T3 showed significantly higher floc volume at the end of 60 and 90 days of culture respectively. By the end of 120 days of the experiment,

Table 4. Yield and resource use efficiency

Treatments	FCR^{Plant}	PFRR	$PFRM_h$
C	$0.49±0.04^{a}$	$0.03±0.010^{c}$	$0.07±0.025^{c}$
T1	$0.35±0.02^{b}$	$0.08±0.006^{a}$	$0.19±0.015^{a}$
T2	$0.31±0.01^{b}$	$0.06±0.004^{ab}$	$0.14±0.011^{ab}$
T3	$0.28±0.02^{b}$	$0.05±0.004^{bc}$	$0.12±0.011^{bc}$

Values in the same column with different superscripts differ significantly ($P<0.05$) for each parameter. One-way ANOVA was used following Duncan multiple range test in SPSS-16.0

Control: 150 no./m^3, T1: 150 no./m^3, T2: 250 no./m^3, T3: 350 no./m^3

the floc volume in T3 was highest, followed by T1 and T2. The floc volume for the present experiment (Fig. 4) was similar to those previously reported by Haridas *et al.* in 2017 and is found to be optimum for the rearing of GIFT tilapia in Biofloc system. The floc level was able to maintain optimum in the range of25-30 ml/l in all the treatments, which might be due to its uptake by fish as well as the gradual accumulation of micron-size floc in the NFT channel.

Alkalinity values in the present study (137.67-147 ppm) were significantly lower in biofloc integrated aquaponics (BFT-NFT) system in comparison to NFT-control (170.67±2.18 ppm). The values in the treatment were reduced with an increase in the stocking density and performed similarly to other Biofloc cultures (Ekasari *et al.,* 2015). The fluctuation in the alkalinity was reported in the Biofloc system compared to clear water (Azim and Little, 2008, Haridas *et al*., 2021; Sontakke *et al.,* 2021). The lower alkalinity levels might be due to the utilisation of the same for pH maintenance and heterotrophic bacteria production (Van Wyk and Scarpa, 1999).The level of phosphorus in the present study was similar to those earlier reported in the aquaponics system by Bittsanszky *et al.* (2016). There was a significant difference between the treatments and control. Within the Treatment; T1 shows the lower value for phosphorus, which can be attributed to the better utilisation by the plants and lower level of those through lower feeding ration as per the density.

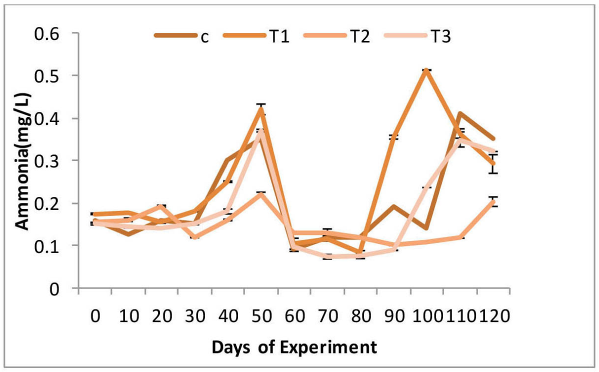

Fig. 2. Total ammonia-nitrogen variation in different treatments

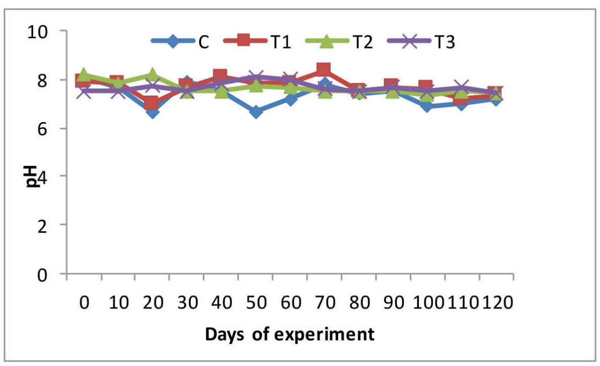

Fig. 3. pH variation in different treatments

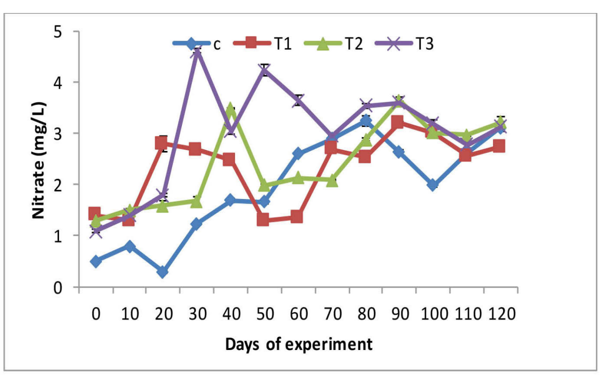

Fig. 4. Nitrate (NO_3-N) variation in different treatments

The mean potassium level at the end of the ex-

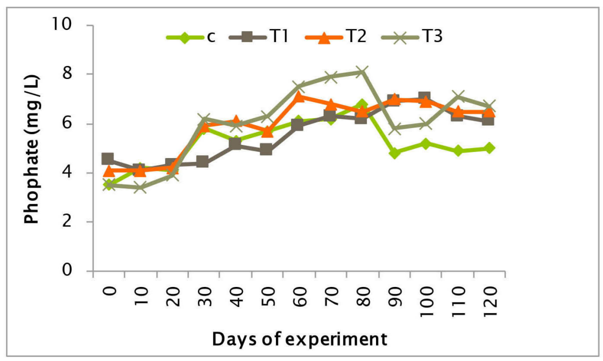

Fig. 5. Phosphate variation in different treatments

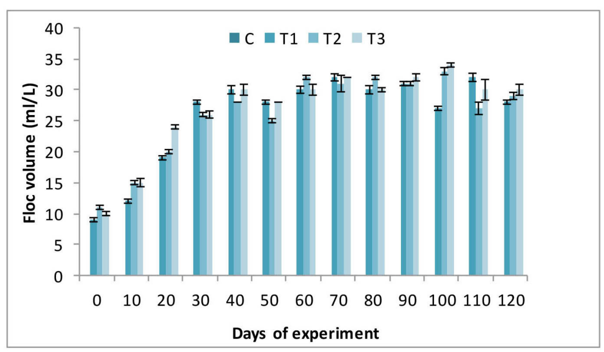

Fig. 6. Floc volume variation in different treatments

periment was highest in T3 followed by T2, T1, and control. Within the treatment, T2 and T3 showed significantly higher potassium levels than T1 at the end of the experiment. An increased level of potassium was observed in all the treatments (BFT-NFT) compared to control, which is attributed to the ability of Biofloc bacteria to accumulate potassium. Within the treatment, an increased level of potassium with increasing stocking density was observed, which might be due to a higher amount of feed used in T3 and T2 compared to control and T1. Similarly, Peter *et al*. (2021) also mentioned an increasing trend in the level of potassium with an increase in the stocking density of fish in recalculating aquaponics systems.

Conclusion

The study demonstrated the possible utilization of Biofloc technology in the aquaponics system to enhance the production performance of conventional aquaponics systems. The result of the present study suggests that the integration of NFT with biofloc technology has the potential to enhance plant and fish growth. A stocking density of fish at the rate $150/m^3$ and plant density of 12 no. $/m^2$ is optimum for an integrated Biofloc system with aquaponics in Nutrient film technique. Further research is required to analyse the composition of bio floc and its influence on the microbial composition of media and nutrient utilization by the plants.

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Ethical Approval

The authors declare that the research work was carried out in compliance with laws, policies and institutional and national guidelines for the care and use of laboratory animals.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authors' contributions

Contribution by all authors as follows

1. Dr. Sanitha Saseendran: Ideas; formulation or evolution of overarching research goals and aims
2. Dr. Kiran Dube Rawat: Verification of the overall replication/ reproducibility of results/experiments and other research outputs
3. Dr. Chandrakant M.H: Development or design of methodology; creation of models
4. Dr. Harsha Haridas: Manuscript corrections and editing
5. Dr. Babitha Rani A.M(corresponding author): Development or design of methodology, Application of statistical and other formal techniques to analyze or synthesize study data

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