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Research Article

Supplementation of biofloc in carp (*Cyprinus carpio* var. *communis*) culture as a potential tool of resource management in aquaculture

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Abstract – Supplementation of biofloc in rearing of common carp (*Cyprinus carpio* var. *Communis* (0.84 ± 0.003 g) was tested in replacing costly feed and environmental amelioration. Neem (*Azadirachta indica*) leave extract in biofloc media has beenknown to be beneficial in controlling pathogenic *Vibrio* sp. in brackish water shrimp. Two studies were conducted for 120 days in outdoor experimental cisterns with reduction of feed from 50% to 100%, with two biofloc media; without (Experiment 1; E1) and with neem extract (Experiment 2; E2). The experimental groups were: biofloc with feeding at 6% body weight (T1E1 and T1E2), biofloc with feeding at 3% body weight (T2E1 and T2E2), biofloc without feeding (T3E1 and T3E2), feeding at 6% body weight (C1) and only biofloc (C2E1 and C2E2). Absolute weight gain in T2E1 (9.96 g) was 8.23% and in T2E2 (9.56 g) was 4.39% higher than C1 (9.14). In spite of 5.04% and 13.98% higher growth respectively in T1E1 (10.50 g) and T1E2 (11.66 g) than C1 (9.97 g), BFT could not compensate the total withdrawal of feed (T3E1 and T3E2) and resulted in 22.22% to 33.33% mortality in T3E2 and T3E1 respectively. Under identical feeding regime, FCR was improved by 52.57% to 53.76% in T2E1 (1.84) and 48.71% to 51.23% in T2E2 (1.99) compared to T1E1 (3.98) and T1E2 (4.08). Absolute weight gain in T2E1 was 8.97 times and in T2E2 was 4.63 times higher than C1. In spite of 18.54% and 7.26% higher growth respectively in T1E1 and T1E2 than C1, BFT could not compensate the total withdrawal of feed (T3E1 and T3E2) and resulted in 22.22% to 33.33% mortality in T3E2 and T3E1 respectively. Under identical feeding regime, FCR was improved by 52.57% to 53.76% in T2E1 and 48.71% to 51.23% in T2E2 compared to T1E1 and T1E2 respectively. Increased N: P ratio of water and soil in E2 favoured fish growth. Neem leave extract in biofloc media should be judicious as it may cause nitrification inhibition.

Keywords: Biofloc / *Azadirachta indica* / *Cyprinus carpio* var. *communis* / feed conversion ratio / mineralizing microbes

1 Introduction

Globally, common carp (*Cyprinus carpio* L.) is the fourth most important cultured fish with a share of 7.7% of total aquaculture fish production (FAO, 2020). Moreover, common carp culture practice is changing from semi-intensive form to more intensive systems (Bakhshi et al., 2018). High stocking density can lead to stress related issues in fish, which are the major concerns in the current context due to high incidence of disease-causing harmful pathogen, low growth and feed utilization performance (Xie et al., 2018). Intensification of aquaculture has been warranted because of increasing per capita demand, which eventually led to increase in pollution load especially in tropical carp culture sector (Mpeza et al., 2013; Tavakol et al., 2017).

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In recent times, biofloc technology (BFT) is getting more attention in aquaculture due to its greater effectiveness in terms of sustainable production (Ahmed et al., 2017) through improvement of water quality, feed efficiency and immunity of fish (Crab et al., 2012; Mirzakhani et al., 2019). Moreover, the technology has a huge potential to conserve land and water and to promote heterotrohic feed resources (Ekasari and Maryam, 2012). Also, the main principle of BFT is based on more efficient use of nutrient input in limited or zero water exchange (Avnimelech, 1999). BFT is mainly focused on prevention of feed-borne toxic nitrogen metabolites (NH_3, NO_2^-, etc.) accumulation through stimulation of heterotrophic microbes by manipulation of carbon/nitrogen ratio (C:N) (Avnimelech, 2009). High proliferation ability of heterotrophic bacteria results in formation offlocs, so called biofloc which contains heterogenous populations of heterotrophic microbes, fungi, plankton, protozoa, nematodes, organic polymers and dead cells (De Schryver et al., 2008; Avnimelech, 2009; Ekasari et al., 2010). Regular consumption of floc by cultured fish can increase feed efficiency (Xu et al., 2012), resource productivity (Wei et al., 2016), and bio-security in aquaculture (Pérez-Fuentes et al., 2016). Also, the biofloc can ameliorate water quality and improve growth performance of common carp under zero water exchange system (Bakhshi et al., 2018). Schneider et al. (2005) reported that 7–13% of improvement nitrogen retention can be possible through optimization of C:N ratio in biofloc system. Biofloc can also favor phosphorus mineralization and the mineralization process becomes limited in maintaining the balance between available nitrogen: phosphorus beyond 0.065 mg l^{-1} (Dinda et al., 2019).

The successful culture of fish in biofloc supplemented system has been reported in common carp (Hargreaves, 2013), channel catfish (Green et al., 2014), Nile tilapia (Ekasari et al., 2015) with promising findings. Mahanand et al. (2013) reported that biofloc technology could able to enhance the good water quality, natural food availability and growth performance of common carp in high-intensity systems. The omnivorous common carp is compatible to ingest and digest bioflocs and able to tolerate high concentrations of suspended material in the water, and low concentrations of oxygen and stress when cultured in intensive BFT system (Najdegerami et al., 2016).

Various medicinal herbs are used in aquaculture to reduce or replace chemicals and drugsdue todevelopment of antibiotic resistance of bacteria (Cabello, 2006). Application of different parts of neem (*Azadirachta indica*) in aquaculture has become a means to control pathogen and disease (Martinez, 2002) to benefit from its bioactive compounds (azadirachtin and nimbin) (Biswas et al., 2002). The presence of natural bioactive compounds, namely azadirachtin in neem highly influence the phosphatase activity (Gopal et al., 2007) and to some extent inhibit the nitrification process in aquaculture (Das et al., 2018). Dinda et al. (2019) reported that neem based biofloc system can inhibit nitrification process in culture of common carp. However, farmers in India have been recently using neem leaves extract as herbal therapeutants in biofloc supplemented aquaculture of both finfish and shrimps (Mandal and Das, 2018, Dinda et al., 2019).

Therefore, the present study was aimed to evaluate the impact of use of biofloc media in biofloc system for culture of common carp fingerlings on growth, proximate composition, water quality and feeding performance at different feeding levels with two experiments to evaluate the supplemental neem extract in culture environment.

2 Materials and methods

2.1 Study area and preparation of experimental system

The present study was conducted in an outdoor experimental system consisting of cylindrical cemented cistern (Area: 0.18 m^2; Volume: 180 liters) (*Cyprinus carpio* var. *communis*) at Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, Chakgaria, Panchasayar, Kolkata-700094 (22°28′46″N and 88°24′4″E). Two biofloc medium experiments were conducted simulta-neously without (E1) and with neem extract (E2). In both main treatment blocs, experimental cisterns were grouped into biofloc with feeding level at 6% body weight (T1E1 and T1E2), biofloc with feeding level at 3% body weight (T2E1 and T2E2), biofloc without feeding (T3E1 and T3E2), no biofloc with feeding at 6% body weight (Control 1; C1). Another control treatment without fish and feed but under BFT in both with and without neem extract was also employed (Control 2; C2E1 and C2E2). All the treatments were triplicated cisterns (Tab. 1). A commercial pellet feed with 25.04% crude protein was purchased from the local market and provided to fish once daily (9-00 AM) at predetermined levels for 120 days. Usually, the protein requirement in fish ranged from 25 to 45% (Davies and Gouveia, 2010), and as the test fish were reared in manured system, 25.04% crude protein feed was provided as supplemental feed.

All cisterns were provided with 15 cm of soil base and filled with ground water (pH: 7.5) prior to stocking experimental fish. The experimental units were manured with semi-decomposed cow dung at a dose of 10,000 kg ha^{-1}. After seven days of manuring, liming and fertilization were performed (agricultural lime at 200 kg ha^{-1}; single super phosphate at 20 kg ha^{-1} and urea at 40 kg ha^{-1}). The cisterns were covered with fine mosquito net and kept undisturbed for another seven days. Continuous aeration was provided to each cistern during entire period of the experiments. When the water color turned into bluish-green, we assumed enough planktonic development to stock experimental fish. Fifteen healthy fingerlings of common carp (0.85 ± 0.05 g, 4.65 ± 0.04 cm) were acclimatized for seven days and stocked with 83 nos m^{-2} to each cistern in both experimental blocks (E1 and E2), except C2E1 and C2E2. Required volume of water (5–10% of total volume of cistern) was added to both the systems to maintain the water level due to evaporation and sampling loss.

Table 1. Experimental design for the present study.

Cyprinus carpio var. *communis*	
E1 (BWN: biofloc without neem supplementation)	E2 (BN: biofloc with neem supplementation)
Biofloc + Feed (6% body weight) + Fish (T1E1)	Biofloc + Feed (6% body weight) + Fish (T1E2)
Biofloc + Feed (3% body weight) + Fish (T2E1)	Biofloc + Feed (3% body weight) + Fish (T2E2)
Biofloc + Fish (T3E1)	Biofloc + Fish (T3E2)
Feed (6%) + Fish (C1)	
Only Biofloc (C2E1) (no fish + no feed)	Only Biofloc (C2E2) (no fish + no feed)

Table 2. Composition of biofloc media used in present study.

Carbohydrate content	M1: Media Composition for experiment E1	M2: Media Composition for experiment E2
Boiled rice water: 25% Sugarcane molasses: 70% Neem leaves: 20%	Boiled rice water: 30.0 liters Sugarcane molasses: 5.0 liters Yeast powder: 1.20 kg Filtered freshwater: 10.0 liters	Boiled rice water: 30.0 liters Neem leaves: 0.40 kg Sugarcane molasses: 5.0 liters Yeast powder: 1.20 kg Filtered freshwater: 10.0 liters

2.2 Preparation of biofloc medium

Two different starting biofloc media, without (M1) and with neem (M2) were prepared through an aerobic process in two separate glass jars (50 liters each) provided with constant aeration (Tab. 2). Within the media composition, sugarcane molasses was used as major external carbon source, whereas, boiled rice water neem leaves extract was considered as low carbon sources for biofloc media development. Supplemental ammonium sulfate was added as source of ammonia at required quantity during the preparation of media. The M1 media composition was used for first experiment (E1) and M2 medium composition was used for second experiment (E2) for in situ development of biofloc. The biofloc medium of 15 ml per cistern was applied over the water surface of respective treatments of both E1 and E2 once daily (10–11 AM) in the morning for the first 45 days of experiment as practiced by the shrimp farmers of the district East Midnapur, West Bengal, India. The shrimp farmers usually apply 100 liters biofloc medium in 1200 m^3 shrimp pond for first 45 days of culture period in biofloc system. The aim of the ex-situ preparation of biofloc media (M1 and M2) was to develop heterotrophic bacterial populations and subsequent biofloc in situ in the cisterns.

2.3 Maintenance of carbon: nitrogen (C: N) ratio

Sugarcane molasses was used as an external organic carbon source to achieve the required C: N ratios by calculating according to the formula given by Avnimelech (2009). Diluted molasses was applied in respective treatments daily at 10.30 hr. with higher aeration to facilitate uniform mixing in water. Initially, C: N input ratios were calculated based on the carbon-nitrogen contents of the feed and the carbon content of the molasses. The commercial feed (25.04% crude protein) for *C. carpio* had a calculated C: N ratio of 9:1, assuming the feed had 50% carbon and 5.60% of nitrogen (Xu et al., 2018). About 0.76 ml to 1.32 ml molasses per gram feed was added to maintain C: N ratio between 12:1–15:1 in the respective treatment groups. However, for T3E1 and T3E2, only sugarcane molasses was the sole source of carbon that has been taken into account in absence of feed supplementation.

2.4 Collection of samples

Collection of water and soil samples were performed aseptically in sterilized polypropylene bottles (200 ml) at 15 days intervals from each cisterns for physico-chemical and bacteriological analyses following the methods described by Arulmoorthy et al. (2014). Soil samples were collected using mini hand grab-sampler and pooled together by thorough mixing, air dried, pulverized with pestle and mortar, sieved through a 150 μm mesh sieve and stored in labeled polythene packets for analyses. Biofloc samples were collected at 15 days interval by using Imhoff cone (1 liter capacity) for proximate composition analysis from the respective treatments and filtered through 100- μm mesh. The cumulative biofloc samples were dried in an oven at 102 °C temperature until constant weight and then preserved in a refrigerator. Biofloc samples were ground and processed for proximate analysis. For carcass analysis of experimental fish, fish was collected at the end of the experiment from each treatment and anesthetized with clove oil (50 μl l^{-1} of water) followed by dissection whereas intestine was removed and whole fish was subjected for proximate composition.

2.5 Analyses of samples

2.5.1 Physico-chemical parameters

Water temperature and pH (HI-98127) and total dissolved solids (TDS) (Eutech – N374) were measured in-situ. Water temperature was measured using the same pH meter. Standard methodologies were followed for estimation of dissolved oxygen (DO), biological oxygen demand (BOD_1), total alkalinity (TA), total hardness (TH), ortho-phosphorus (Ortho-P), ammonia-nitrogen (NH_3-N), nitrite-nitrogen (NO_2-N) and nitrate-nitrogen (NO_3-N) of water (Wetzel and Likens, 1991; APHA, 1995). Soil pH, organic carbon (SOC), available nitrogen (SAN) and available phosphorus (SAP) were determined following the methods described by Jackson (1959).

2.5.2 Proximate composition of feed, biofloc and fish carcass

Proximate analyses of experimental feed (Tab. 3) and biofloc (on dry matter basis) (Tab. 4) were performed as per the standard method of AOAC (2005). Dried biofloc samples were ground and processed for proximate analysis. The level of moisture was determined in hot air oven at 105 °C temperature for overnight until constant weight obtained. Crude protein (CP) and ether extract (EE) were estimated using micro-Kjeldahl method (KEL PLUS, Pelican Equipments, India) and Soxhlet extraction method (SOCS PLUS, Pelican Equipments, India), respectively. Crude fiber (CF) of the diets (fat free samples) was analyzed by using FIBRA PLUS (Pelican Equipments, India). The dried feed and whole fish sample were subjected to 550 °C for 5 h in a Muffle furnace (Macro Scientific, India) to measure the total ash (TA) content. The nitrogen-free extract (NFE) of the diets was calculated using following formulae: Nitrogen-free extract (NFE, g kg^{-1}) = [1000 – (g CP kg^{-1} + g CF kg^{-1} + g TA kg^{-1} + gCFkg^{-1})]. Digestible energy (DE) of the diets was calculated as per the following formula given by Halver (1976): DE (kcal 100 g^{-1}) = [4 × CP (g 100 g^{-1}) + 9 × EE (g 100 g^{-1}) + 4 × NFE (g 100 g^{-1})]. The protein (P) to energy (E) ratio was calculated by using following formula: P:E (mg protein. kcal DE^{-1}) = (CP% × 1000)/DE. Gross energy (GE) of the diet was measured by an automated bomb calorimeter (5E-AC/PL; Changsha Kaiyuan Instruments Corporation Pvt. Ltd., China) and expressed in kcal 100 g^{-1}. Carcass composition of the experimental fish was calculated on % wet weight basis.

Table 3. Proximate composition of commercial feeds used for *C. carpio*.

Proximate composition (% dry matter basis) of feed for *C. carpio**	
Moisture (%)	7.4
[1]CP (%)	25.04
[2]EE (%)	5.89
[3]CF (%)	7.11
[4]TA (%)	12.11
[5]NFE (%)	57.48
[6]DE (kcal/100 g)	383.09
[7]P : E (mg protein.kcal DE^{-1})	65.36
[8]GE (kcal.100 g^{-1})	436.57

*Feed for *C. carpio* was purchased from local market, Kolkata, India. Abbreviation: [1]CP (%), Crude protein; [2]EE (%), Ether extract; [3]CF (%), Crude fibre; [4]TA (%), Total ash; [5]NFE (%), Nitrogen-free extract; [6]DE (kcal.100 g^{-1}), Digestible energy; [7]P : E (mg protein . kcal DE^{-1}), Protein : Energy ratio; [8]GE (kcal.100 g^{-1}), Gross energy.

2.5.3 Microbial population

Enumeration of aerobic heterotrophic bacteria (THB) was done by following the standard methods (APHA, 1995). Estimation of nitrogen fixing bacteria (NFB), ammonifying bacteria (AB), ammonia oxidizing bacteria (AOB) and denitrifying bacteria (DNB) were done by following the methods described by Alexander (1978), phosphate solubilizing bacteria (PSB) by the method of Subba-Rao (1977) and cellulose decomposing bacteria (CDB) the method of Sarkhel and Das (2005).

2.5.4 Growth response, feeding efficiency and mortality of *C. carpio*

Fish growth was recorded with fortnightly weighings. Absolute weight gain (AWG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), apparent net protein utilization (ANPU%) were calculated as follows:

Table 4. Growth response and mortality rate (Mean ± Standard error) of *C. carpio* in biofloc system.

Parameters	T1E1	T2E1	T3E1	T1E2	T2E2	T3E2	C1
Fish weight (g/fish)	10.50 ± 1.15^{c}	10.79 ± 1.18^{c}	5.28 ± 0.51^{a}	11.66 ± 1.31^{d}	10.41 ± 1.20^{c}	5.25 ± 0.52^{a}	9.97 ± 1.08^{b}
[1]AWG (g/fish)	9.66 ± 0.14^{c}	9.96 ± 0.16^{d}	4.44 ± 0.12^{a}	10.81 ± 0.24^{e}	9.56 ± 0.18^{c}	4.39 ± 0.12^{a}	9.14 ± 0.14^{b}
[2]SGR (%)	2.09 ± 0.36^{c}	2.13 ± 0.32^{d}	1.53 ± 0.24^{a}	2.18 ± 0.34^{e}	2.09 ± 0.32^{c}	1.52 ± 0.26^{a}	2.07 ± 0.26^{b}
[3]FCR	3.98 ± 0.34^{d}	1.84 ± 0.47^{a}	–	4.08 ± 0.36^{d}	1.99 ± 0.726^{b}	–	3.88 ± 0.38^{c}
[4]PER	1.31 ± 0.45^{a}	2.59 ± 0.74^{d}	–	1.40 ± 0.49^{b}	2.53 ± 0.72^{c}	–	1.26 ± 0.38^{a}
[5]ANPU (%)	10.68 ± 3.45^{b}	21.68 ± 0.02^{e}	–	10.28 ± 3.42^{c}	19.81 ± 3.06^{d}	–	10.24 ± 3.46^{a}
Mortality rate (%)	13.88 ± 0.01^{b}	16.67 ± 0.02^{c}	33.33 ± 0.01^{e}	8.33 ± 0.01^{a}	8.33 ± 0.01^{a}	22.22 ± 0.02^{d}	16.67 ± 0.01^{c}

Note: Means superscripted with different letters in each row are significantly different ($P < 0.05$).
Abbreviation: [1]AWG (g/fish), Absolute weight gain; [2]SGR (%), Specific growth rate; [3]FCR, Feed conversion ratio; [4]PER, Protein efficiency ratio; [5]ANPU, Apparent net protein utilization.

Weight gain = (Final wet body weight- Initial wet body weight)

$$\text{Specific growth rate (\%)} = \frac{\ln(\text{final wet body weight}) - \ln(\text{initial wet body weight})}{\text{Number of days}} \times 100$$

Feed conversion ratio = [Total dry feed fed (dry weight in g) / Weight gain (wet weight in g)]

Protein efficiency ratio = [Live body weight gain (wet weight in g) / Crude protein fed (dry weight in g)]

$$\text{Apparent net protein utilization} = \frac{(\text{Final carcass protein} - \text{Initial carcass protein})}{\text{Protein fed (dry weight in g)}} \times 100.$$

2.6 Statistical analyses

Data were submitted to the two way analysis of variance (Montgomery, 2001) with interactions. This has been applied to test the significance in difference among the treatments with regards to the efficacy of biofloc over feed based treatment and interaction of neem extract in neem based biofloc over all the treatments. Post-hoc analysis (Duncan's multiple range test; DMRT) were applied to find out the homogeneity among the mean of every possible pair of treatment combinations (SPSS Statistics v. 20.0). The statistical model applied for the two way ANOVA was: $Y_{ijk} = T_i + C_j + (T^*C)_{ij} + e_{ijk}$; where, T_i = treatment at ith number, C_j = condition at jth number, e_{ijk} = error. Pearson's correlation coefficient was applied to examine relationship between available nutrient parameters in soil and with selective physico-chemical parameters.

3 Results

All the parameters related to growth and nutrient utilization including body weight, AWG, SGR, feed intake, FCR, PER and ANPU were found to be significantly ($P < 0.05$) affected by biofloc application and feeding level in both E1 and E2 (Tab. 4). Growth rate increment of fish was significantly ($P < 0.05$) less in T3E1 (1.53 ± 0.24%) and T3E2 (1.52 ± 0.26%) compared to T1E1, T2E1, T1E2 and T2E2 (2.09–2.18%). The SGR increased in all the treatment groups except C1E1 and C1E2 with highest and lowest absolute growth attained in T2E1 and T3E1, and, T1E2 and T3E2 respectively. Moreover, significantly higher body weight was attained in T1E2 (11.66 ± 1.31 g) compared to T1E1 (10.50 ± 1.15 g). Though treatment difference remained significant ($P < 0.05$), insignificant difference ($P > 0.05$) was observed between T1E1 and T2E1; T1E2 and T2E2. Similar trends were also observed in ABG and SGR in fish. Feed intake capacity was naturally lower both in T2E1 and T1E2 compared to control due to predetermined feeding level. FCR was higher in T1E1 and T1E2 and, lower in T2E1 and T2E2 compared to C1. PER and ANPU significantly increased in T2E1 and T2E2 as compared to T1E1, T1E2 and C1.

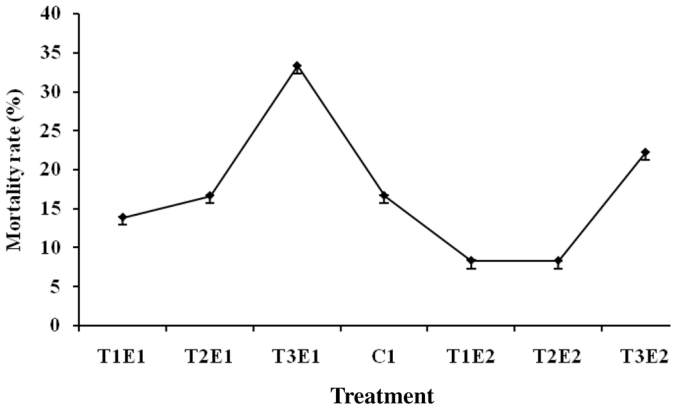

Fig. 1. Mortality rate (%) of *C. carpio* in different treatments.

However, biofloc alone could not be sufficient in supporting growth and survival rates of the test fish either in E1 or in E2 were lower with mortality rates 33.33% and 22.22% in T3E1 and T3E2 respectively (Fig. 1). Biofloc volume was highest in T1E2 and was lowest in C2E1 (Tab. 5) with significant ($P < 0.05$) difference among the treatments of both E1 and E2.

Analyses of proximate composition of biofloc indicated that crude protein content was increased, whereas, crude lipid, carbohydrate and ash content declined in all the treatments during the study period. Mean protein content ranged from 27.98% to 33.55% and was highest in T1E2 and lowest in C2E1. Carbohydrate content ranged from 4.96% to 9.87% and significantly ($P < 0.05$) differed among the treatments. Lipid content ranged from 1.06% to 3.02% and significantly differed ($P < 0.05$) among the treatments (Tab. 5). The calculated DE value was higher in the treatments of E2 compared to E1. Mean DE value ranged from 369.24–384.01 kcal. 100 g^{-1} and significantly differed among the treatments ($P < 0.05$; Tab. 4). Mean P:E ratio ranged from 7.40–8.84 mg protein. kcal DE^{-1} and significantly differed among the treatments ($P < 0.05$; Tab. 4).

Water temperature ranged between 25 and 27 °C during the study period. Water and soil pH remained alkaline in all the treatment groups of both E1 and E2 throughout the study period and ranged between 7.03–7.67 and 7.13–7.61 respectively (Tab. 6). NH_3-N of water gradually decreased up to 60th day followed by increasing trend in all the treatment groups except in C1 (Fig. 2). NH_3-N in general, was higher in all the treatment groups of E2 compared to the treatment groups of E1 and it was significantly higher both in T1E1, T2E1; T1E2 and T2E2 as compared to C1. The mean NH_3-N concentration ranged from 0.086 mgl^{-1} to 0.191 mgl^{-1} and significantly differed among the treatments ($P < 0.05$; Tab. 6). NO_2-N of water gradually increased in all the treatment groups of both E1 and E2 with highest concentration recorded in T1E1 and lowest in C2E1 and C2E2 (Fig. 3). The mean NO_2-N concentration ranged from 0.002 mgl^{-1} to 0.007 mgl^{-1} and significantly differed among the treatments ($P < 0.05$; Tab. 6). NO_3-N of water remained significantly higher in the treatments of E2 compared to E1 (Fig. 4). The mean NO_3-N concentration

Table 5. Biofloc volume and proximate composition of biofloc (% dry weight basis) inboth E1 and E2.

	(Mean ± Standard Error)							
Parameters	T1E1	T2E1	T3E1	C2E1	T1E2	T2E2	T3E2	C2E2
Biofloc volume (ml. l^{-1})	15.54 ± 0.74^{c}	16.01 ± 0.82^{d}	14.70 ± 0.55^{b}	13.82 ± 0.38^{a}	16.86 ± 0.82^{e}	16.54 ± 0.81^{d}	16.18 ± 0.74^{d}	14.61 ± 0.57^{b}
Proximate composition of biofloc (% dry weight basis)								
Moisture (%)	54.20 ± 1.24^{d}	52.60 ± 0.56^{c}	55.93 ± 1.94^{d}	57.66 ± 2.31^{e}	42.84 ± 6.95^{a}	50.80 ± 0.09^{b}	52.4 ± 1.29^{c}	55.8 ± 1.58^{d}
[1]CP (%)	30.40 ± 0.83^{b}	32.38 ± 0.33^{c}	29.63 ± 1.39^{a}	27.98 ± 1.58^{a}	32.30 ± 0.64^{b}	33.55 ± 0.30^{b}	31.05 ± 1.03^{b}	28.42 ± 1.26^{a}
[2]CF (%)	9.85 ± 1.48^{f}	9.87 ± 1.56^{f}	9.87 ± 1.40^{f}	9.78 ± 1.41^{e}	9.29 ± 1.83^{d}	5.71 ± 1.90^{c}	5.46 ± 1.82^{b}	4.96 ± 1.65^{a}
[3]EE (%)	2.89 ± 0.33^{f}	2.49 ± 0.40^{e}	2.24 ± 0.36^{c}	2.36 ± 0.34^{d}	3.02 ± 0.29^{b}	1.06 ± 0.35^{a}	1.17 ± 0.39^{b}	1.29 ± 0.43^{b}
[4]TA (%)	0.84 ± 0.03^{e}	0.75 ± 0.04^{d}	0.62 ± 0.02^{b}	0.56 ± 0.02^{a}	0.84 ± 0.04^{e}	0.72 ± 0.02^{d}	0.67 ± 0.01^{c}	0.65 ± 0.01^{c}
[5]NFE (%)	56.02 ± 1.34^{b}	54.51 ± 1.28^{a}	57.64 ± 1.28^{b}	59.32 ± 1.36^{c}	54.55 ± 1.42^{a}	58.96 ± 1.38^{c}	61.65 ± 1.45^{d}	64.68 ± 1.42^{e}
[6]DE (kcal.100 g^{-1})	371.69 ± 0.12^{c}	369.97 ± 0.13^{a}	369.24 ± 0.11^{a}	370.44 ± 0.12^{b}	374.58 ± 0.14^{d}	379.58 ± 0.12^{e}	381.33 ± 0.13^{f}	384.01 ± 0.11^{g}
[7]P: E (mg protein. kcal DE^{-1})	8.18 ± 0.01^{e}	8.75 ± 0.03^{g}	8.02 ± 0.02^{c}	7.55 ± 0.02^{b}	8.62 ± 0.02^{f}	8.84 ± 0.01^{g}	8.14 ± 0.04^{d}	7.40 ± 0.03^{a}

Note: Means superscripted with different letters in each row are significantly different ($P < 0.05$).
Abbreviation: [1]CP (%), Crude protein; [2]CF (%), Crude fibre; [3]EE (%), Ether extract; [4]TA (%), Total ash; [5]NFE (%), Nitrogen-free extract; [6]DE (kcal.100 g^{-1}), Digestible energy; [7]P: E (mg protein. Kcal DE^{-1}), Protein: Energy ratio.

ranged from 0.173 mgl^{-1} to 0.339 mgl^{-1} and significantly differed among the treatments ($P < 0.05$; Tab. 6).

Total available nitrogen (TAN) continued to increase in all the treatment groups of both E1 and E2 (Fig. 5) and it was comparatively higher in the treatments with neem extract (E2) than E1. The mean TAN ranged from 0.260 mgl^{-1} to 0.535 mgl^{-1} and significantly differed among the treatments ($P < 0.05$; Tab. 6). Ortho-phosphate of water gradually increased in T1E1, T2E1, T1E2 and T2E2, whereas, the trend gradually declined in T3E1 and T3E2 (Fig. 6). However, it was comparatively higher in the biofloc medium without neem compared to E2 (medium with neem extract). The mean ortho-phosphate concentrations ranged from 0.05 mgl^{-1} to 0.081 mgl^{-1} and significantly differed among the treatments in both E1 and E2 ($P < 0.05$; Tab. 6). Significant difference in the values of N: P ratio was observed in water and soil of both E1 and E2 ($P < 0.05$; Tab. 6).

Total aerobic heterotrophic bacterial population (THB) in water gradually increased in all the treatments with highly significant treatments difference ($P < 0.05$) in E1 and E2. The highest population was encountered 6.97 CFU (No × $10^3 ml^{-1}$) in T1E1 and 6.16 CFU (No × 10^3 ml^{-1}) in T1E2 which was 9.56–39.71% and 9.31–39.18% higher than rest of the treatments in water and soil, respectively in E1 and E2 (Figs. 7 and 8). THB, AB, AOB, NFB, DNB, CDB and PSB populations were higher in the treatment groups in E1 compared to E2 (Tab. 7). The AB, AOB, NFB and DNB populations increased gradually followed by a declining trend from 90th day in all the treatment groups except in C2E1 and C2E2 in which such decline was encountered from 45th day onwardsin both water and soil. The mean density of AB ranged from 1.88 to 4.44 CFU (No × 10^3 ml^{-1}) in water and 3.25 to 4.99 CFU (No × 10^3 ml^{-1}) in soil with significant difference among the treatments of both E1 and E2 ($P < 0.05$; Tab. 7).

The mean population of AOB ranged from 2.21 to 5.67 CFU (No × 10^3 ml^{-1}) in water and 3.06 to 6.27 CFU (No × 10^3 ml^{-1}) in soil with significant difference among the treatment groups of both E1 and E2 ($P < 0.05$; Tab. 7). The mean density of NFB ranged from 2.32 to 5.31 CFU (No × 10^3 ml^{-1}) in water and 3.15 to 6.23 CFU (No × 10^3 ml^{-1}) in soil with significant difference among the treatment groups of both E1 and E2 ($P < 0.05$; Tab. 7). The mean population density of DNB ranged from 2.71 to 5.37 CFU (No × 10^3 ml^{-1}) in water and 2.93 to 6.13 CFU (No × 10^3 ml^{-1}) in soil with significant difference among the treatment groups of both E1 and E2 ($P < 0.05$; Tab. 7). CDB population in water and soil continuously increased in the entire treatment group except in T3E1, T3E2, C2E1 and C2E2, it declined during 2nd half of the study period. The mean population of CDB ranged from 3.43 to 5.81 CFU (No × 10^3 ml^{-1}) in water and 3.50 to 5.84 CFU (No × 10^3 ml^{-1}) in soil with significant difference among the treatments of both E1 and E2 ($P < 0.05$; Tab. 7). PSB population in water and soil tended to increase in all the treatments upto 60th day of experiment period, whereas, the population declined in C2E1 and C2E2 during the 2nd half of the study period. The mean population of PSB ranged from 2.43 to 4.88 CFU (No × 10^3 ml^{-1}) in water and 3.07 to 5.40 CFU (No × 10^3 ml^{-1}) in soil with significant difference among the treatments of both E1 and E2 ($P < 0.05$; Tab. 7).

Table 6. Physico-chemical parameters (mean ± standard error) of water and soil in *C. carpio* biofloc system.

Parameters	T1E1	T2E1	T3E1	C2E1	T1E2	T2E2	T3E2	C2E2	C1
Water pH	7.24–8.24	7.29–8.21	7.41–8.22	7.78–8.27	7.31–8.14	7.28–8.15	7.28–8.16	7.51–8.15	7.47–8.15
[1]TDS (mg l^{-1})	270 ± 30.31^{h}	255 ± 26.79^{g}	171.3 ± 4.46^{c}	158.5 ± 17.32^{b}	236.5 ± 23.26^{f}	219.3 ± 21.92^{e}	160.08 ± 5.53^{b}	151.5 ± 17.92^{a}	201.1 ± 18.36^{d}
[2]DO (mgl^{-1})	7.87 ± 0.33^{a}	7.95 ± 0.33^{a}	8.19 ± 0.23^{b}	8.94 ± 0.34^{c}	9.20 ± 0.27^{d}	8.39 ± 0.41^{c}	8.41 ± 0.32^{c}	7.83 ± 0.29^{a}	7.89 ± 0.24^{a}
[3]TA (mgl^{-1})	129.85 ± 4.51^{b}	133.11 ± 4.17^{c}	137.52 ± 4.60^{d}	140.19 ± 4.78^{e}	122.30 ± 2.92^{a}	130.19 ± 5.16^{b}	132.00 ± 4.87^{c}	138.00 ± 5.30^{d}	130.04 ± 3.99^{b}
[4]TH (mgl^{-1})	445.15 ± 32.28^{d}	417.63 ± 33.87^{c}	349.93 ± 33.49^{a}	409.74 ± 39.61^{b}	441.19 ± 29.95^{d}	410.59 ± 35.46^{b}	402.74 ± 39.92^{b}	409.41 ± 46.35^{b}	486.74 ± 40.97^{e}
[5]Ortho-P (mgl^{-1})	0.081 ± 0.0^{g}	0.074 ± 0.0^{f}	0.061 ± 0.0^{d}	0.051 ± 0.0^{a}	0.073 ± 0.0^{s}	0.066 ± 0.0^{f}	0.057 ± 0.0^{e}	0.05 ± 0.0^{a}	0.058 ± 0.0^{c}
[6]NH_3-N (mgl^{-1})	0.173 ± 0.03^{h}	0.139 ± 0.02^{e}	0.109 ± 0.01^{c}	0.086 ± 0.01^{a}	0.191 ± 0.03^{i}	0.148 ± 0.02^{f}	0.128 ± 0.01^{d}	0.103 ± 0.01^{b}	0.168 ± 0.02^{g}
[7]NO_2-N (mgl^{-1})	0.007 ± 0.0^{f}	0.005 ± 0.0^{d}	0.004 ± 0.0^{c}	0.002 ± 0.0^{a}	0.005 ± 0.0^{d}	0.003 ± 0^{b}	0.003 ± 0.0^{b}	0.002 ± 0.0^{a}	0.006 ± 0.0^{e}
[8]NO_3-N (mgl^{-1})	0.304 ± 0.03^{g}	0.253 ± 0.03^{e}	0.226 ± 0.02^{d}	0.173 ± 0.01^{a}	0.339 ± 0.04^{h}	0.281 ± 0.04^{f}	0.249 ± 0.03^{e}	0.178 ± 0.01^{b}	0.199 ± 0.02^{c}
[9]TAN (mgl^{-1})	0.484 ± 0.05^{h}	0.397 ± 0.04^{f}	0.340 ± 0.03^{c}	0.260 ± 0.01^{a}	0.535 ± 0.06^{i}	0.431 ± 0.05^{g}	0.379 ± 0.04^{e}	0.283 ± 0.01^{b}	0.373 ± 0.04^{d}
[10]TAN:P	5.99 ± 0.54^{d}	5.29 ± 0.53^{b}	5.76 ± 0.74^{c}	5.20 ± 0.34^{a}	7.24 ± 0.64^{g}	6.39 ± 0.64^{e}	6.81 ± 0.83^{f}	5.86 ± 0.44^{c}	6.33 ± 0.59^{e}
[11]Soil pH	7.33–8.43	7.10–8.37	7.03–7.67	7.13–7.53	7.27–8.20	7.10–7.97	7.20–8.00	7.13–7.63	7.17–7.63
[12]SOC (mg g^{-1})	2.71 ± 0.38^{g}	2.28 ± 0.29^{e}	1.56 ± 0.16^{c}	2.17 ± 0.31^{d}	2.54 ± 0.34^{f}	2.22 ± 0.28^{d}	1.48 ± 0.16^{b}	2.17 ± 0.31^{d}	0.9 ± 0.03^{a}
[13]SAP (mg kg^{-1})	72.76 ± 5.05^{e}	69.83 ± 4.79^{d}	61.41 ± 2.40^{b}	52.25 ± 0.86^{a}	71.66 ± 4.66^{e}	68.97 ± 4.53^{d}	59.21 ± 1.76^{b}	51.90 ± 0.81^{a}	66.76 ± 4.59^{c}
[14]SAN (mg kg^{-1})	421.24 ± 36.31^{e}	365.94 ± 33.31^{d}	253.94 ± 16.15^{c}	178.58 ± 11.07^{a}	437.15 ± 40.92^{f}	375.61 ± 36.49^{d}	260.85 ± 14.25^{c}	194.62 ± 10.30^{b}	178.58 ± 11.07^{a}
[15]SAN:SAP	5.76 ± 0.32^{h}	5.19 ± 0.26^{f}	4.14 ± 0.22^{c}	3.43 ± 0.21^{a}	6.04 ± 0.41^{i}	5.40 ± 0.37^{g}	4.49 ± 0.23^{d}	3.75 ± 0.18^{b}	4.59 ± 0.27^{e}

Note: Means superscripted with different letters in each row are significantly different ($P < 0.05$).

Abbreviation:[1]TDS (mg l^{-1}),Total dissolved solids; [2]DO (mg l^{-1}), Dissolved oxygen; [3]TA (mg l^{-1}), Total alkalinity; [4]TH (mg l^{-1}), Total hardness; [5]Ortho-P (mg l^{-1}), Ortho-phosphorous; [6]NH_3-N (mg l^{-1}), Ammonia-nitrogen; [7]NO_2-N (mg l^{-1}), Nitrite −nitrogen; [8]NO_3-N (mg l^{-1}), [9]Nitrate-nitrogen, [10]TAN (mg l^{-1}), Total available nitrogen; [11]TAN:P, ratio of Total available nitrogen and phosphorous; [12]SOC (mg g^{-1}), Soil organic carbon; [13]SAP (mg kg^{-1}), Soil available phosphorous; [11]SAN (mg kg^{-1}), Soil available nitrogen; [15]SAN:SAP, Ratio of Soil available nitrogen and Soil available phosphorous.

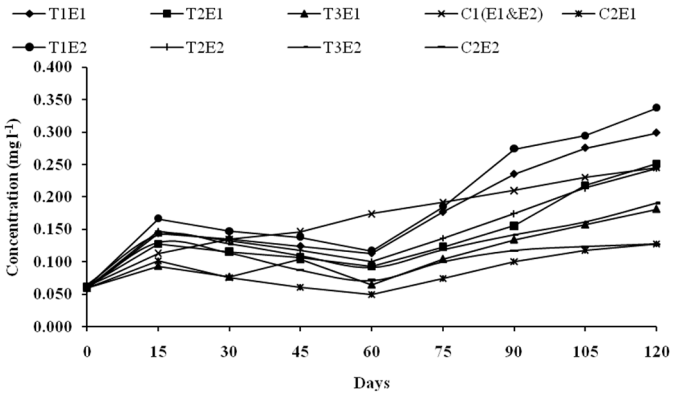

Fig. 2. Temporal changes in NH_3-N of water in all the treatments of E1 and E2.

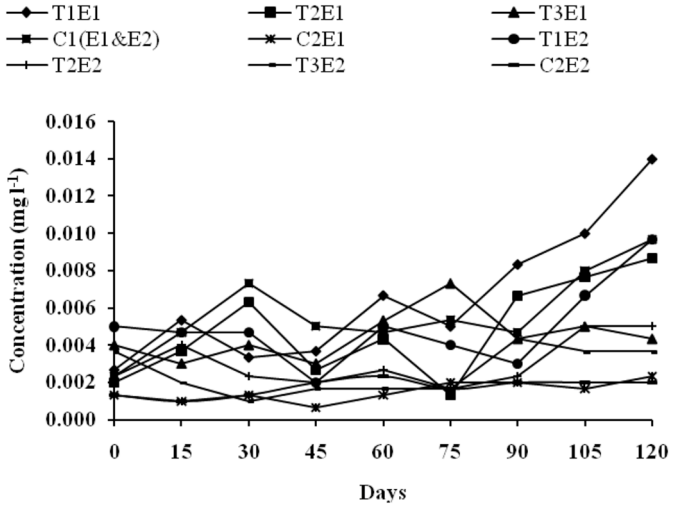

Fig. 3. Temporal changes in NO_2-N of water inall the treatments of E1 and E2.

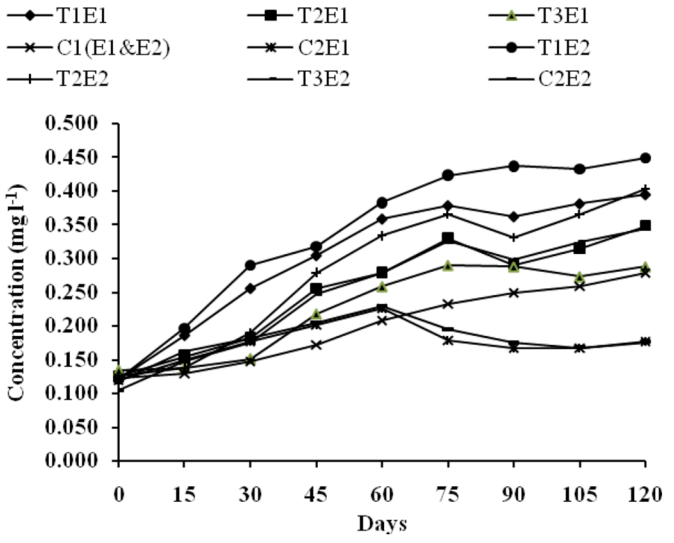

Fig. 4. Temporal changes in NO_3-N ofwaterin all the treatments of E1 and E2.

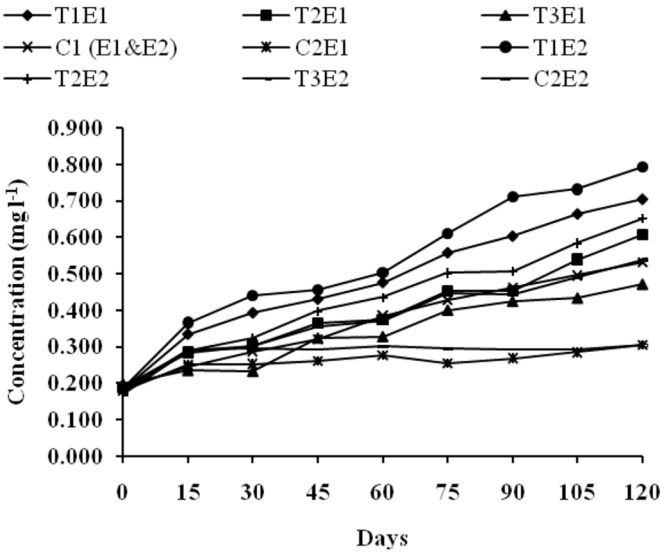

Fig. 5. Temporal changes in TAN of water inall the treatment groups in E1 and E2.

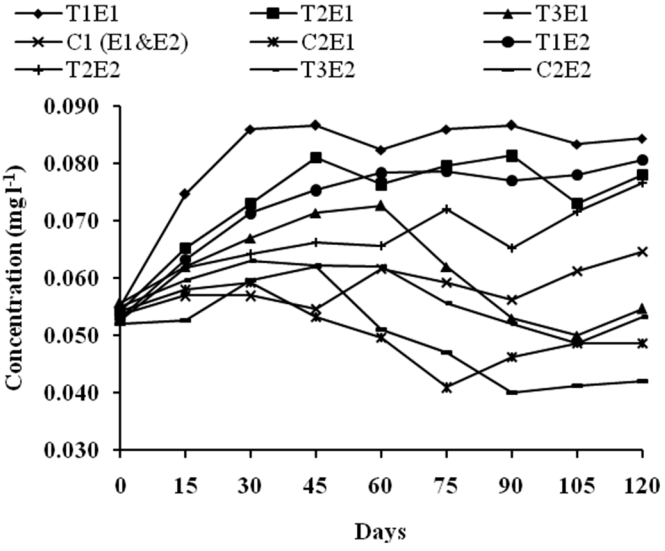

Fig. 6. Temporal changes in ortho-P ofwaterin all the treatments of E1 and E2.

4 Discussion

As the absolute weight gain of the test carp over the initial weight was 2.79 and 5.17 times in only biofloc supported treatment without feed supplementation (T3E2 and T3E1), it was indicative of the potential of biofloc in supporting *Cyprinus carpio* L. However, it was higher respectively by 1.46 and 1.18 times where feed was applied at 6% (T1E1 and T1E2) and 3% (T2E1 and T2E2) of body weight daily compared to only neem supplemented biofloc applied treatment (T3E2) against 1.17 and 1.24 times in without neem supplemented treatments (T3E1). However, biofloc alone could not be sufficient in supporting growth and survival of fish in either of the media with or without neem as the

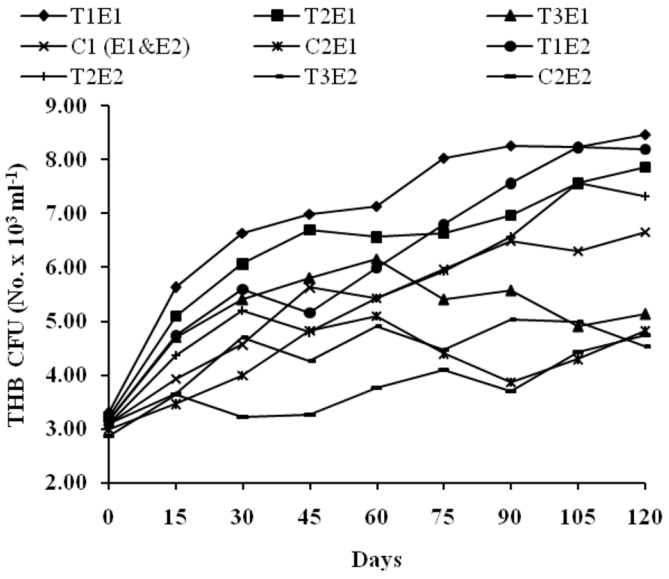

Fig. 7. Temporal changes of THB in water inthe treatments of E1 and E2.

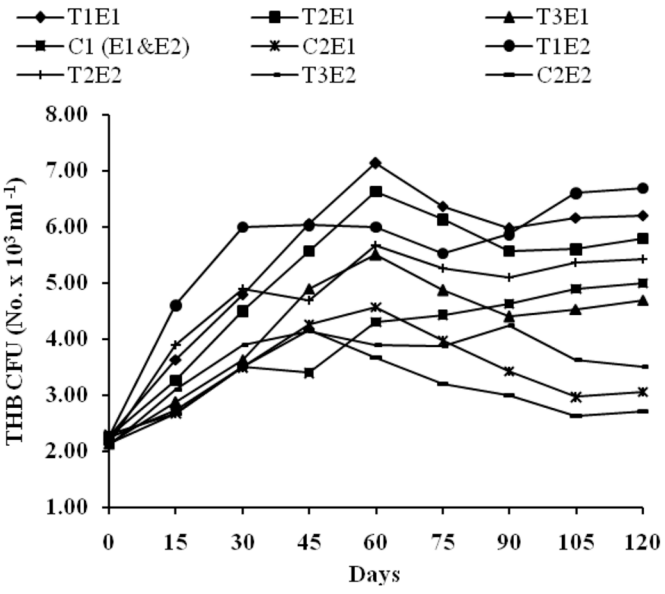

Fig. 8. Temporal changes of THB in soil in the treatments of E1 and E2.

Table 7. Microbial populations in the both experiments E1 and E2.

		Mean ± Standard Error [CFU (No × 10^3 ml^{-1})]								
System		T1E1	T2E1	T3E1	C2E1	T1E2	T2E2	T2E2	C2E2	C1
Water	[1]THB	6.97 ± 0.55^{i}	6.30 ± 0.46^{g}	5.14 ± 0.28^{d}	4.20 ± 0.22^{b}	6.16 ± 0.56^{g}	5.59 ± 0.47^{f}	4.41 ± 0.21^{c}	3.75 ± 0.41^{a}	5.34 ± 0.19^{e}
	[2]AB	4.44 ± 0.48^{f}	3.66 ± 0.39^{e}	2.99 ± 0.27^{c}	2.37 ± 0.23^{b}	3.93 ± 0.45^{e}	3.27 ± 0.43^{d}	2.40 ± 0.24^{b}	1.88 ± 0.15^{a}	3.01 ± 0.28^{c}
	[3]AOB	5.67 ± 0.56^{h}	4.73 ± 0.41^{g}	3.93 ± 0.32^{e}	3.05 ± 0.15^{b}	4.32 ± 0.55^{f}	3.69 ± 0.55^{d}	3.17 ± 0.34^{b}	2.21 ± 0.14^{a}	3.36 ± 0.21^{c}
	[4]NFB	5.31 ± 0.55^{f}	4.17 ± 0.37^{e}	3.57 ± 0.32^{d}	2.65 ± 0.65^{b}	3.85 ± 0.36^{d}	3.23 ± 0.32^{c}	2.91 ± 0.28^{b}	2.32 ± 0.23^{a}	3.72 ± 0.23^{d}
	[5]DNB	5.37 ± 0.47^{f}	4.59 ± 0.38^{e}	3.90 ± 0.28^{d}	3.34 ± 0.26^{c}	4.10 ± 0.40^{d}	3.36 ± 0.31^{c}	3.00 ± 0.22^{b}	2.71 ± 0.08^{a}	4.05 ± 0.35^{d}
	[6]CDB	5.81 ± 0.56^{g}	5.17 ± 0.49^{f}	4.15 ± 0.26^{d}	3.27 ± 0.17^{a}	5.34 ± 0.46^{f}	4.72 ± 0.42^{e}	3.99 ± 0.33^{c}	3.43 ± 3.43^{b}	3.98 ± 0.32^{c}
	[7]PSB	4.88 ± 0.55^{g}	4.28 ± 0.43^{f}	3.54 ± 0.31^{d}	2.49 ± 0.19^{a}	4.11 ± 0.43^{e}	3.61 ± 0.39^{d}	2.90 ± 0.25^{b}	2.43 ± 0.26^{a}	3.36 ± 0.36^{c}
Soil	[1]THB	7.45 ± 0.65^{g}	6.89 ± 0.57^{f}	5.57 ± 0.39^{c}	4.43 ± 4.43^{b}	6.21 ± 0.76^{e}	5.65 ± 0.72^{c}	4.43 ± 0.40^{b}	3.90 ± 0.24^{a}	5.88 ± 0.51^{d}
	[2]AB	4.99 ± 0.35^{h}	4.46 ± 0.26^{f}	3.87 ± 0.19^{c}	3.30 ± 0.19^{a}	4.59 ± 0.29^{g}	4.23 ± 0.21^{e}	3.69 ± 0.13^{b}	3.25 ± 0.16^{a}	4.03 ± 0.21^{d}
	[3]AOB	6.27 ± 0.45^{h}	5.04 ± 0.29^{f}	4.28 ± 0.16^{d}	3.39 ± 0.15^{b}	5.46 ± 0.38^{g}	4.69 ± 0.29^{e}	3.55 ± 0.13^{c}	3.06 ± 0.20^{a}	4.24 ± 0.19^{d}
	[4]NFB	6.23 ± 0.46^{h}	5.40 ± 0.34^{g}	4.26 ± 0.21^{d}	3.73 ± 0.19^{c}	4.88 ± 0.35^{f}	4.16 ± 0.22^{d}	3.48 ± 0.08^{b}	3.15 ± 0.11^{a}	4.30 ± 0.21^{e}
	[5]DNB	6.13 ± 0.46^{g}	5.24 ± 0.33^{f}	4.08 ± 0.24^{e}	3.57 ± 0.24^{c}	4.64 ± 0.34^{e}	3.97 ± 0.25^{d}	3.31 ± 0.15^{b}	2.93 ± 0.09^{a}	4.13 ± 0.17^{e}
	[6]CDB	5.84 ± 0.55^{h}	5.24 ± 0.43^{f}	4.26 ± 0.21^{d}	3.31 ± 0.12^{a}	5.52 ± 0.42^{g}	4.90 ± 0.31^{e}	3.90 ± 0.18^{c}	3.50 ± 0.19^{b}	3.94 ± 0.24^{c}
	[7]PSB	5.40 ± 0.52^{h}	5.04 ± 0.47^{g}	4.17 ± 0.36^{e}	3.41 ± 0.25^{b}	5.51 ± 0.45^{i}	4.72 ± 0.37^{f}	3.60 ± 0.22^{c}	3.07 ± 0.21^{a}	3.90 ± 0.32^{d}

Note: Means superscripted with different letters in each row are significantly different ($P < 0.05$).
Abbreviation: [1]THB [CFU (No × 10^3 ml^{-1})], Total heterotrophic bacteria; [2]AB [CFU (No × 10^3 ml^{-1})], Ammonifying bacteria; [3]AOB [CFU (No × 10^3 ml^{-1})], Ammonia oxidizing bacteria; [4]NFB [CFU (No × 10^3 ml^{-1})], Nitrogen fixing bacteria; [5]DNB [CFU (No × 10^3 ml^{-1})], Denitrifying bacteria; [6]CDB [CFU (No × 10^3 ml^{-1})], Cellulose decomposing bacteria; [7]PSB [CFU (No × 10^3 ml^{-1})], Phosphate solubilizing bacteria).

mortality rate was substantial (22.22 to 33.33%) in the latter, which may be due to not application of biofloc in C2E1 and C2E2 in after first 45 days of total experiment period. The findings are in agreement with Dinda et al. (2019) who reported biofloc alone is insufficient to guarantee the level of growth and survival required for high-density fish farming.

A comparison of the treatments T1E1, T1E2, and C1 on 6% feeding level with and without BFT respectively revealed that BFT provided 18.54% and 7.26% more growth in T1E1 and T1E2 as compared to C1 in presence and absence of neem leave extract, suggesting that BFT can be quite efficient in increasing growth and productivity in common carp. The findings are in conformity with the results of earlier studies (Pérez-Fuentes et al., 2013; Kamilya et al., 2017; Dinda et al., 2019). As body weight, absolute weight gain and specific growth rate differed significantly ($P < 0.05$) among the treatments with insignificant ($P > 0.05$) difference between with (E2) and without neem extract (E1) biofloc without feed

(T3E2 and T3E1, respectively), there was no effect of neem extract in the biofloc medium in contributing fish growth. However, insignificant difference between T1E1 and T2E1 and, T1E1 and T2E2 established that presence of neem extract under feeding regime supported 50% feed reduction (T2E1 and T2E2) without any significant decrease in the fish growth. Therefore, neem supplementation in BFT with feed supplementation appeared to perform better in terms of weight gain and reduction of supplemental feed cost.

However, FCR was significantly higher by 2.16 and 2.05 times in T1E1 and T1E2 compared to T2E1 and T2E2 (Tab. 5). Therefore, under any of the biofloc treatments, production cost towards feed could be reduced at least by 50% without significant decrease in fish growth. Moreover, under identical feeding regime (6% body weight day^{-1}), compared to C1, FCR was slightly improved by 2.66% and 5.01% in both T1E1 and T1E2. Though, FCR significantly differed among the treatments ($P < 0.05$), presence (E2) or absence of neem extract (E1) under 50% reduction of feed in T2E1 and T2E2 had no significant impact on FCR ($P > 0.05$). Moreover, neem extract in biofloc under normal feeding rate (T1E1 and T1E2) did not exert any impact on PER as difference with C1 was insignificant ($P > 0.05$); though under reduced feeding rate, such comparison was significant ($P < 0.05$). Therefore, *in-situ* biofloc has substantial protein sparing capacity which could be able to substantially scale down the rate of costly feed application (Xu et al., 2012).

Reduction of pH with either of the biofloc treatments in comparison to the control was due to inorganic carbon consumption by autotrophic and heterotrophic bacteria that form the microbial biomass (Pérez-Fuentes et al., 2016; Martins et al., 2017). Martínez et al. (2014) reported that organic loading resulted in decline of pH in water. Such reduction in water pH resulted in a decline of alkalinity during the corresponding phase as they were positively correlated ($r = 0.71$ and 0.89). Moreover, because of organic loading during the first half, BOD_1 remained high and it declined after the biofloc application stopped after 45 days. Several studies (Das and Jana, 2003; Schryver et al., 2008; Martínez et al., 2014) also recorded such situation where organic loading had a direct bearing upon the BOD load of the system. Initially, total hardness of water was fairly high (624–655 mg l^{-1}), however, as the culture systems matured, there were drastic falls of such hardness and became congenial (240–350 mgl^{-1}) for fish culture after one month of investigation. Total dissolved solids and conductivity of water in either of the biofloc system increased over time because of the enrichment of organic loading and nutrients in the medium compared to the control treatment. This has been corroborated with the fact that concentration of any of the inorganic nutrients also increased simultaneously in those treatments. Wu and Chou (2003) also reported a direct relationship between nutrient loading and conductivity of water.

Significant increase in all the nutrients (TAN, NH_3-N, NO_3-N and ortho-P) in the treatments where feed was applied irrespective of the types of biofloc was perhaps because of the metabolic wastes and decomposition of the uneaten feed and dead materials. As the applied feed contained 25% crude protein, substantial quantity of ammonia might have been resulted in because of the de-amination of amino acids in energy metabolism. Adhikari et al. (2014) have demonstrated that supplementary feed acted as a source of both nitrogen and phosphorus in fish culture systems. Inorganic nitrogen concentration of water in BFT treatments with neem were comparatively higher compared with those without neem, indicating that supplemental nitrogen input could be the case through the boiled neem leaves in the medium (Vinaygam et al., 2006). BFT application also proved to be efficient in increasing the mineralization potential of the systems as evidenced from the more rate and potential of transformation of any of the inorganic forms of nitrogen in BFT treatments. However, neem supplementation in BFT treatments displayed negative impacts upon nitrogen mineralizing microbes as evidenced from the significantly less population size compared to the treatments without neem and treatment differences were significant ($P < 0.05$). However, positive relationship was established in the nutrient status between any of the biofloc supplemented treatment groups and C1. The relationship between total inorganic nitrogen (TIN) and N:P of water was established with a strong positive relationship in any of the bioflocs identical to C1 where fish were reared with supplementary feed in absence of biofloc ($R^2 = 0.985$ for T1E1; $R^2 = 0.962$ for T2E1; $R^2 = 0.985$ for T3E1; $R^2 = 0.945$ for C2E1; $R^2 = 0.967$ for T1E2; $R^2 = 0.968$ for T2E2; $R^2 = 0.963$ for T3E2 and $R^2 = 0.957$ for C2E2).

As expected soil organic carbon increased substantially in biofloc supported systems particularly feed was appliedsource of carbon. Such increase in organic pool in the sediment might have supported the phosphate mineralizing microbial community and resulted in a strong positive relationship between organic carbon and available phosphorus in soil of E1 and E2 ($R^2 = 0.677$ for T1E1; $R^2 = 0.640$ for T2E1; $R^2 = 0.709$ for T1E2 and $R^2 = 0.743$ for T2E2). However, such relationship was also established in C1 with 6% feed application in absence of biofloc with much higher degree of significance (87%).

Higher abundance of aerobic heterotrophic bacterial population in all the treatment groups of both BFT (E1 and E2) confirmed that external source of carbon enhanced such population through amelioration of C:N ratio which in turn supported higher production of biofloc and microbial protein as well. Compared to neem supplemented biofloc system, higher abundance of THB in biofloc medium without neem validated the antimicrobial properties of neem leaves in biofloc medium might have inhibited nitrification which resulted in low N:P ratio. This in accordance with the earlier findings of Das (2008), who reported a strong nitrifying inhibition of neem extract in aquatic productions systems. However, in general, strong positive relationships were established between total available-N (TAN) and TAN:P of water and SAN and SAN : SAP in soil maintained strong positive relationships ($R^2 = 0.77$–0.97) (Fig. 9). The increasing trend of N:P ratio in both BFT systems supported nitrogen and phosphorus mineralization, being in conformity with earlier findings of Ahmad et al., (2017).

5 Conclusion

The findings of the study strongly indicated the potential of BFT in reduction of dependence on compound feed in common carp. The neem (*Azadirachta indica*) supplementation in BFT improved the ecological health of the culture system with

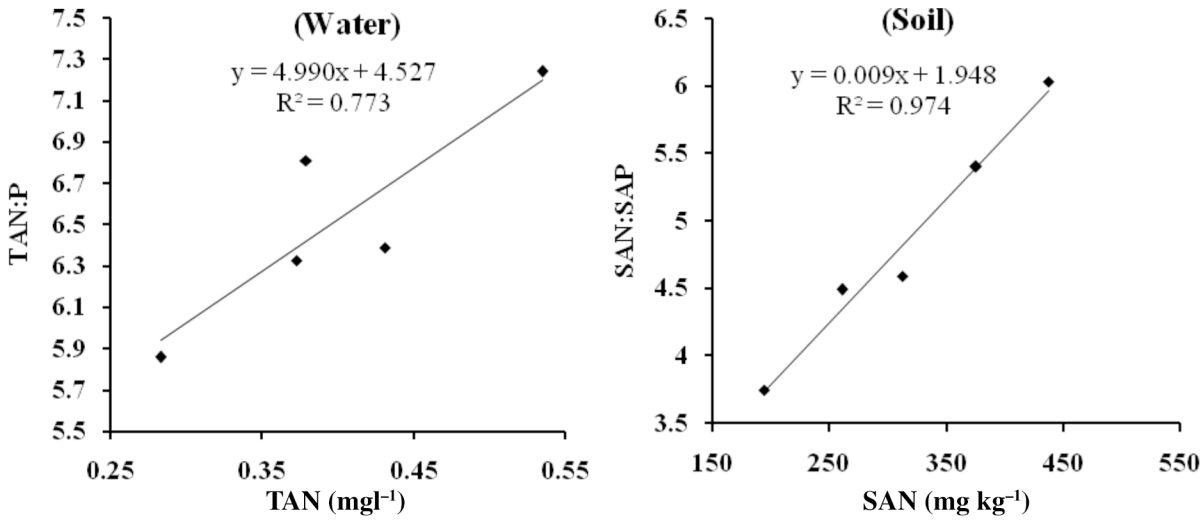

Fig. 9. Linear relationship between TAN *vs* TAN:P ratio in water and SAN *vs* SAN:SAP ratio in soil in neem supplemented biofloc (E2) system.

regards to physico-chemical and nutritional properties of water and soil. Besides, the present study concluded that heterotrophic potential within the systems with biofloc application with or without neem supplementation has been augmented excepting nitrogen mineralizers in some cases. However, application of neem leave extract in preparation of biofloc media should be judicious as it can limit nitrification.

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