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Biointegration of Rohu (*Labeo rohita*) Fry and Lemon Grass (*Cymbopogon citratus*) in a Recirculating Aquaponic System

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A 75-day experiment was conducted to optimize fish and plant component ratio in a recirculating aquaponic system. Three treatments having different component ratio of rohu (*Labeo rohita*) fry to lemon grass (*Cymbopogon citratus*) *viz.* T_1 (3:1), T_2 (4.5:1) and T_3 (6:1) were assigned as treatments. All the treatments were compared for fish growth, plant growth, survival of fish, and various water quality parameters were observed to determine the best component ratio which maintains better water quality and gives better growth. The water quality parameters like temperature, pH, dissolved oxygen, total alkalinity, and total hardness were found to be within the acceptable limits for fish as well as plant growth. While total ammonia nitrogen (TAN) varied in the range of 0.09 to 0.17 mg L^{-1}, the nitrite and nitrate values ranged from 0.06 to 0.08 mg L^{-1} and 2.74 to 5.30 mg L^{-1} respectively. Different component ratio showed significant effect on fish growth in terms of length and weight gain, survival rate, specific growth rate (SGR). Among the treatments, the higher growth rate of fish was observed in T_1 (2.54±0.01 g) followed by T_2 (2.25±0.03 g) and T_3 (2.10±0.02 g) but on comparing the total fish biomass produced in the system, the treatment T_3 yielded maximum production as the fish biomass was the highest in T_3 (121.08 g) followed by T_2 (96.18 g) and T_1 (73.81 g). The highest plant growth was achieved in T_3 (19.94±0.10 g) followed by T_2 (19.35±0.07 g) and T_1 (19.04±0.12). Therefore, considering total fish biomass production and plant growth together in the system, it can be concluded that the component ratio of 6:1 (T_3) can be recommended for culturing rohu fry and lemon grass in recirculating aquaponic system.

(***Key words:*** *Aquaponic system, Component ratio, Lemon grass, Rohu)*

Two most crucial determinants for sustainable aquaculture in future are the availability of quality water and land. About 2.41 million hectares of water bodies are available for freshwater aquaculture in India. But, day by day, these resources are depleting due to agriculture uptake resulting into the scarcity of water (NFDB, 2009). In addition to this, aquaculture practices are utilising huge quantity of water resources and also polluting the natural aquatic ecosystems by discharging the aquaculture wastes like feed derived wastes mainly from intensive aquaculture system; and thus, increase the conflict with other stake holders. Therefore, the aquaponics could be one of the solutions to save both water and land. Aquaponics is the bio-integration of recirculating aquaculture with hydroponics in one production system (Endut *et al*., 2010; Rakocy *et al*., 2006; Thomas *et al*., 2019). It is based on natural biological processes such as nitrification and phytoremediation (Fronte *et al*., 2016; Nuwansi *et al*., 2019). The balance between nutrient generation and removal reduces the need for water quality monitoring. Nutrients which are released by the fish through excretion or microbial breakdown of organic waste mainly from the feed waste consists of about 70-80% nitrogen (N) (Krom *et al*., 1995) are absorbed by plants as nutrients and moisture for their growth and helping in purification of water in which fish lives. Microbial process keeps both the fish and plants healthy. This creates a sustainable ecosystem where both fish and plants can thrive using less water and space producing no wastewater and pollutants compared to conventional methods (Angkha *et al*., 2019; Harry *et al*., 2014). Endut *et al*. (2010) stated that the optimum conditions like HLR, plants to fish ratio, oxygen levels and water temperature are vital to maximize fish and plant production, nutrient recovery, and minimize the water exchange in RAS system.

Inorganic nitrogen in the form of ammonium (NH_4^+) is the major nutrient taken up by the roots of higher plants (Vaillant *et al*., 2004). So, there is less reliance on nitrification for ammonia removal when sufficient plants

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are present in the aquaponic systems. The fish-to-plant component ratio is an important aspect for aquaponics and a proper ratio creates a balanced production system through nutrient uptake and assimilation into plant biomass (Nuwansi *et al*., 2017; Shete *et al*., 2015). The rate of change in nutrient concentration can be influenced by varying the component ratio of plants to fish (Rakocy *et al*., 2006). To improve the effectiveness of the aquaponic system, it must be sized correctly in order to maintain optimum balance between nutrient production from fish culture and nutrient uptake by the plant component. Insufficient amount of nutrients from fish will result in slow growth of plants. Large plant growing area may improve water quality but will also lead to slower plant growth rate and reduced production of plant crops (Buzby and Lin, 2014). Therefore, maintaining proper fish and plant ratio is important in aquaponics.

Indian fish farming mainly depends on carps which contribute about 85% of the total fish production (Laxmappa, 2014). Among the Indian major carps, rohu (*Labeo rohita*) is one of the most preferred species and commands a higher price in the market. Lemon grass is (*Cymbopogon citratus*) widely used as a culinary herb in Asian cuisines and also as medicinal herb in India. It has a subtle citrus flavour and can be dried and powdered or used fresh. It is commonly used in teas, soups, and curries. Lemon grass oil is used as a pesticide and a preservative. Research shows that lemongrass oil has antifungal properties (Tzortzakis and Economakis, 2007). Appendini and Hotchkiss (2002) stated that lemongrass also has antimicrobial property.

The objective of this study was to determine the optimum fish and plant component ratio for efficient working of an aquaponic system in terms of fish yield, plant production, and nutrient removal with rohu as fish species and lemon grass as plant species in gravel bed hydroponic system.

MATERIALS AND METHODS

The aquaponic recirculating system was designed and setup in the wet laboratory at ICAR-CIFE, Powerkheda Centre. The system consisted of 12 individual, identical aquaponic units allowing replication of experimental treatments. Each aquaponic recirculating system consisted of a fish tank, 1 hydroponic tank filled with gravel, a submersible pump with pipe and fittings for pumping water, etc. Pipelines made up of polyvinyl chloride were installed to connect the fish culture tank and hydroponic tanks to recirculate the water (Fig. 1). Circular tubs of 65 L capacity each were selected for fish rearing. For experimental purpose, the water volume maintained in the tanks was 60 L. A submersible water pump (Sobo WP1650 1500 L h^{-1} at 1.5 m head) was installed in the fish tank for water circulation. PVC pipes having 15 mm diameter were used for circulating the water in the entire system. Halves of a HDPE barrel each having size of 91 X 57 cm with a depth of 29.4 cm were used for hydroponics tank. Hydroponic tanks were filled with the gravel of size ranging from 5 to 15 mm. Water from the fish tank to the hydroponics unit was regulated using a submersible pump and water from the hydroponic tank returned to the fish tank by gravity with the help of 15 mm diameter PVC drain pipe.

L. rohita fry were collected from earthen nursery pond located at the experimental site. After collecting, fish were given bath treatment with 5 ppm $KMnO_4$. Dead and stressed fish were discarded. Bath treated fish were transferred to round fish rearing tanks provided with continuous aeration and acclimatized to the experimental site condition for 1 day by feeding solely plankton in addition to the artificial pelleted feed containing 32% protein as per satiation of fish.

The experimental design consisted of three treatments with each treatment having three randomly assigned replications. Different fish to plant component ratios of 3:1, 4.5:1, 6:1 were assigned as three treatments: T_1 (30 fishes), T_2 (45 fishes), and T_3 (60 fish), respectively. Control (C, without plants) was stocked with 30 fishes in 60 l plastic tub with three replicates.

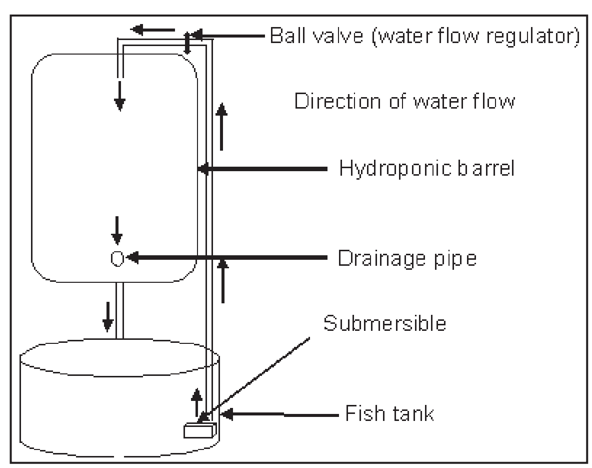

Fig. 1. *Schematic diagram of experimental aquaponic system*

The weight and length of rohu fry at stocking was 0.16 g and 1.7 cm, respectively. Plants were transplanted to experimental grow-bed after cutting up to 20 cm length at a stocking density of 28 plants m^{-2}. 10 nos. of lemon grass were planted in each treatment.

The spawn were provided with commercial feed (32% protein) at 6 to 10% of body weight twice a day (10.00 am and 5.00 pm). Sampling of fish was carried out at 15 days interval for assessment of growth (weight) and health. The daily feed ration was adjusted accordingly. The length of fish was measured with the help of a graduated ruler while the weight was recorded with the help of an electronic balance. Growth parameters *viz*., specific growth rate (SGR), feed conversion ratio (FCR), feed efficiency ratio (FER), and protein efficiency ratio (PER) were estimated. Sampling of plants was done at the end of experiment by measuring the height and weight of plants. Water quality parameters *viz.*, temperature, pH, dissolved oxygen, total hardness, total alkalinity, ammonia (NH_4^+), nitrite, nitrate were analysed on weekly basis using the standard methods (APHA, 2005).

The data were subjected to analysis using statistical package SPSS version 16 in which one-way ANOVA and Duncan's multiple range test were performed at significance level of 95% ($p<0.05$) confidence limit to know the significant difference between the treatments for different parameters.

RESULTS AND DISCUSSION

Water quality parameters

The water quality parameters observed during the experimental period are presented in Table 1. Temperature of water during the study period was found to be in acceptable limits to *Labeo rohita* and lemon grass (23.64 to 23.86°C), and pH of the water was in the range of 7.91 to 8.13 which is optimum for fish, plant, and growth of the nitrifying bacteria. The optimum range of pH for nitrification is 7.0 to 9.0 (Chen *et al*., 2006). Dissolved oxygen was maintained between 6.47 to 6.60 mg L^{-1} which is optimum for fish growth, plant growth, and biofilter performance. Dissolved oxygen levels should be maintained above 5 mg L^{-1} for optimum growth of warm water fish (Masser *et al*., 1999). Rakocy *et al*. (2006) and Shete *et al*. (2017) stressed the importance of maintaining high DO levels in aquaponics for the health of root. Goto *et al*. (1996) reported that low DO concentration may interrupt the nitrification cycle and insufficient nitrate can directly impact the plant growth. Total alkalinity and total hardness levels were found to be within the acceptable limits for fish as well as plants culture. The alkalinity values ranged from 244.18 to

Table 1. *Water quality parameters observed during experimental period*

Parameters	C	T_1	T_2	T_3
Temperature (°C)	23.64±0.63	23.82±0.57	23.86±0.60	23.86±0.56
pH	8.13±0.01	7.91±0.09	7.99±0.07	7.95±0.12
DO (mg L^{-1})	6.60±0.07	6.49±0.03	6.51±0.10	6.47±0.06
Total alkalinity (mg L^{-1})	254.26±3.25	244.18±6.47	245.52±5.85	248.26±4.96
Total hardness (mg L^{-1})	251.06±1.16a	257.62±2.40ab	259.74±3.00b	261.72±3.15b
Ammonia (NH_4^+) (mg L^{-1})	0.17±0.02b	0.09±0.02 a	0.12±0.02ba	0.13±0.02ba
Nitrite (mg L^{-1})	0.08±0.01	0.06±0.01	0.06±0.01	0.07±0.01
Nitrate (mg L^{-1})	5.30±0.94b	2.74±0.43a	3.38±0.56ba	4.25±0.68ba

Mean values with same superscript did not show any significant difference ($p>0.05$)

254.26 mg L^{-1}. Suitable alkalinity range for aquaculture is 20 to 300 mg L^{-1} (Buttner *et al*., 1993). Total hardness of the water was found to be comparatively lower in the control group than the treatment groups; however, all the values were within the desired levels for the culture of rohu. Hardness values ranged from 251.06 to 261.72 mg L^{-1}. In most waters, bicarbonate and carbonate ions are principal buffers and act as buffering agents against sudden pH fluctuations. Ammonia was significantly higher in the control than the treatments. High levels of ammonia in control were also observed by Hussain *et al*. (2015) and Nuwansi *et al*. (2016) in aquaponic system. Among the treatments, T_3 showed comparatively higher ammonia level than T_2 and T_1. Hussain *et al*. (2014) also observed higher ammonia accumulation when fish were stocked at high density in an aquaponic system. Total ammonia nitrogen (TAN) varied in the range of 0.09 to 0.17 mg L^{-1} throughout the experiment period. The suggested value of ammonia in a recirculating aquaculture system is less than 1.00 mg L^{-1} (Nijhof and Bovendeur, 1990; van Rijn and Rivera, 1990). Even after daily water exchange in the control tanks, TAN values were higher than that of the treatment tanks which shows the filtration efficiency of the aquaponic system. Seawright *et al*. (1998) found that the ammonia production and subsequent nitrification increased with higher stocking densities. Nitrite values were ranged from 0.06 to 0.08 mg L^{-1}. Nitrate values ranged from 2.74 to 5.30 mg L^{-1} including in the control. Ammonia and nitrite-N are toxic to fish but nitrate is relatively harmless and is the preferred form of nitrogen for growing higher plants (Rakocy *et al*., 2006). Verhagen *et al*. (1995) reported that plant roots are more competitive for ammonium than the oxidizing bacterial species (*Nitrosomonas europaea*).

Fish growth parameters

Maintenance of different fish to plant component ratio showed different growth rates in rohu (Table 2). Total weight and length gain in rohu was higher in control. Among the treatments, T_1 with 3:1 ratio showed higher weight gain and length gain as compared to other treatments.

The lowest growth of fish observed in treatment T_3 (6:1) could be the result of more number of fish per system which led to less space for movement and growth. Influence of stocking density on growth as reported earlier in the rearing system was also observed in the present study as the growth decreased with increasing densities, obviously, it is due to the increased competition for living space and food. Growth of fish can be affected by violating behaviour requirements for space through endocrine responses or disruption to feeding efficiency (Pankhurst and Vander Kraak, 1997).

Food given was 10% of the body weight during the first month and it was gradually reduced to 6% following the protocol reported by Jena *et al*. (2005). The lowest FCR was recorded in T_1 and C, and increased FCR in T_2 and T_3. The reason for this increment could be the food which was more efficiently utilized in the lower stocking density as indicated by a significantly lower ($p<0.05$) value of FCR as compared to the high stocking density. The same trend was also reported by Sharma

Table 2. *Fish growth parameters observed during the experimental period*

Parameters	C	T_1	T_2	T_3
Initial weight (g)	0.15±0.00a	0.16±0.00a	0.16±0.00a	0.16±0.00a
Final weight (g)	2.57±0.00c	2.54±0.01c	2.25±0.03b	2.10±0.02a
Initial length (cm)	1.72±0.00a	1.72±0.00a	1.71±0.00a	1.72±0.00a
Final length (cm)	5.43±0.00c	5.41±0.01c	5.36±0.01b	5.20±0.01a
Survival (%)	97.77±1.01a	96.66±1.09a	94.81±0.07a	96.11±1.05a
SGR (% day^{-1})	3.39±0.02c	3.31±0.02b	3.21±0.01a	3.15±0.02a
FCR	2.17±0.00a	2.15±0.01a	2.31±0.03b	2.41±0.01c
FER	0.46±0.00c	0.46±0.00c	0.43±0.01b	0.41±0.00a
PER	1.43±0.00c	1.45±0.01c	1.35±0.02b	1.29±0.00a
Final biomass (g)	75.42±0.81a	73.81±1.31a	96.18±1.74b	121.08±5.74c

Mean values with same superscript did not show any significant difference (p>0.05) and Chakrabarti (2003) during rearing of rohu and catla spawn in RAS system. Higher stocking densities usually result in higher feed conversion ratios due to the reduced feed intake and decreased efficiency of feed conversion. The same trend was observed in total weight gain, length gain, SGR, FER, and PER which were higher in low stocking density of fish than in higher stocking density as also reported by Shelton *et al*. (1981). Increasing stocking density had a profound negative impact on the growth of grass carp, *Ctenopharyngodon idella*, in small impoundments. SGR values are comparable with the results of Samad *et al*. (2017) who studied the density based fingerling production of rohu in pond. High survival rates observed during the experiment ranged from 94.81 to 97.77%. High survival was also reported by Hussain *et al*. (2014) and Shete *et al*. (2013a, 2013b and 2016) in aquaponic system. Similarly, high survival (80 to 93%) of rohu larvae was also observed in RAS system by Sharma and Chakrabarti (2003).

Plant growth parameters

Initial and final growth parameters of the plant (lemon grass) are presented in Table 3. When fish are cultured, only a small proportion (25-30%) of the feed is converted to useable energy (Rakocy *et al*., 1993). The balance of nutrients is excreted in solid and dissolved fractions. Dissolved nutrients accumulate in the recirculation system with low water exchange and high feeding rates to levels which approximate hydroponic nutrient solutions (Endut *et al*., 2010). Higher stocking density of fish caused high accumulation of excrements and metabolic wastes from the fish and led to significantly higher concentration of nitrogen compounds (Jha and Barat, 2005). These dissolved nutrients are then recovered by the plants. Also, the daily application of fish feed provides continuous supply of nutrients to plants. According to Rakocy *et al*. (2004), aquaculture effluent provides most of the nutrients required by plants if the optimum ratio between daily feed input and plant growing area is maintained. Lennard and Leonard (2006) reported 90.9% removal of nitrate using gravel bed as media for lettuce production. Therefore, gravel bed hydroponic sub-system was used during the aquaponic study for growing lemon grass. Plant growth is an important criteria to correlate the suitability or efficiency of aquaponic recirculation system. Fish growth and plant growth were affected by different fish: plant component ratios. In terms of plant growth, T_3 (6:1) showed better performance than 3:1 and 4.5:1 ratio. Lower growth performance of plant in 3:1 ratio could be due to less nutrients. Higher plant growth was observed in T_3 which was stocked with more number of fish. Hussain *et al*. (2014) reported that the plant production increased with increase in fish ratio due to increase in availability of nutrients.

In the present study, the highest plant growth was achieved in T_3 (19.94±0.10 g) followed by T_2 (19.35±0.07 g) and T_1 (19.04±0.12), and the higher growth rate of fish was observed in T_1 (2.54±0.01 g) compared to T_2 (2.25±0.03 g) and T_3 (2.10±0.02 g). However, the total fish biomass production was the highest in T_3 (121.08 g) followed by T_2 (96.18 g) and T_1 (73.81 g). Hence, considering the growth of fish and total biomass production in the system, the component ratio of 6:1 (T_3) can be recommended for culturing rohu fry and lemon grass in recirculating aquaponic system. Results showed that as the growth of plants was increased, the growth of the fish was decreased significantly ($p>0.05$) when the density of fish increased. The survival percentage of the fish decreased with increasing density of fish. However, further studies with increased density of fish may be taken up for optimization of the fish (rohu) and plant (lemon grass) component ratio in recirculating aquaponic system. In an integrated system, production performance of all the crops should be considered at optimum levels. The optimum component ratio can be determined by a compromise between fish and plant

Table 3. *Initial and final plant (lemon grass) growth parameters*

Parameters	T_1	T_2	T_3
Initial weight (g)	5.47±0.07^{a}	5.59±0.04^{a}	5.43±0.11^{a}
Final weight (g)	19.04±0.12^{a}	19.35±0.07^{b}	19.94±0.10^{c}
Initial length (cm)	20±0.00^{a}	20±0.00^{a}	20±0.00^{a}
Final length (cm)	73.08±0.29^{a}	75.26±0.43^{b}	78.59±0.32^{c}

Mean values with same superscript did not show any significant difference ($p>0.05$)

productions and water quality maintenance. In the present bio-integrated aquaponic system of rohu fry and lemon grass culture, best performance of both the fish and plant was found in T_3 (6:1) component ratio. Thus, the component ratio of 6:1 (fish: plant) can be recommended for culturing rohu fry and lemon grass in aquaponics.

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