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OPTIMIZING THE PLANTING DENSITY OF LETTUCE (*Lactuca sativa*) WITH TILAPIA (*Oreochromis niloticus*) IN A RECIRCULATION AQUAPONIC SYSTEM

A.Q.M. Robiul Kawser[*1], MD. Amzad Hossain[1], MST. Fahamida Yeasmin[1]

ABSTRACT

An experiment was conducted for a period of 60 days to optimize the planting density of Lettuce (*Lactuca sativa*) with Tilapia in a shallow water recirculation aquaponic system. Four different planting density/m^2 i.e. 15 plants/m^2, 20 plants/m^2, 25 plants/m^2 and 30 plants/m^2 of hydroponic trough was used as four different treatments whereas, the stocking density of Tilapia (*Oreochromis niloticus*) was 100 fish/m^3 for all the treatments. Fish was reared in 300 L size tank and fed up to satiation twice a day with commercially available floating pellet with 30% protein level. The size of each of the hydroponic trough was 30 L which were made up with GI steel sheet. Styrofoam was used to cover up the hydroponic trough and made necessary number of hole according to the planting density of different treatment to support the plants in water medium. During the experimental period different water quality parameters were monitored regularly. Growth and yield of Lettuce were measured by means of number of leafs, plant height (cm), leaf area (cm^2), dry matter (g) and wet yield (kg/m^2). Whereas, Tilapia's growth performances were evaluated on the basis of weight gain (g), production (kg/m^3), specific growth rate (% per day) and feed conversion ratio. At the end of the experiment, it was observed that Lettuce production were highest in T_3 (25 plants/m^2), although the growth performance was significantly better in T_1 and T_2 because of higher nutrient availability in these hydroponic trough. Fish production was significantly higher in T_3 and T_4 compared to T_1 and T_2, although, highest production were observed in T_4. Regarding both Lettuce and Tilapia production with suitable water quality parameters, it was therefore, optimized that, 25 plants/m^2 area of hydroponic trough was ideal to support 100 Tilapia/m^3 in a shallow water based recirculation aquaponic system.

Key words: Aquaponic, Lettuce (*Lactuca sativa*), Tilapia (*Oreochromis niloticus*), Production

[1*]Departement of Aquaculture, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur 1706, Bangladesh, E-mail: robiulkawser@bsmrau.edu.bd
Corresponding Author

INTRODUCTION

Aquaculture is the fastest growing food production sector in the world. Compare to poultry and cattle, fish provide protein of high quality (Sargent and Tacon, 1999). Therefore, fish and other fishery products can be the key to combat malnutrition problem in Bangladesh. Fish provides 60% of national animal protein consumption and 11 percent of the total population are engaged in fisheries sector in Bangladesh (DoF, 2014). The per capita fish consumption in Bangladesh was 19.30 kg whereas the requirement is 21.90 kg (DoF, 2014). Bangladesh is facing serious problem of loss of agricultural land due to horizontal expansion of aquaculture. In that case development of recirculating aquaponic system can reduce the pressure on land and utilized the resource properly.

Aquaponics is a sustainable food production system that combines conventional aquaculture, with hydroponics (cultivating plants in water) in a symbiotic environment. The production of fish and vegetables through the integration of fish aquaculture and plant production has been demonstrated (Fitzsimmons, 1991; Fitzsimmons, 1992; Rakocy *et al.*, 2004; McMurtry *et al.*, 1997; Chaves, 2000; Sabidov, 2004; Castro *et al.*, 2006; Diver, 2006). In aquaculture, effluents accumulate in the water, increasing toxicity for the fish. This water is led to a hydroponic system where the by-products from the aquaculture are broken down by nitrogen fixing bacteria, then filtered out by the plants as nutrients, after which the cleaned water is recirculate back to the animals. Recirculation aquaculture systems (RAS) represent a new and unique way to farm fish. Instead of the traditional method of growing fish outdoors in open ponds and raceways, this system rears fish at high densities, in indoor tanks with a controlled environment. Recirculating systems filter and clean the water for recycling back through fish culture tanks. New water is added to the tanks only to make up for splash out and evaporation and for that used to flush out waste materials. Recirculated water can be passed through a hydroponic system. Plants are grown as in hydroponics systems, with their roots immersed in the nutrient-rich effluent water. This enables them to filter out the ammonia that is toxic to the aquatic animals, or its metabolites. After the water has passed through the hydroponic subsystem, it is cleaned and oxygenated, and can return to the aquaculture vessels. This cycle is continuous. Now a day, the natural production of fish has been decreased alarmingly for various factors. The practitioners implemented improved technology to increase fish production per unit area within minimum period of time. They also introduced various culture methods on the basis of varying characteristics of water bodies. All those aquaculture effort are mainly conducted in ponds. However, land available for construction of ponds is limited in our country. Therefore, new aquaculture techniques need to be introduced. In Bangladesh, the world's most densely populated country, most farmers use agrochemicals to enhance food production and storage life, though the country lack oversight on safe levels of chemicals in foods for human consumption. To combat these issues efforts have been made in Bangladesh to produce low-cost aquaponics system. Although popular articles are available in this regards however, scientific information on the aquaponic system in Bangladesh is limited. But it is important to determine the number of plants/ m^2 of a specific species is optimum to neutralize the waste materials produced in fish rearing tank. This study aimed to find out the optimum planting density of lettuce (*Lactuca sativa*) for a definite fish stock without compromising the vegetable production.

MATERIALS AND METHODS

Experimental design

The experiment was conducted for a period of 60 days from 20th September to 20th November, 2015 in department of aquaculture field research area of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU). The experiment was designed into four treatment groups (T_1, T_2, T_3 and T_4) consist of four different planting density of Lettuce (*Lectuca sativa*) each having three replications (Table 1). Size of the fish rearing tanks was 300 liter where fish were stocked at a density of 100 fish/m^3 in each of these tanks. Commercially made floating pellets (30% protein) was used to fed the fish.

Table 1: The experiment was conducted with four treatments, each treatment having three replicates as follow.

Treatment	Planting Density of Lettuce	Replication	Tilapia stocking density	Tilapia tank size	Vegetable tray size
T_1	15 plants/m^2	3	100 fish/m^3	300 L	30 L
T_2	20 plants/ m^2	3	100 fish/m^3	300 L	30 L
T_3	25 plants/ m^2	3	100 fish/m^3	300 L	30 L
T_4	30 plants/ m^2	3	100 fish/m^3	300 L	30 L

Stocking and rearing of fish species

Juveniles of Nile tilapia (*Oreochoromis niloticus*) of 10.0 ± 0.7 g size was collected from a commercial nursery and transported to BSMRAU campus and reared in a 500L tank until use. In the beginning of the experiment, fish was weighed individually, selected and distributed into each of the 300L tanks at a stocking density of 100 fish/m^3. Each of the tanks was connected to a vegetable culture tray. Fish was fed with commercially available pellet. The fish was fed to satiation within 10 minute twice daily (0900 and 1500 hr.), six days a week. All tanks were uniformly aerated. Tank was connected to a recirculation system with a suitable recirculation speed of 2 L/min.

Setting vegetable tray

Vegetables were grown in trays made of GI sheets. A general floating bed hydroponic method was followed (Lennard and Leonard, 2006). The rectangular size trays were of 300 L capacity. The trays were filled with 20 L of water as a growing media. Then trays were covered by Styrofoam sheet which was used to support the plant in aquaponic system. A water pump of 12 watt capacity was used in each fish rearing tanks to lift the water into the vegetable tray. Appropriate tubing was used to connect the vegetable trays with the fish culture tanks. Lettuces seeds were sown in nursery beds and allow growing for 15 days in traditional ways before transplanting into the experimental aquaponic systems. Plantlets of lettuce were then transplanted from the nursery beds to vegetable tray at the planting densities mentioned in the experimentation design in the previous section.

Fish Sampling

Fish was sampled fortnightly and growth and survival monitored. At the end of the rearing period, all fish in a tank was counted for survival data. Length and weight of 10 fish from each tank was taken to know the growth performance.

Growth parameters of fish

Growth of fry in length (cm) and weight (g) was measured and the following parameters were used to evaluate fry growth:

The specific growth rate (SGR) was calculated as: SGR = 100 (ln mean final weight) - (ln mean initial weight) ÷ culture days. Feed conversion ratio (FCR) was calculated as: FCR = total feed given ÷ total wet weight gain. % Weight gain (g) was calculated as: 100 ((Mean final weight - Mean initial weight)/ Mean initial weight).

Growth of vegetable

Lettuce growth was monitored on the basis of number of leaf, plant height (cm), leaf area (cm^2), dry matter (g) and fresh vegetables production (kg/m^2).

Monitoring water quality

Maximum-minimum temperatures were recorded daily in each tank. For water parameters, samples from the inflow pipe and fish culture tanks were collected. Dissolved oxygen (DO) and pH was measured using Hach Oxygen and pH meters, respectively (Hach Co., Loveland, Colorado). Total ammonia nitrogen (NH_3-N), nitrite nitrogen (NO_2-N), nitrate nitrogen (NO_3-N) and total phosphorus (TP) was determined following the methodology of APHA (1989).

Data analysis

Data obtained from this experiment were subjected to one-way analysis of variance (ANOVA) while least significant difference (LSD) test was used to compare treatment means. All statistical analyses were performed using the statistical software package (Statistics 10).

RESULTS AND DISCUSSIONS

Water Quality Parameters

During the experimental period the average temperature of fish rearing tanks and vegetables growing medium was ranges from 24 to 28^0C. pH of the fish rearing tank and vegetables growing medium was ranges from 6.4 to 6.8 and 5.2 to 5.8, respectively. Percentage removal of dissolved oxygen (DO), total ammonia nitrogen (TAN), nitrite nitrogen (NO_2-N), nitrate nitrogen (NO_3-N) and total phosphorus (TP) was measured to determine the water quality (Table 2). Dissolved oxygen level during this experimental period was ranges from 5.1 to 5.8 mg/l. Significant difference was observed in TAN, nitrite–N, nitrate–N and total phosphorus concentrations among different planting density of *Lactuca sativa*. The percentage removal of water quality parameters was increased with increasing in plants number/m^2 of hydroponic bed until the maximum was reached at 25 plants/ m^2 in T_3. Further increasing plant number did not significantly increase the percent removal of ammonia and phosphorus level. Highest ammonia level during this experimental period was 2.1±0.4 mg/l at a planting density of 15 plants/ m^2. DoF (2008) have reported that the range of suitable dissolved oxygen for fish culture would be 5-8 mg/1. Aminul (1996) has stated that the pH values from 6.7 to 7.5 and water temperature of 25°C to 35°C was the best suited for fish production. El-Sayed (1999) has reported that *O. niloticus* can tolerate ammonia level up to 3.5ppm. Therefore, the ammonia level during the present study was within the suitable range for fish production. Considering the above discussion it can be conclude that T_3 was the best among different planting density to remove harmful products from fish rearing tank.

Table 2: Different water quality parameter of recirculation aquaponic system.

Treatment		Water quality parameters				
		DO	TAN	NO_2-N	NO_3-N	TP
	Influent (mg/l)	5.1	13.4	0.71	20.2	16.6
T_1	Effluent (mg/l)	1.4	2.1	0.32	6.3	6.8
	Removal (%)	72.5^b	84.3^c	54.9^d	68.8^b	59.0^c
	Influent (mg/l)	5.8	13.1	0.65	20.1	16.5
T_2	Effluent (mg/l)	1.4	1.9	0.23	6.1	6.1
	Removal (%)	75.8^a	85.5^b	64.6^c	69.7^b	63.0^b
	Influent (mg/l)	6.1	13.3	0.66	20.2	16.5
T_3	Effluent (mg/l)	1.7	1.0	0.16	5.5	5.7
	Removal (%)	72.1^b	92.5^a	75.8^b	72.8^a	65.5^a
	Influent (mg/l)	5.8	13.6	0.62	19.7	16.1
T_4	Effluent (mg/l)	1.5	1.1	0.16	5.5	5.6
	Removal (%)	74.1^c	91.9^a	74.2^a	72.1^a	65.2^a

Means with different superscript in a column are significantly different ($P \leq 0.5$)

Growth and Yield of Lettuce (*Lactuca sativa*)

Growth of Lettuce (*Lactuca sativa*) was determined on the basis of number of leaves, plant height (cm), leaf area (cm^2) and dry matter content. It was observed that, the growth of lettuce was significantly different at different planting density. The growth rate of plants was significantly higher in T_1 and T_2 compare to T_3 and T_4. This was due to higher amount of nutrient availability to each plant from fish rearing tanks at a low planting density in T_1 and T_2 compare to T_3 and T_4. But the total production/m^2 area of hydroponic bed was significantly higher in T_3 and T_4 (Table 3). This was due to higher planting density per m^2 area of hydroponic bed in T_3 and T_4 compare to T_1 and T_2. As the number of plants per m^2 increases, it reduces the nutrient availability to each plant. So the balance between nutrient availability and number of plants/m^2 is crucial although critical. Without hampering the total production of vegetables, a stocking density of 100 Tilapia/m^3 can support 25 plants/m^2 of hydroponic unit where the maximum yield was 2.2 kg of Lettuce/m^2 in T_3. Further increase in the number of plants did not yield higher production. This may be due to less nutrient availability to each plant as the higher planting density and nutrient availability is inversely correlated.

Table 3: Effect of planting density on the growth and yield of Lettuce (*Lactuca sativa*).

	Growth parameters				Yield (kg/m^2)
Treatment	No. of leaves	Plant height (cm)	Leaf area (cm^2)	Dry matter (g)	
T_1	16.6^a	18.4^a	78.5^a	117.7^a	1.5^b
T_2	15.9^{ab}	16.3^{bc}	74.2^b	117.3^a	1.7^b
T_3	15.2^b	16.7^{bc}	73.5^b	115.1^b	2.2^a
T_4	15.0^b	15.9^c	71.4^c	111.8^c	1.9^a

Means with different superscript in a row are significantly different ($P \leq 0.5$)

Wilson (2006) has reported that, in floating bed system Lettuce (*Lactuca sativa*) production was 4.47 kg/m^2 from 80 L size hydroponic unit. Plants grow best and uptake nutrients at a lower pH (5.5-6.5) (Resh 2001). Specifically, lettuce will grow well in a pH range of 5.5-6.5 (Resh, 2001). At a stocking density of 2 kg/m^3 of Tilapia, the lettuce production was 4.32 kg/m^2 (Licamele, 2009).

Growth and Survival of Tilapia (*O. niloticus*)

The average initial weight of *O. niloticus* was 10±0.70 g in all the treatments (Table 1). The average final weight of *O. niloticus* connected with different hydroponic trough differ significantly ($P \leq 0.05$). Maximum final weight, % weight gain and production were highest in T_3 where plants were stocked at 25 numbers/m^2 and did differ significantly ($P \leq 0.05$) with T_1. Although, no significant difference ($P \geq 0.05$) was observed in SGR and FCR value of different treatments. 100 percent survival rate was observed in T_3 and T_4 which was significantly higher ($P \leq 0.05$) than T_1 and T_2.

Table 4: Fish growth performance and survival rate at different planting density after 60 days of rearing period.

	T_1	T_2	T_3	T_4
Initial Weight (g)	10±0.7^a	10±0.7^a	10±0.7^a	10±0.7^a
Final Weight (g)	65.5±2.2^b	66.8±1.7^b	70.7±1.5^a	71.0±1.1^a
Weight gain (g)	55.5±1.5^b	56.8±1.0^b	60.7±0.8^a	61.0±0.4^a
Weight gain (%)	555±2.8^d	567±1.0^c	607±1.0^b	610±1.0^a
Production (kg/m^3)	6.1±1.1^b	6.4±1.3^{ab}	7.07±1.1^a	7.1±1.4^a
Survival rate (%)	96±2.0^c	98±1.0^b	100±1.0^a	100±1.0^a
SGR (% per day)	3.13±0.8^a	3.17±1.0^a	3.25±0.6^a	3.26±0.9^a
FCR	1.8±0.5^a	1.6±0.7^a	1.6±0.7^a	1.7±0.6^a

Means with different superscript in a row are significantly different ($P \leq 0.5$)

Cruz and Laudencia (1978) have studied the protein requirements of Nile tilapia (*Oreochromis niloticus*) fingerlings and concluded that 20-30% crude protein required in the ration for optimum growth. Production of *O. niloticus* in a recirculating aquaponic system was 6.38 kg/m^3 during 10 weeks of experimental period (Rakocy *et al.*, 2006). Cruz and Ridha (2001) have conducted an experiment on growth and survival rates of Nile tilapia (*Oreochromis niloticus*) juveniles reared in a recirculating system and found that the survival rate was 100% in both cases. Mohamed (2009) has conducted an experiment on the effect of dietary protein level on the growth performance and body composition of monosex Nile tilapia (*O. niloticus*) reared in fertilized tank and observed 97% survival. Resley *et al.* (2009) have obtained SGR value of 4.7 (% day-1) in the growth and survival of juvenile cobia, in a recirculating aquaculture system. Kamal *et al.* (2006) have obtained SGR value of 1.6 (% day-1) in an aquaponic production of Nile tilapia (*Oreochromis niloticus*) and Bell pepper (*Capsicum annuum*) in recirculating aquaponic system. Rakocy *et al.* (2006) have found SGR value of 4.4 (% day-1) in an intensive Nile tilapia and basil aquaponic production system.

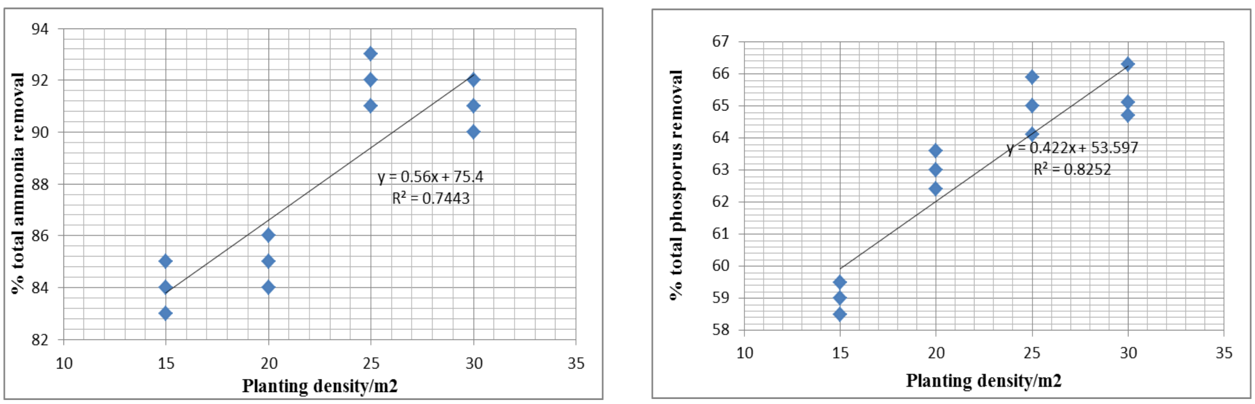

Figure 1: Correlation between Lettuces planting density/m2 with total ammonia removal (%) and total phosphorus removal (%) in fish rearing tanks.

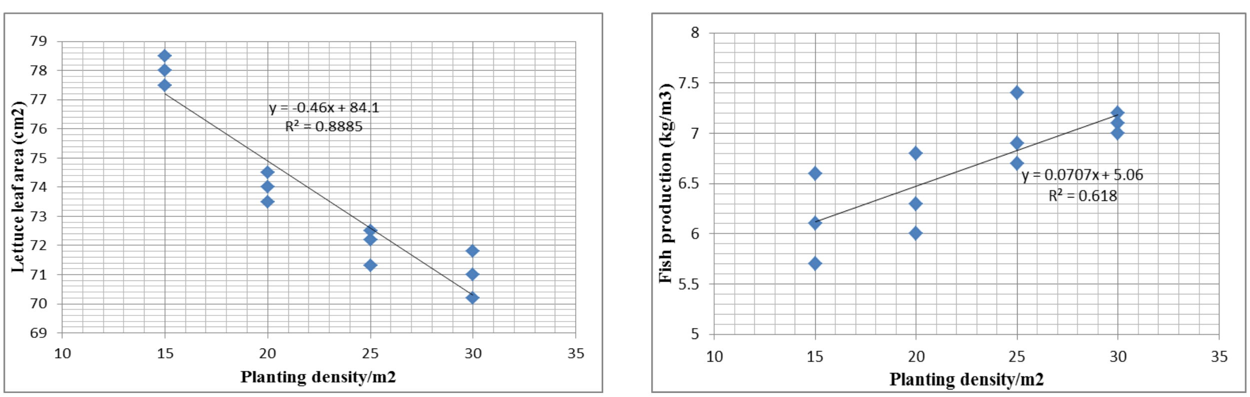

Figure 2: Correlation between Lettuces planting density/m^2 with lettuce leaf area (m^2) and fish production (kg/m^3).

Correlation coefficient of lettuce planting density/m^2 and percentage removal of total ammonia and total phosphorus was + 0.86 and + 0.91, respectively (Figure 1). Therefore, a strong correlation exists between planting density of lettuce and removal of harmful product for fish. So, with the increase of number plants/m^2, the water quality parameters tend to become favorable for fish culture. But with the increase of planting density/m^2 of hydroponic trough the amount of available nitrogen and phosphorus required for lettuce growth reduce which is signify by the strong negative correlation between planting density/m^2 and lettuce leaf area (cm^2) and the value of correlation coefficient was - 0.94 (figure 2). As planting density/m2 and water quality is positively correlated there a positive correlation is exists between the planting density of lettuce and production (kg/m^3) of fish and the value observed in this experiment was + 0.79.

CONCLUSIONS

Lettuce (*Lactuca sativa*) growth was significantly higher in T_1 and T_2 but the total production (kg/m^2) was highest in T_3. Although, no significant difference was observed in the lettuce production of T_3 and T_4. However, grayish color of the leaves in T_4 indicates the deficiency of nitrogen and phosphorus availability in hydroponic unit was started to prominent. Production of Tilapia (*O. niloticus*) was highest in T_4 but did not differ significantly with T_3. Therefore, it can be conclude that, a lettuce density of 25 plants/m^2 can efficiently utilize the waste product produce from 100 Tilapia/m^3 stocking density in a shallow water recirculation aquaponic system.

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