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Optimization of Planting Density of Indian Spinach in a Recirculating Aquaponics System Using Nile Tilapia

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Abstract. An experiment was conducted for a period of 10 weeks to compare the effect of planting density on the growth and yield of Indian spinach (*Basella alba*) and Nile tilapia *(Oreochromis niloticus)* in a recirculating aquaponics system. Indian spinach was planted at four densities (4 plants/m^2, 8 plants/m^2, 12 plants/m^2 and 16 plants/m^2). Stocking density of Nile tilapia (Av. body wt. 32.5 g) was 45 fish/tank (water capacity 300 L) in all planting densities. The highest weight gain, percent weight gain, specific growth rate and protein efficiency ratio of fish were obtained at planting density of 12 plants/m^2. Feed conversion ratio was also lowest at this density. Number of leaves per plant, plant length, plant weight and yield of Indian spinach were the highest at 12 plants/m^2. It was concluded that the plant density of 12 plants/m^2, for Indian spinach integrated with 45 fish/tank was suitable for production of both vegetable and Nile tilapia in a recirculating aquaponics system.

Keywords: Indian spinach, Nile tilapia, planting density, growth, feed utilization, aquaponics

Introduction

Aquaponic is a type of integrated fisfh farming systems in which the plants utilize fish waste as a source of nutrients and thus purify polluted water. The system minimizes the potentially negative environmental impact which would be caused by fish culture water (Lennard and Goddek, 2019; Savidov *et al*., 2005). This interction in the compact system can reduce the need for filters, fertilization, mechanical maintenance, water monitoring and water changes as compared to aquaculture or hydroponics alone (Rakocy *et al*., 2004). In most aquaponics systems, fish are fed with a highprotein diet (Rakocy, 2012; Rakocy *et al*., 2006). Therefore, the fish excrete waste that contain potentially toxic nitrogen compounds, intcuding predominantly ammonia (NH_3). These compounds are first mineralized into nitrite and then nitrate by nitrifying bacteria (*Nitrosomonas and Nitrobacter*) respectively. The plants and the associated micro-organisms utilize the nitrogen compounds from the water, serving for growth as bio-filter of nutrients and thereby reduce the need for active biological and/or chemical filtration and other water treatment. As the plants remove pollutants from the water, they also reduce the need to replace used water from the fish tanks (Goddek *et al*., 2015).

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Small to medium-scale aquaponics systems require relatively small space, thus suitable in homes, backyards, basements, balconies and rooftops to increase personal and community food security (Rakocy, 2012). Selection of fish species and plant variety for any aquaponics system is important. Nile tilapia (*Oreochromis niloticus*) is among the best candidates for culture in aquaponics, because of its suitable features for aquaculture such as resilience, ease of domestication and faster growth rate compared to other short cycled fish species (Rayhan *et al*., 2018; Kawser *et al*., 2016; Moya *et al*., 2014). Green leafy vegetables grow generally well in the hydroponics, sub system although most profitable varieties are Chinese cabbage, lettuce, basil, tomatoes, okra, cantaloupe and bell peppers (Bailey and Ferrarezi, 2017; Love *et al*., 2015; Graber and Junge, 2009). Indian spinach (*Basella alba*)is a popular summer leafy vegetable, which belongs to the family Basellaceae. Its nutritive value is very high with a good content of minerals, vitamins and substantial amount of fibre (Sanni, 1983). Plant spacing is important in crop production to maximize the yield. Densely planted crops obstruct the proper growth and development of plant.

On the other hand, wider spacing ensures the basic requirements are met but decreases the total number of plants as well as total yield, especially for leafy plants. Yield may be increased up to 25% by using optimum spacing (Danner *et al.,* 2019; Bansal and Verreth, 1995). Planting density is important for aquaponics because, it is crucial to know how many plants are sufficient to utilize wastes produced in the fish culture tank. However, most of the reported studies have been conducted on stocking density of fish in aquaponics system but not the planting density of vegetable (Rayhan *et al.,* 2018; Kawser *et al.,* 2016; Watanabe *et al.,* 2002; Cruz, 2001). Therefore, the primary goal of this study was to evaluate the effect of planting density on the growth and yield of Indian spinach and Nile tilapia in a recirculating aquaponics system.

Materials and Methods

The present experiment was conducted for a period of 10 weeks (from July 28 to October 5, 2018) to find out the efficacy of integrated farming of Indian spinach and Nile tilapia in a recirculating aquaponics system. The experiment was conducted in the backyard Aquaponics Shed of Faculty of Fisheries, BSMRAU, Gazipur, Bangladesh.

Establishment of recirculating aquaponics system. The design of aquaponics system was comparable to recirculating systems, incorporating a hydroponics component and replacing bio-filter and devices for removing fine and dissolved solids (Fig. 1). Fish were reared in round plastic tanks (300 L water capacity). The fish rearing tanks were placed on ground level under a shed. Vegetable trays, made of steel sheets with volume of 0.15 m^3 and dimensions of 1.25 m × 0.85 m × 0.15 m size were placed above a stage under shed. All the vegetable growing trays were filled with small size gravel pieces (8-15 mm). The experiment was conducted in triplicate.

Separate recirculation system was established for each set of fish rearing tank and vegetable tray. At first the fish rearing tank was filled with water. A sub mersible water pump of 12 watt capacity (water flow 3.0 l/min) was set into the tank to pump water to the vegetable tray through a plastic pipe. Water from the vegetable trays returned to the fish rearing tanks by gravitational force. Thus, a recirculating aquaponics system was established.

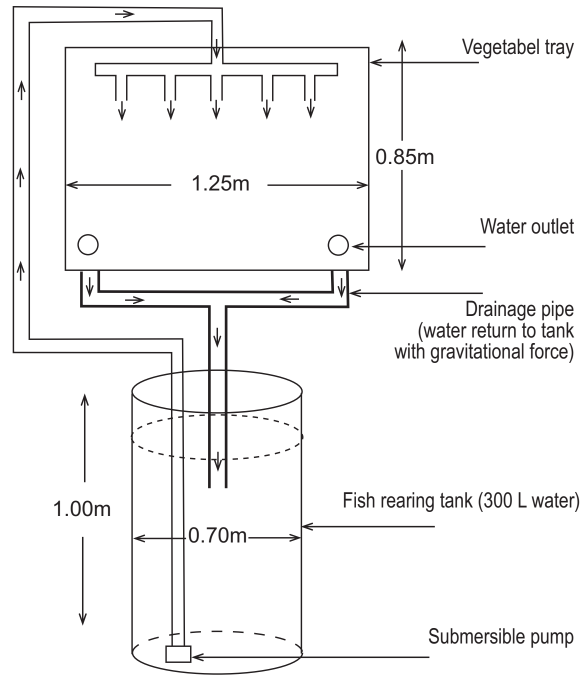

Fig. 1. Schematic representation of an aquaponics unit. In total 15 such units were used.

Stocking and rearing of Nile tilapia. A total of 850 male monosex Nile tilapia were collected from a local commercial fish hatchery (Sagor Motso Hatchery, Mymensingh). The fish were transported to the experimental site in oxygenated polythene bag. Then the fish were kept in 500 L tanks for 7 days for conditioning. The fish were weighed individually, selected on the basis of individual body weight and distributed as 45 fish into each of the 300 L tanks in such a way that the total weights of fish in all tanks were similar. All tanks were uniformly aerated with aquarium aerators (Hailea, ACO 308). Fish were fed to satiation with commercially available floating Tilapia Pellets (Saudi-Bangla Fish Feed Ltd., Mymensingh) containing 11.3% moisture, 30.0% protein, 3.8% crude lipid and 10.2% ash.

Cultivation of Indian spinach. Indian spinach seeds were collected from local market. Then the seeds were sowed in the adjacent agriculture land. Then15 days, old Indian spinach plants were transplanted in aquaponics units. Before transplantation, roots of plant were washed with water to remove the adhering soil. The planting densities were, 4 plants/m^2 (spacing 25.0 cm x 17.0 cm), 8 plants/m^2 (spacing 13.5 cm x 9.5 cm), 12 plants/m^2 (spacing 9.5 cm x 6.5 cm) and 16 plants/m^2

(spacing 7.5 cm x 5.0 cm). No supplemental fertilizer was used during plant cultivation except the nutrient received from the effluent coming from fish rearing tanks.

Sampling of fish. Fish were sampled fortnightly and growth and survival monitored. At the end of 10 weeks rearing period, fish were counted and killed by anaesthetization with MS-222 (3-aminobenzoic acid ethyl ester, 100 mg/L). Length and weight of 10 fish per tank were taken to determine the growth performance. Ten car casses (dead body) from each tank were pooled, washed with distilled water and stored at -20 °C for whole body chemical composition analysis.

Indian spinach data collection. Numbers of leaves of 4 randomly chosen plants from each tray were counted after 24, 42, 56 and 70 days after planting (DAP). All the leaves of each plant were counted, except smallest young leaves. A average of leaves of 4 plants gave the number of leaves per plant. Then, length was measured in cm by a meter scale. The average of length of 4 plants gave the plant length.

The first harvesting of Indian spinach was done from all trays after 24 DAP. The plants were cut manually by a pair of scissors at a length of 15.2 cm from the bed level. Yield (biomass) was measured by collectively weighing all the plants harvested. The plants were allowed to grow and the subsequent three harvests were done after 42, 56 and 70 DAP and the yields on harvesting dates and total yield were recorded. Plant weight was measured in g by an electronic balance (Simadzu, Japan, ATX 324). The average of weight of 4 plants gave the plant weight.

Monitoring water quality. Water quality parameters in rearing tanks were measured fortnightly between 8.0 and 9.0 a.m. during the study period following the standard methods of APHA (2012). Water temperature (°C) was recorded using a thermometer (9337U20, Thomas) and pH by a digital pH meter (HQ11D, HACH) at the spot. Chemical analysis of water sampled in leveled 250 mL black plastic bottles, was done in the laboratory of the Department of Aquaculture, BSMRAU. pH of tanks water was measured by using a digital pH meter (HQ40D, Hach Co., Colorado, USA). Ammonia (mg/L), nitrate (mg/L), nitrite (mg/L) and phosphate (mg/L) were determined with a spectrometer (DR 5000, Hach Co., Colorado, USA). A digital DO meter (HQ_30D, Hach Co., Colorado, USA) was used to determine the dissolved oxygen content of water.

Chemical analysis of sample. The proximate composition of Indian spinach, fish and feed samples was determined according to standard methods given by the Association of Official Analytical Chemists (AOAC, 1995).

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

$$\text{Weight gain (\%)} = \frac{\text{Mean final weight (g)-Mean initial weight(g)}}{\text{Mean initial weight (g)}} \times 100$$

$$\text{Specific growth rate (\%/day)} = \frac{\ln Wt - \ln W_o}{t} \times 100$$

where;

W_t and W_o are the average weight of fish at time t and 0, respectively and t is the culture period in day.

$$\text{Condition factor CF (\%)} = \frac{\text{Fish weight in g}}{(\text{Fish length in cm})^3} \times 100$$

$$\text{Survival rate (\%)} = \frac{\text{Number of fish at the end}}{(\text{Number of fish stocked})} \times 100$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Weight of feed consumed by fish (g)}}{\text{Total wet weight gain of fish (g)}}$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Gain in body mass (g)}}{\text{Protein intake (g)}}$$

Statistical analysis. The data were analyzed statistically by one-way analysis of variance (ANOVA) using statistical software Statistix 10 (Analytical Software, Tallahassee, FL), where significant differences in means were compared by Least Significant Difference (LSD) option of the package. Normality and homogeneity were tested with Shapiro-Wilk test and Levene test, respectively. The significance level was determined at $P<0.05$.

Results and Discussion

Water quality parameters in fish rearing tanks are presented in Table 1. The average water temperature varied between 28.2 ± 0.2 and 29.0 ± 0.2 °C and was not significantly different among treatments. As a well-adapted fish, Nile tilapia have tolerance of a wide range of temperature 20-35 °C for growth where the optimum temperature is reported between 26 and 28 °C within which productivity can be assumed at maximum (Crab *et al.,* 2009; De Schyver *et al*., 2008). According to Colt (2006) optimal water temperature for. Life of Nile tilapia is 28 °C. On the other hand, nitrifying bacteria grow well in temperature 28-30 °C (Wongkiew *et al*., 2017). Therefore, the temperature found in this experiment was appropriate for fish culture and nitrification process in aquaponics system.

The average pH level of water during the experimental period ranged from 7.25±0.2 to 7.67±0.1. However, water pH was not significantly different among the treatments (P>0.05), pH in water body generally regulates considerably the water chemistry. Any sudden fluctuation of pH causes the death of many aquatic species. Boyd (1998) reported that the suitable pH range for fish production was 7.3 to 8.4. Nile tilapia can tolerate a pH range of 4 to 11 and pH between 6.5 and 9.0 is the desirable range for Nile tilapia culture (Huet, 1972). Therefore, in the present study, the pH value was suitable for Nile tilapia growth. The nitrifying bacteria growth is inhibited below a pH of 6.5 with an optimum pH at 7.8 depending on species and temperature (Tyson *et al.,* 2007). Therefore, the range of pH value found in present study was also good for nitrification.

The optimum ammonia level in a recirculatory system should be less than 1.0 mg/L (Van Rijn and Rivera, 1990). In the present study, the ammonia-nitrogen level of tank water ranged between 0.49±0.1 and 0.74±0.1 mg/L (Table 1). The overall ammonia contents in all treatments increased with the progress of culture, however, within the suitable limit for Nile tilapia. However, there was no significant difference (P>0.05) among water ammonia-nitrogen levels between sampling dates during the experimental period. It indicated that the value of ammonia was within safe limits for fish (Kamal, 2006). Nitrate level of tank water ranged between 35.00± 1.2 and 36.95±1.3 mg/L. Tank water nitrate in a sampling day was not significantly differing (P>0.05) during the experimental period. According to Rakocy *et al.* (2004) optimum range of nitrate was 26.7 mg/L to 54.7 mg/L for batch culture during basil production trials with Nile tilapia. Licamele (2009) recorded concentrations of 50 mg/L NO_3-N in an aquaponics integrated system with lettuce (*Lactuca sativa*) and Nile tilapia (*Oreochromis niloticus*) were safe for culture.

Nitrogen is important component for plant growth and spinach obtains this nitrogen from fish excreta in the form of ammonia and /or other nitrogen compounds (Lennard and Goddek, 2019; Srimongkol *et al*., 2019). Either too elevated or too low nitrogen availability can limit the plant growth (Goddek *et al*., 2015; Savidov *et al*., 2005). In the experiment the nitrogen concentration was similar in all treatments, because the fish density was similar. The spinach growth was highest at planting density of 12 plants/m^2, therefore we can conclude that this planting density matched the available N. In contrast, planting densities of 4 and 8 plants/m2 plants probably received excessive amounts of nitrogen that limited the growth. Again at planting density of 16 plants/m^2 , the plants lacked nitrogen that limited the plant growth. Kamal (2006) recommended concentration of nitrite in culture system to be less than 0.15 mg/L for culture of Nile tilapia in a mini pond, although Atwood *et al.* (2001) found that the 96 h LC50 of nitrite were 81 and 8 mg/L in small (4.4 g) and large (90.7 g) Nile tilapia, respectively. In basil production trials (Rakocy *et al.* 2004) used range of nitrate from 0.4 mg/L to 1.1 mg/L.

Table 1. Average of water quality parameters through the experimental period (N = 6)

Parameters	Planting density of Indian spinach (plants/m^2)			
	4	8	12	16
Temperature (°C)	28.77 ± 0.21	28.22 ± 0.26	28.59 ± 0.22	29.00 ± 0.26
pH	7.25 ± 0.21	7.67 ± 0.15	7.51 ± 0.24	7.45 ± 0.06
DO (mg/L)	5.66 ± 0.12	5.52 ± 0.10	5.46 ± 0.09	5.72 ± 0.15
NH_3-N (mg/L)	0.74 ± 0.18	0.52 ± 0.25	0.64 ± 0.19	0.49 ± 0.16
NO_2-N (mg/L)	0.12 ± 0.02	0.13 ± 0.02	0.12 ± 0.04	0.11 ± 0.05
NO_3-N (mg/L)	35.35 ± 1.32	35.00 ± 1.28	36.95 ± 1.38	36.01 ± 1.26
PO_4-P (mg/L)	2.85 ± 0.47	2.93 ± 0.53	2.89 ± 0.29	3.04 ± 0.20

From the above statements, the level of nitrite in the present study was within the acceptable range for fish.

In the present study, the phosphate level of tank water ranged between 2.85±0.47 to 3.04±0.20 mg/L (Table 1). Phosphate levels in water was not significantly diferent (P>0.05) during the experimental period.Boyd (1998) reported that tolerable limit of phosphates for fish in aquaponics system is between 0.20 and 1.15 mg/L. Stefan *et al.* (2013) conducted a research on vegetable production in an integrated aquaponics system with rainbow trout and spinach and recommended that phosphate concentration in aquaponics system ranges from 1.5 mg/L to 3.7 mg/L. So, the phosphorous level of the present study was within suitable range.

In the present study, dissolved oxygen (DO) level in rearing water ranged between 5.46±0.09 to 5.72±0.15 mg/L (Table 1). There was no significant difference (P>0.05) in DO in different treatments during the experimental period. Maintenance of DO is important for fish health and aerobic micro-organisms in the biofilter (Hussain *et al*., 2015). Nile tilapia needs 5 mg/L DO for optimal growth, and in 2.5 mg/L or below shown significant growth retardation of fish (Masser *et al*., 1999). Similarly, the nitrifying bacteria become ineffective in converting harmful ammonia to less harmful nitrite at DO levels below 2 mg/L (Masser *et al*., 1999). These reports indicate that the levels of DO in all the treatments were within favorable range for Nile tilapia.

Growth response of Nile tilapia. The average weight gain of Nile tilapia was comparable in all treatments, roughly 40.7 to 46.3 g (Table 2). Significant higher weight gain was observed at the planting density of 12 plants/m^2, while lower weight gain was observe 16 plants/m^2 planting and densityof Indian spinach. Data on fish length followed the similar trend. The average percent weight gain of Nile tilapia varied 126±1.4 to 141±1.4% in all treatments, significantly higher percent weight gain observed at the planting density of in 12 plants/m^2 and lower at the planting density of 16 plants/m^2. Therefore, the fish production was at its highest, where also the plant production was higher, that is at the plant density of 12 plants/m^2 . Because more plants had purified water properly and fish got clean and NH_3-free or low-N water that enhanced the growth of fish.

Kamal (2006) obtained maximum growth of Nile tilapia when cultured with bell pepper at a planting density of 10 plants/m^2. Effendi *et al.* (2017) observed an increase of the Tilapia biomass due to addition of romaine lettuce in the culture system, because the plants used the nitrogen (mainly the NH_4) as a source of nutrients. This led to a better quality of the water. Wongkiew *et al.* (2017) observed that introducing lettuce in the fish culture system decreased the nutrient concentration in the system. In the present study, the maximum growth of Nile tilapia with 12 Plants/m^2 was similar to above findings. The survival rate of Nile tilapia was high at all planting densities of Indian spinach and only a single fish died (Table 2). Survival of fish observed in the present study was high, which was similar to those reported for earlier Nile tilapia in aquaponics system (Wang *et al.,* 2016; Bakhsh and Chopin, 2012; Al-Hafedh *et al.,* 2008).

Overall specific growth rate SGR were comparable, however, significantly higher SGR of Nile tilapia was observed at 12 plants/m^2 planting density of Indian spinach, whereas SGR in 4 plants/m^2 and 8 plants/m^2 planting were satistically same to 12 plants/m^2 (Fig. 2).

Table 2. Growth data of Nile tilapia in different planting densities after 70 days of rearing

Parameters	Planting density of Indian spinach (plants/m^2)			
	4	8	12	16
Initial weight (g)	32.55 ± 0.62	32.52 ± 0.29	32.66 ± 0.35	32.29 ± 0.22
Final weight (g)	74.66^b ± 1.19	74.70^b ± 2.45	79.00^a ± 0.08	72.99^c ± 2.94
Weight gain (g)	42.11^b ± 1.82	42.18^b ± 2.15	46.34^a ± 0.14	40.70^c ± 2.96
Weight gain (%)	129.37^b ± 1.41	129.70^b ± 1.41	141.89^a ± 1.41	126.05^c ± 1.41
Initial length (cm)	8.30 ± 0.04	8.17 ± 0.26	8.26 ± 0.41	8.05 ± 0.24
Final length (cm)	15.79^b ± 0.01	15.84^b ± 0.43	16.25^a ± 0.1	15.89^b ± 0.07
Length gain (cm)	7.49^c ± 0.06	7.67^b ± 0.16	7.99^a ± 0.31	7.84^{ab} ± 0.16
Condition factor	1.89^b ± 0.02	1.85^b ± 0.03	2.12^a ± 0.02	1.82^b ± 0.04
Survival rate (%)	100.0	100.0	100.0	98.9 ± 1.6

* Means in a raw with different superscript are significantly different (P<0.05).

Lower SGR observed in 16 plants/m^2 planting density. Midmore (2011) obtained a slightly higher SGR value 2.03 (%/day) with Nile tilapia in Honduras using pellet and fertilizer. Hussain *et al.* (2015) observed SGR value of GIFT strain ranged from 2.04 to 2.30 (%/day) fed with formulated diet, which is higher than the values of present study. Kamal (2006) obtained SGR value of 1.6 (% /day) in an aquaponics production of Nile tilapia (*O. niloticus*) and Bell pepper (*Capsicum annuum*) in recirculating aquaponics system. In the present study the SGR value was slightly lower than the findings of SGR value of fish in aquaponics system obtained by Kamal (2006), which may be due to differences in stocking density, culture period, climate condition and culture environment.

The condition factor (CF) indicates the healthy fish and is based on the hypothesis that heavier fish of a given length are in better condition (Bagenal and Tesch, 1978). The CF of Nile tilapia in the present study ranged from 1.82 ± 0.04 to 2.12 ± 0.02 (Table 2). Significant higher CF (2.12 ± 0.02) was observed in 12 plants/m^2 and the lowest CF (1.82 ± 0.04) was observed in 16 plants/m^2, respectively. Ayode (2011) reported that higher condition factor of fish indicates good health.

Feed utilization. The highest feed conversion ratio (FCR) was observed in 8 plants/m^2 planting density of Indian spinach, while the lowest FCR was found in 12 plants/m^2 planting density (Fig. 3). The FCR value in 12 plants/m^2 planting density indicated better feed utilization because of more plant purified water and fish got clean or low ammonia water and effectively utilize the feed. Watanabe *et al*. (2002) in a study with Nile tilapia found FCR values of 1.5-2.0. However, nitrogen content of water was similar in all treatments. The FCR values for Nile tilapia in the present aquaponics system was better than that of other studies.

Significant highest protein efficiency ratio (PER, 1.61) was observed in 12 plants/m^2 while the lowest (1.41) PER was observed in 16 plants/m^2, (Fig. 3). PER of Nile tilapia was 1.17 and 1.23 using floating and sinking pellets, respectively in a recirculating aquaponics system (Cruz, 2001). PER of *Cyprinus carpio* was 1.34 in an aquaponics system with spinach as a hydroponic crop (Hussain *et al*., 2015). Using castor seed meal as feed ingredient for Nile tilapia PER was 1.52 in a recirculating aquaponics system (Balogun *et al.,* 2005). PER of the present study was similar when compared to most of the previous studies and a planting density of 12 plants/m^2 created a favourable environment for protein utilization in fish.

Proximate composition of whole body carcasses. More or less similar values of the average moisture, protein, lipid and ash of Nile tilapia were found in all treatments (Table 3). Whole body carcass composition of tilapia is mostly affected by the nutrient composition of diet especially the protein (Foh *et al.,* 2011). In the present experiment, as fish in all treatments received the same food, therefore, it is not surprising that the whole body carcass composition was not diferent.

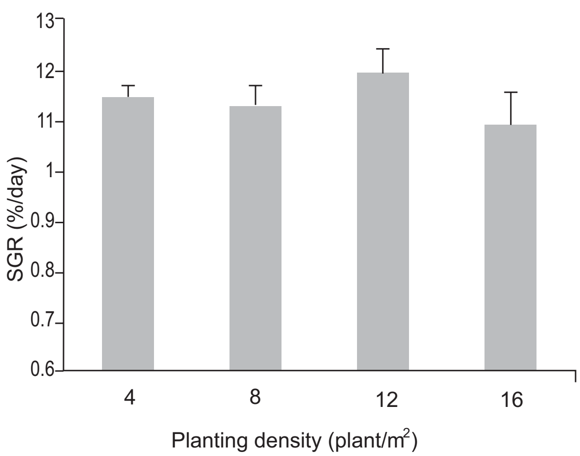

Fig. 2. Specific growth rate of tilapia in different planting densities, means with different letters are significantly different (P<0.05).

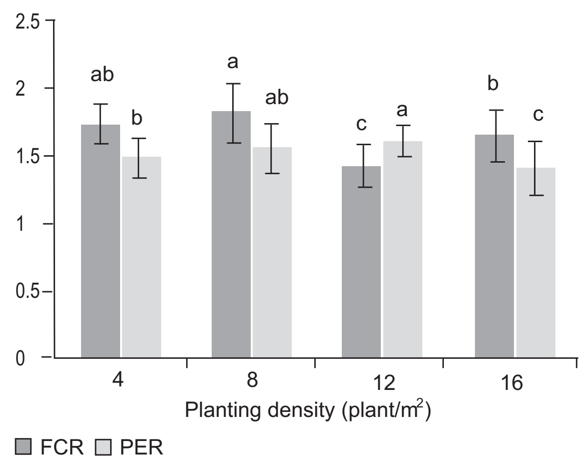

Fig. 3. FCR and PER of tilapia in different planting densities. Means with different letters are significantly different (P<0.05).

Table 3. Proximate composition of whole body carcasses of tilapia fed with floating pellets in different planting densities

Planting density of Indian spinach (plants/m^2)	Parameters			
	Moisture%	Protein%	Lipid%	Ash%
4	73.56 ± 0.58	18.17 ± 0.18	5.03 ± 0.21	1.79 ± 0.12
8	73.61 ± 0.14	18.43 ± 0.41	5.74 ± 0.01	1.72 ± 0.22
12	74.36 ± 0.04	18.81 ± 0.01	5.87 ± 0.27	1.88 ± 0.04
16	73.59 ± 0.07	18.13 ± 0.01	5.85 ± 1.35	1.54 ± 0.09

Vegetable growth performance. Number of leaves per plant of Indian spinach varied significantly due to different planting densities after 24, 42, 56 and 70 DAP (Table 4). Number of leaves per plant was the highest at 12 plants/m^2 planting density in each of the sampling dates. Similarly, plant length and plant weight were also the highest in 12 plants/m^2 in all the sampling dates.

In the present study, number of leaves, length, and weight per plant were significantly different and those values were significantly higher in 12 plants/m^2 planting density. In an aquaponics system plant gets nitrogen from fish waste as the form of ammonia which then converts to nitrite and then nitrate by the process of nitrification.

The yield of Indian spinach per treatment varied significantly due to different planting density after 24, 42, 56 and 70 DAP, which are shown in (Table 5). Total yield of Indian spinach per square meter varied significantly due to different planting density (Table 5). The highest total yield (5.3 Kg/m^2/70 days) was recorded at planting density of 12 plants/m^2, while the lowest (0.777 Kg/m^2/70 days) yield was found in 4 plants/m^2. At the planting density of 12 plants/m^2 plants got suitable amount of nitrogen for their growth on the other hand excessive and inadequate amount of nitrogen hampered the growth as shown in 4 plants/m^2 and 16 plants/m^2. Kamal (2006) studied the aquaponics production of Nile tilapia *(O. niloticus)* and Bell pepper *(C. annuum)* in re-circulating water system. The results showed that fish cultured with 10 plants/m^2 gave the best significant (P<0.05) fish production and yield of marketable Bell pepper. Therefore, a planting density of 12 plants/ m^2 can be considered as optimum for vegetable production.

The present study indicates that weight gain, percent weight gain, specific growth rate, condition factor, food

Table 4. Growth data of Indian spinach in different planting densities observed after different periods of cultivation

Planting density of Indian spinach (plants/m2)	Days after planting			
	24 Day	42 Day	56 Day	70 Day
Number of leaves per plants				
4	4.08^d ± 0.12	14.08^c ± 0.12	7.75^d ± 0.17	17.75^c ± 0.17
8	11.12^b ± 0.17	21.12^{ab} ± 0.17	11.22^b ± 0.03	21.22^{ab} ± 0.03
12	12.37^a±0.17	22.37^a ± 0.17	14.16^a ± 0.05	24.16^a± 0.05
16	10.18^c ± 0.26	20.18^{bc} ± 0.26	9.21^c ± 0.04	19.21^b ± 0.04
Length per plant (cm)				
4	48.25^d ± 2.47	60.25^c ± 3.18	76.56^c ± 3.97	92.56^c ± 3.97
8	60.96^b ± 0.30	76.96^{ab} ± 0.30	89.90^b ± 0.04	105.90^b ± 0.04
12	65.71^a ± 1.83	81.71^a ± 1.83	108.97^a ± 2.56	124.97^a ± 2.56
16	55.57^c ± 0.29	71.57^b ± 0.29	65.68^d ± 1.54	81.68^d ± 1.54
Weight per plant (g)				
4	24.70^d ± 1.47	40.70^d ± 1.47	56.50^c ± 1.23	72.50c ± 1.23
8	52.96^b ± 0.22	68.96^b ± 0.22	81.87^b ± 2.12	97.87^b ± 2.12
12	69.12^a ± 2.12	85.12^a ± 2.12	137.87^a ± 2.53	153.87^a ± 2.53
16	32.42^c ± 0.28	48.42^c ± 0.28	49.23^d ± 1.92	65.23^d ± 1.92

Note: Values are given with mean± standard deviation. Different letters in the same columns (for a parameter) are significantly different at 5%.

Table 5. Yield (Kg/m^2) of Indian spinach in different planting densities observed on different dates

Planting density of Indian spinach (plants/m^2)	Days after planting				
	24 Day	42 Day	56 Day	70 Day	Total
4	0.098^c ± 0.005	0.162^d ± 0.005	0.226^d ± 0.004	0.291^d ±0.004	0.777^d ± 0.005
8	0.423^{bc} ± 0.001	0.551^c ± 0.001	0.654^c ± 0.016	0.782^c ± 0.016	2.411^d ± 0.008
12	0.829^a ± 0.025	1.021^a ± 0.025	1.651^a ± 0.030	1.846^a ±0.030	5.347^a ± 0.002
16	0.518^b ± 0.004	0.774^b ± 0.004	0.787^b ± 0.030	1.043^b ±0.030	3.122^{bc} ±0.015

Note: Values are given with mean ± standard deviation. Value in the same column bearing different letters is significantly different at 5%.

conversion ratio, and protein efficiency ratio of Nile tilapia were affected by planting density of Indian spinach and the best results were obtained in 12 plants/m^2 planting density of Indian spinach. Similarly in case of vegetable growth and yield the present study indicates that number of leaves per plant, plants length per plant, plants weight per plant and yield of Indian spinach were best in 12 plants/m^2.

Conclusion

This study revealed that a planting density of 12 Indian spinach/m^2 integrated with 45 Nile tilapia/300 L water was suitable for biomass production of both Indian spinach and Nile tilapia. Farmers and household owners can utilize this plant to fish ratio for effective running of an aquaponics system for growing Indian spinach and tilapia.

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