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Optimizing Koi Carp, *Cyprinus carpio* var. *Koi (Linnaeus, 1758)*, Stocking Density and Nutrient Recycling With Spinach in an Aquaponic System

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Abstract

Fish waste water nutrient recycling in an aquaponic system was studied under different stocking densities of Koi Carp, *Cyprinus carpio* var. *koi*, along with spinach, *Beta vulgaris* var. *bengalensis*. Fish growth performance, plant growth, nutrient dynamics, and nutrient removal and their dependence on different stocking densities, namely 1.4, 2.1, and 2.8, were observed, of the different combinations, fish stocked at 1.4 kg/m^3 had the best growth. Percent nutrient removal (NO_3–N, PO_4–P, and K) was significantly higher at 1.4 kg/m^3. Thus, 1.4 kg/m^3 stocking density can be suggested as optimum for Koi Carp production in spinach aquaponic systems.

Aquaculture has emerged as a major food producing sector, and it is now a major global industry with total annual production exceeding 63.6 million m.t. (FAO 2011). Aquaculture practices require freshwater which is a scarce resource. They can potentially discharge waste water containing organic matter, ammonia, nitrate, and phosphate. Releasing these nutrients to receiving water bodies creates environmental pollution. Untreated aquaculture water discharge not only contaminates rivers, but can also spread infections to downstream fish culture ponds (Thein et al. 2007). Organic matter in the discharged effluent reduces dissolved oxygen levels and further contributes to sediment buildup. Moreover, high nutrient loading degrades water quality by stimulating excessive phytoplankton production (Joyner 1992). Therefore, inorganic nitrogen and phosphate removal is essential for aquaculture wastewater to protect receiving waters from eutrophication and for potential water reuse (Endut et al. 2009).

Conventional methods have been used for removing nutrients and toxic substances in recirculating aquaculture such as sand filters and mechanical filters for particulate matter removal. Trickling filters, fluidized bed reactors, and rotating biological contactors are used to control and treat dissolved nutrients. These methods are used for oxidation of organic matter, nitrification, or denitrification (Van Rijn 1996). All these methods have the disadvantage of high energy demand, sludge production, and frequent maintenance. Aquaponics is also a tool for removing environmental pollution, which is useful compared to other recirculating aquaculture systems. Aquaponic systems are recirculating aquaculture systems with hydroponic plants without soil (Rakocy et al. 2006). Aquaponics work on the principle of nitrogen cycle, wherein dissolved waste generated from the production system is effectively converted to plant nutrients by beneficial nitrifying bacteria. Plants can utilize these nutrients for their growth. Recirculating aquaculture systems already produce large volumes of fish in a small footprint without aquaponics. Aquaponics does allow for increased production using the same volume of water and nutrient input. By this technique, it is possible to reduce wastes and associated environmental impacts, and at the same time generate fish and plant crops (Naegel 1977; Quillere et al. 1995; Ghaly et al. 2005; Rakocy et al. 2006). Aquaponics has the potential to reduce environmental pollution caused by aquaculture effluents.

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Koi Carp, *Cyprinus carpio* var. *koi*, is an ornamental fish with high market demand because of its color patterns. It has great demand in South East Asian countries, and being hardy in nature, it is highly suitable for garden pools and aquariums. Limited information on Koi Carp culture in aquaponics system is available. Spinach, *Betta vulgaris* var. *bengalensis*, commonly known as Palak or beet leaf, is a native green vegetable to the Indochina region. It is a cool seasonal crop, requiring a mild climate for good production. It is tolerant to slightly alkaline soils and highly tolerant to salinity. Spinach is considered a popular salad ingredient, as it is rich in iron and a good source of folic acid. It can be grown hydroponically in greenhouse production (Rosik-Dulewska and Grabda 2002). The objective of this research was to determine an optimal fish to plant ratio using Koi Carp fingerlings and beet leaf in a recirculating aquaponic system.

Materials and Methods

Experimental Design

The aquaponic recirculating system was designed and setup in the wet laboratory of Aquaculture Division of CIFE, Mumbai, India. The system consisted of 18 individual, identical aquaponic units, allowing experimental treatment replication. Nutrient dynamics, nutrient removal and stocking density standardization of Koi Carp under aquaponic systems were studied by conducting an experiment on Koi Carp fingerlings stocked at three different stocking densities for 60 d.

The experimental design consisted of five treatments, each having three assigned replicates. Different stocking densities of Koi Carp, that is, 1.4 kg/m^3 (40/tank), 2.1 kg/m^3 (60/tank), and 2.8 kg/m^3 (80/tank) were assigned in three treatments as T1, T2, and T3, respectively, with spinach plantlets in hydroponic tanks at 28 plants/m^2. S1 and S2 were the treatments with stocking density of Koi Carp fingerlings at 2.1 kg/m^3 (60/tank) and 2.8 kg/m^3 (80/tank), respectively, without spinach plants to compare nutrient accumulation (nitrate, potassium, phosphate) in the absence of plants. Control (C) led at the same rate was set with stocking density of Koi Carp at 1.4 kg/m^3 (40/tank) without plants in hydroponic tanks with three replicates. Flow rate was maintained as 1.5 L/min (constant flow) in all treatments and controlled throughout the experiment. Spinach growth in the aquaponic system was compared with traditional agriculture practice treatment (A), which was grown on manured soil. Initially, the system was operated for 2 wk with fish to enhance ammonia level for nitrifying bacteria growth. After 14 d (2 wk), spinach plantlets were transplanted to the hydroponic component of the system.

Each aquaponic recirculating system consisted of a fish tank, two hydroponic tanks filled with gravel, a submersible pump with pipe arrangement, and ball valves to regulate the water flow (Fig. 1). Pipelines (15 mm) made of polyvinyl chloride were installed to connect the fish culture tank and hydroponic tanks to recirculate water.

Rectangular tanks of 180 L ($81.2 \times 57 \times 38.8$ cm) capacity each were selected for growing fish. Water was filled up to 120 L. The fish tank was installed with a submersible water pump (Sobo, Zhongshan, China, WP1650 1500 L/h at 1.5 m head) for water circulation. Two halves of a HDPE barrel each having size of 91×57 cm with a depth of 29.4 cm were connected with one fish tank and used for hydroponics studies. Hydroponic tanks were filled with gravel ranging from 5 to 15 mm. Constant water flow from the fish tank to the hydroponics tank was regulated by 15 mm diameter ball valves provided at both sides of hydroponic tank and water from the hydroponic tank returned by gravity force to the fish tank through a 15 mm diameter polyvinyl chloride (PVC) drain pipe.

Fish

Fingerling Koi Carp were procured from commercial fish breeders. Before stocking, fish were disinfected by bath treatment of 5 ppm $KMnO_4$. Fingerling Koi Carp were stocked according to assigned stocking density. Average weight and length of fish were 4.58 ± 0.03 and 4.23 ± 0.03 cm. Fingerlings were provided artificial pelleted sinking feed with protein (32%), moisture (7.07%), either extract (8.31%), at 2% body weight twice a day.

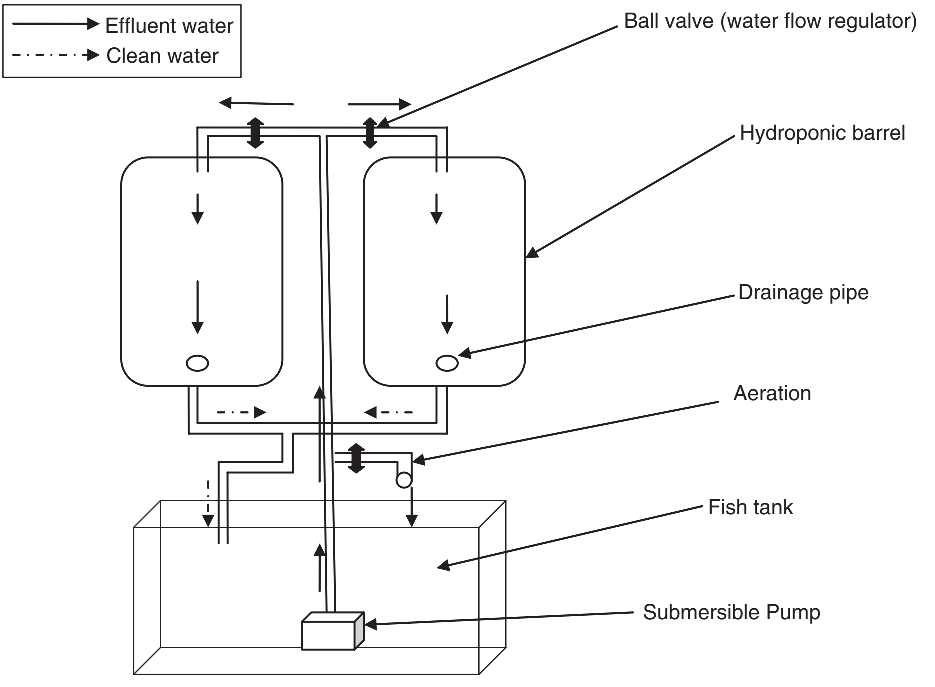

FIGURE 1. *Schematic diagram of experimental aquaponic system.*

Plants

Spinach seed was sown in nursery trays (52.5 × 27 cm) made of plastic and consisting of 140 cavities, filled with coconut husk medium, and allowed to grow for 15 d before transplanting into the experimental aquaponic systems. Plantlets of spinach were transplanted from the nursery tray to hydroponics tanks at $28/m^2$. The plant size at stocking was 7.05 ± 0.01 cm.

Sampling

Fish sampling was carried out at 15 d intervals for growth (length and weight) assessment and health check. Plant growth was monitored every 15 d by measuring the plant height, leaf length, and leaf width. After 60 d, spinach plants were harvested.

Sample Analysis

Water quality variables were analyzed during the experiment at 10 d intervals. Sampling was conducted between 08:30 and 09:30 at each sampling date, and samples were kept in a refrigerator at 4 C in labeled polythene bottles for chemical analysis. Water temperature was measured by thermometer, and pH was measured using universal pH indicator. Dissolved oxygen, free CO_2, hardness, alkalinity, ammonia, nitrite, and nitrate were analyzed by standard methods outlined in APHA (2005). Sodium, potassium, and calcium in the water samples were estimated by flame atomic emission spectrometry (FAES) using a flame photometer (Elico CL 378, Hyderabad, India).

Magnesium, iron, and zinc were analyzed by digesting water samples using Supra Pure concentrated acids (Merck, Darmstadt, Germany) in a microwave-based digestion system (Microwave 3000, Anton Parr, Graz, Austria). Digested samples were diluted to 50 mL each and subjected to analysis by atomic absorption spectrophotometer (Analyst 800, Perkin Elmer, Waltham, MA, USA) using flame atomization.

Statistical Analysis

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 a significance level of

TABLE 1. *Physico-chemical variables and nutrient dynamics for different treatments over a period of 60 d.*[1]

Variables	C	T1	T2	T3	S1	S2
Temperature (C)	$24.03^a \pm 0.62$	$24.08^a \pm 0.62$	$24.08^a \pm 0.62$	$24.0^a \pm 0.59$	$24.08^a \pm 0.60$	$24.06^a \pm 0.57$
Salinity (ppt)	$1.60^a \pm 0.08$	$1.60^a \pm 0.08$	$1.61^a \pm 0.08$	$1.60^a \pm 0.08$	$1.60^a \pm 0.08$	$1.60^a \pm 0.08$
pH	$7.33^a \pm 0.02$	$7.33^a \pm 0.02$	$7.32^a \pm 0.01$	$7.33^a \pm 0.01$	$7.33^a \pm 0.02$	$7.34^a \pm 0.01$
DO (mg/L)	$6.66^b \pm 0.23$	$6.79^b \pm 0.21$	$6.11^{ab} \pm 0.0.32$	$5.77^a \pm 0.28$	$6.10^{ab} \pm 0.28$	$5.65^a \pm 0.29$
Free CO_2 (mg/L)	$4.49^a \pm 0.17$	$4.44^a \pm 0.15$	$4.59^a \pm 0.15$	$5.18^a \pm 0.43$	$4.81^a \pm 0.20$	$5.06^a \pm 0.41$
Hardness (mg/L)	$349.64^a \pm 3.73$	$347.95^a \pm 3.52$	$347.86^a \pm 3.52$	$348.71^a \pm 3.75$	$348.83^a \pm 3.75$	$348.80^a \pm 3.37$
Alkalinity (mg/L)	$235.57^a \pm 5.51^a$	$239.71^a \pm 4.05$	$238.62^a \pm 6.25$	$237.29^a \pm 5.77^a$	$238.14^a \pm 4.95$	$238.0^a \pm 5.48$

[1]Mean values with same superscript did not show any significant difference ($P > 0.05$).

($P < 0.05$) at 95% confidence limit to know the significant difference between the treatments and control means for different variables.

Results and Discussion

Water Quality Variables and Nutrient Dynamics

Water temperature during the study varied within a range of 23–26.5 C, with no marked variation among the treatments and control. Mean dissolved oxygen in all treatments and control varied significantly ($P \leq 0.05$). Dissolved oxygen was highest in T1 (6.79 ± 0.21), C (6.66 ± 0.23); whereas, the lowest dissolved oxygen concentration was in T3 (5.77 ± 0.28) and S2 (5.87 ± 0.31). During the experiment, dissolved oxygen varied from 7.15 to 4.9 mg/L. The concentration of free CO_2 varied from 3.92 to 5.93 mg/L and did not show any significant difference among treatments and Control ($P > 0.05$) (Table 1).

Water quality variables were within the ranges suitable for Koi Carp culture. Temperature, pH, dissolved oxygen, and free CO_2 in all treatments and Control were within the ranges suitable for rearing Koi Carp. Total alkalinity and total hardness were higher in the treatments and control groups, but all the values were within the desired levels.

Ammonia nitrogen (NH_4^+–N) varied significantly ($P \leq 0.05$) and was highest in S2 (0.27 ± 0.03) followed by S1 (0.19 ± 0.02), C (0.14 ± 0.020), T3 (0.10 ± 0.02), T2 (0.072 ± 0.02), and T1 (0.051 ± 0.02) (Table 2). Ammonia ranged from 0.05 to 0.27 mg/L. The suggested value of ammonia in a recirculating aquaculture system should be less than 1.00 mg/L (Nijhof and Bovendeur 1990; Van Rijn and Rivera 1990). Ammonium nitrogen (NH_4^+–N) was significantly higher in treatments without plants (S1 and S2) compared to treatments with plants (T1, T2, and T3). Lower NH_4^+–N in the treatments with plants indicated the efficiency of spinach plants to uptake nutrients from the culture wastewater in the aquaponics system. Lin et al. (2002) reported 86–98% removal of ammonium nitrogen (NH_4^+–N) from constructed wetlands system receiving aquaculture waste water. Ammonium (NH_4+) is a major source of inorganic nitrogen taken up by the roots of higher plants (Vaillant et al. 2004). There may be less reliance on nitrification for ammonia removal when sufficient plants are present in the aquaponic systems.

The nitrite–N concentration varied significantly ($P \leq 0.05$) among treatments, with highest value observed in S2 (0.165 ± 0.03) followed by S1 (0.128 ± 0.02), C (0.103 ± 0.02), T2 (0.060 ± 0.01), T3 (0.056 ± 0.01), and T1 (0.047 ± 0.01) (Table 2). Nitrite–N was significantly different among treatments. Less nitrite was observed in integrated systems (T1, T2, and T3) because plants and bacteria removed ammonia efficiently. Verhagen et al. (1995) reported plant roots are more competitive for ammonium than oxidizing bacterial species (*Nitrosomonas europaea*). Increasing the nitrite content in water exerts more stress on the fish and leads to growth suppression, tissue damage, and mortality (Lewis and Morris 1986). In our study, all variables were in the range suitable for Koi Carp culture. Nitrate–N levels were significantly different among treatments. Treatments S2, S1, and C showed higher nitrate levels compared to treatments with plants (T1,

Table 2. *Physico-chemical variables and nutrient dynamics for different treatments over a period of 60 d.*[1]

Variables (mg/L)	C	T1	T2	T3	S1	S2
Ammonia	$0.14^{bc} \pm 0.02$	$0.05^{a} \pm 0.02$	$0.072^{ab} \pm 0.01$	$0.10^{ab} \pm 0.02$	$0.19^{c} \pm 0.02$	$0.27^{d} \pm 0.03$
Nitrite–N	$0.103^{ab} \pm 0.01$	$0.047^{a} \pm 0.01$	$0.060^{a} \pm 0.01$	$0.056^{a} \pm 0.01$	$0.128^{bc} \pm 0.02$	$0.165^{d} \pm 0.03$
Nitrate–N	$16.63^{bc} \pm 3.85$	$3.24^{a} \pm 0.59$	$4.25^{a} \pm 0.89$	$9.93^{ab} \pm 1.91$	$17.86^{bc} \pm 4.02$	$23.44^{c} \pm 5.27$
Phosphate	$1.18^{ab} \pm 0.29$	$0.58^{a} \pm 0.11$	$0.71^{a} \pm 0.14$	$1.03^{ab} \pm 0.28$	$1.30^{ab} \pm 0.28$	$1.708^{c} \pm 0.42$
Potassium	$20.17^{b} \pm 0.43$	$13.94^{a} \pm 1.10$	$14.28^{a} \pm 1.04$	$14.26^{a} \pm 1.02$	$20.31^{b} \pm 0.63$	$20.71^{b} \pm 0.70$
Calcium	$128.65^{b} \pm 1.04$	$124.83^{a} \pm 0.54$	$125.75^{a} \pm 0.52$	$125.33^{a} \pm 0.61$	$128.64^{b} \pm 1.05$	$128.55^{b} \pm 1.05$
Magnesium	$52.37^{b} \pm 0.26$	$44.98^{a} \pm 1.89$	$45.28^{a} \pm 1.78$	$45.34^{a} \pm 1.78$	$52.47^{b} \pm 0.29$	$52.470^{b} \pm 0.30$
Sodium	$348.97^{b} \pm 6.39$	$321.83^{a} \pm 1.66$	$322.15^{a} \pm 1.60$	$321.83^{a} \pm 1.89$	$356.98^{b} \pm 8.40$	$358.85^{b} \pm 7.39$
Iron	$0.047^{b} \pm 0.00$	$0.022^{a} \pm 0.01$	$0.0250^{a} \pm 0.01$	$0.025^{a} \pm 0.01$	$0.053^{b} \pm 0.00$	$0.0051^{b} \pm 0.00$
Zinc	$0.103^{b} \pm 0.00$	$0.067^{a} \pm 0.01$	$0.066^{a} \pm 0.01$	$0.067^{a} \pm 0.01$	$0.11^{b} \pm 0.00$	$0.10^{b} \pm 0.00$

[1]Mean values with same superscript did not show any significant difference ($P > 0.05$).

T2, and T3) (Table 2). This showed spinach plants effectively removed nitrates.

Nitrate–N is relatively harmless, and it is the preferred form of nitrogen for growing higher plants (Rakocy et al. 2006). Nitrate–N is not generally of great concern to target species such as Koi Carp, and aquatic species can tolerate extremely high nitrate–N concentration that is more than 25 mg/L. Ebeling et al. (1993) found many fish produced in aquaculture systems can tolerate nitrate concentrations exceeding 25 mg/L. Poxton and Allouse (1982) recommended NO_3–N concentrations should not exceed 50 mg/L in waters used for culturing of fish and shellfish. In this study, NO_3–N concentrations were in the favorable ranges in all treatments and control.

The mean phosphate concentration varied significantly ($P \leq 0.05$) among all treatments. The highest phosphate concentration was in S2 (1.71 ± 0.42), followed by S1 (1.30 ± 0.28), C (1.18 ± 0.29); whereas the lowest value was observed in T1 (0.58 ± 0.11) (Table 2). Phosphate (PO_4–P) levels were significantly different among the treatments, and levels were lower in the treatments T1, T2, and T3 compared to S1 and S2 because of the phosphate utilization by spinach plants. Mean potassium concentration varied significantly ($P \leq 0.05$) among treatments, and levels were higher in treatments without plants.

Mean calcium and magnesium were significantly different ($P \leq 0.05$) among treatments and control. The higher concentration of calcium and magnesium was because of the hardness of source water. Treatments with plants (T1, T2, and T3) assimilated Ca and Mg in minute quantities, and observation of this study supports the findings of Resh (2004) who reported that at pH above 7, nutrient availability for plant uptake may be restricted, due to precipitation of Ca^{2+}, Mg^{2+}, Fe^{2+}, and Mn^{2+} to insoluble and unavailable salts. Mean sodium varied significantly ($P \leq 0.05$) among treatment. Sodium concentration was also high, because subsoil water site was located close to Versova coast. Although abundance of sodium in a natural water, spinach plants were grown well because the spinach is tolerant to salinity, and supports findings (Richards 1954) that reported spinach can be grown in high salt concentrations (130 mM), and also Robinson et al. (1983) reported salt stress does not result in any major reduction in the photosynthetic potential of the spinach leaf. Mean iron had negligible variation, and it ranged between 0.02 and 0.053 mg/L. Mean zinc concentration was significantly different ($P \leq 0.05$) among treatments (Table 2).

Percentage Removal of Nutrients

The percentage nutrient removal at the end of 60 d was significantly different ($P \leq 0.05$) among treatments. Highest nitrate removal was in T1 (80.01%) followed by T2 (75.07%), and lowest was in T3 (57.83%). Percentage of phosphate removal was significantly different among treatments ($P \leq 0.05$). The highest percent phosphate was removed in T1 (53.18%), followed by T2 (48.58%), and the lowest was observed in T3 (39.14%). Endut et al. (2009) also found total

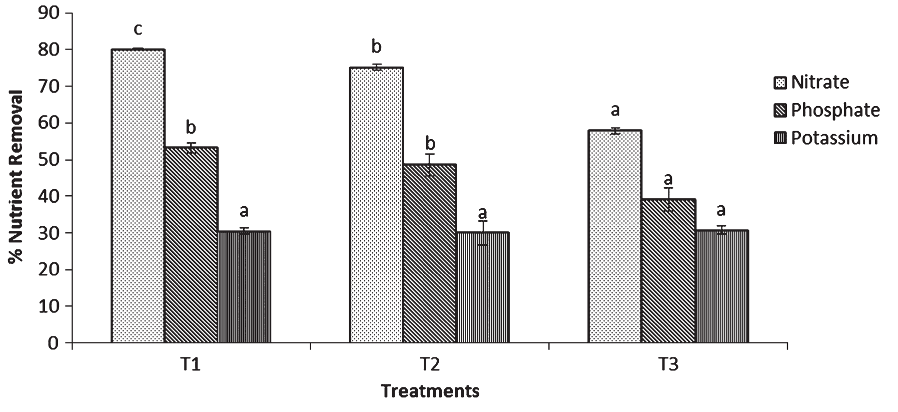

FIGURE 2. *Percent nutrient removal at the end of 60 d in different treatments. (Same superscript did not show any significant difference [P > 0.05]).*

phosphorus removal rates varied from 43 to 53% in aquaponic systems. Potassium removal did not show any significant difference among treatments ($P > 0.05$). Percent removal of potassium varied from 29.99 to 30.46% (Fig. 2).

In this study, the percentage of nitrate removal in aquaponic systems was also studied (Fig. 2). Highest removal was observed in T1 followed by T2 and T3. This is because stocking density chosen in T3 (2.8 kg/m^3) was higher and in turn there was more nutrient release as compared to T1 and T2. Seawright et al. (1998) found the same results of ammonia production, and subsequent nitrification was clearly increasing with higher stocking densities. From the above results, 1.4 kg/m^3 (T1) can efficiently fulfill the nutrient requirements of spinach plants ($28/m^2$), and also higher density of plants may be needed to improve nitrate removal aquaponic systems with higher fish densities. Lennard and Leonard (2006) reported 90.9% removal of nitrate using gravel bed as media for lettuce production. Ghaly et al. (2005) examined using hydroponically grown barley for removal of NO_3–N from aquaculture wastewater, and reported NO_3–N reductions ranging from 54.7 to 91.0%.

Percentage removal of phosphate was observed in decreasing order in T1, T2, and T3. Highest phosphate removal was in T1 and T2, while the lowest removal was in T3. Lower percentage removal of phosphate found in T3 was because of higher phosphate accumulation in the water because of high stocking density of fish as compared to other treatments. Similarly, Lin et al. (2002) reported construction of a wetlands system receiving aquaculture effluent effectively removed 32%–71% phosphate. Ghaly et al. (2005) examined using hydroponically grown barley for removal of PO_4–P from aquaculture wastewater and reported 91.8%–93.6% PO_4–P removal.

Potassium concentration was significantly different among the control and treatments (Fig. 2). Percentage potassium removal was studied at the end of the experiment, which showed potassium removal was from 29.50 to 28.52%. Ghaly et al. (2005) examined using hydroponically grown barley for potassium removal from aquaculture wastewater and reported potassium reduction ranging from 99.6 to 99.8%. Dontje and Clanton (1999) reported 25%–71% potassium removal in recirculating aquaculture systems using cattails, reed canary grass, and tomatoes grown in sand beds. Mant et al. (2003) achieved 24.9% potassium removal using *Salix viminalis* grown in a gravel hydroponic system to treat primary settled sewage. In this study, potassium was not supplemented from an outside source to the aquaponic system. Ground water used for the study contained 18.8 mg/L potassium.

Effect of Stocking Density on Growth Rate of Koi Carp Under Aquaponic System

The body weight of the Koi Carp at harvest among all treatments including control varied

TABLE 3. *Growth performance of Koi Carp under different stocking densities in different treatments and control over a period of 60 d.*[1]

	Treatments					
Variables Stocking density	C 1.4 kg/m^3 (without plant)	T1 1.4 kg/m^3 (with plant)	T2 2.1 kg/m^3 (with plant)	T3 2.8 kg/m^3 (with plant)	S1 2.1 kg/m^3 (without plant)	S2 2.8 kg/m^3 (without plant)
Fish growth variables						
Initial weight (g)	$4.22^a \pm 0.01$	$4.24^a \pm 0.01$	$4.23^a \pm 0.01$	$4.22^a \pm 0.01$	$4.24^a \pm 0.02$	$4.24^a \pm 0.04$
Initial length (cm)	$4.70^a \pm 0.06$	$4.66^a \pm 0.09$	$4.56^a \pm 0.01$	$4.46^a \pm 0.03$	$4.53^a \pm 0.09$	$4.60^a \pm 0.06$
Final weight (g)	$6.81^d \pm 0.09$	$7.03^e \pm 0.03$	$5.69^c \pm 0.13$	$5.39^b \pm 0.06$	$5.50^b \pm 0.03$	$5.07^a \pm 0.02$
Final length (cm)	$6.86^b \pm 0.12$	$6.90^b \pm 0.13$	$6.10^a \pm 0.06$	$5.83^a \pm 0.09$	$6.26^a \pm 0.09$	$5.93^a \pm 0.03$
Percentage weight gain	$61.20^d \pm 0.63$	$65.68^d \pm 1.28$	$34.56^c \pm 3.07$	$27.72^b \pm 1.19$	$29.62^b \pm 0.78$	$19.57^a \pm 0.23$
SGR	$0.795^d \pm 0.01$	$0.841^d \pm 0.013$	$0.493^c \pm 0.038$	$0.407^b \pm 0.02$	$0.432^b \pm 0.01$	$0.298^a \pm 0.00$
FCR	$2.370^a \pm 0.02$	$1.952^a \pm 0.29$	$3.909^b \pm 0.37$	$4.687^c \pm 0.25$	$4.416^{bc} \pm 0.11$	$6.493^d \pm 0.06$
FER	$0.42^b \pm 0.01$	$0.54^b \pm 0.01$	$0.26^a \pm 0.09$	$0.21^a \pm 0.03$	$0.22^a \pm 0.01$	$0.15^a \pm 0.00$
PER	$1.31^d \pm 0.01$	$1.40^d \pm 0.02$	$0.81^c \pm 0.08$	$0.67^b \pm 0.03$	$0.70^{bc} \pm 0.02$	$0.48^a \pm 0.00$
Survival rate	100^a	100^a	100^a	$98.33^b \pm 0.33$	100^a	$98^b \pm 0.58$

SGR, specific growth rate; FCR, feed conversion ratio; FER, feed efficiency ratio; PER, protein efficiency ratio.
[1]Mean values with same superscript did not show any significant difference ($P > 0.05$).

significantly ($P \leq 0.05$). The highest growth was observed in T1 (7.03 ± 0.03 g) followed by C (6.81 ± 0.01 g), while S2 (5.07 ± 0.02 g) had significantly lower growth (Table 3).

Fish stocking density is one of the sensitive factors determining productivity of a culture system as it affects growth rate, size variation, and mortality. To find optimal stocking density is one of the basic factors of aquaponic systems. In this study, Koi Carp fingerlings stocked at different densities (1.4, 2.1, and 2.8 kg/m^3) had different growth rates. Total weight gain, length gain, percentage of weight gain, SGR, FER, and PER were higher in T1 and C, which were stocked at the rate of 1.4 kg/m^3 followed in decreasing order by T2, S1, T3, and S2 and results showed increased density reduced growth rate. Similar studies conducted by Shelton et al. (1981) showed increasing stocking density had a profound negative impact on the growth of grass carp, *Ctenopharyngodon idella*, in small impoundments.

Fish fed with 2% body weight in all treatments and control. Similarly Licamele (2009) reported 5 kg of *Oreochromis niloticus* fed 2% biomass per day throughout the culture period of lettuce. Feed Conversion Ratio (FCR) varied significantly in all the treatments ($P \leq 0.05$). The FCR was better in T1 and C as compared to all other treatments. At higher stocking densities, T3, S2 and S1, FCR were comparatively higher because fish did not consume 2% feed given to them, and that might be because of stress in higher stocking densities. During the last three samplings, this was the possible reason for increased FCR. Similarly, results in this study are supported by the findings of Bernier and Peter (2001). Reduced food intake levels and/or disruption of the feeding behavior is a common feature of the behavioral response to stress in fish. Declining fish growth rate and feed utilization with increasing levels of stocking densities was observed by Vijayan and Leatherland (1988), while increase in FCR with increased stocking density was reported by Imanpoor et al. (2009) in common carp, *C. carpio*, and Moradyan et al. (2012) in rainbow trout alvines, *Oncorhynchus mykiss*. The FCR of T1 and C is comparable with the results of Relic et al. (2012) who studied the aspects of welfare of common carp in tanks under recirculating aquaculture systems by stocking 40 fingerlings in 120 L of water.

Survival rates during the experiment were significantly higher in treatments T1, T2, S1, and C (100%), but T3 and S2 had 98% survival. Similarly, high survival rates were found by Shete et al. (2013) while studying the growth of goldfish, *Carassius auratus*, at different water circulation periods in a recirculating aquaculture system.

TABLE 4. *Growth performance of spinach in different treatments over a period of 60 d.*[1]

	Treatments			
Variables	A (in soil)	T1	T2	T3
Initial height (cm)	$7.06^{a} \pm 0.09$	$7.10^{a} \pm 0.058$	$7.03^{a} \pm 0.09$	$7.02^{a} \pm 0.03$
Final height (cm)	$30.86^{d} \pm 0.29$	$25.50^{a} \pm 0.25$	$26.33^{b} \pm 0.17$	$29.66^{c} \pm 0.35$
Leaf length initial (cm)	$5.20^{b} \pm 0.06$	$5.03^{a} \pm 0.04$	$4.96^{a} \pm 0.03$	$5.07^{ab} \pm 0.04$
Leaf length final (cm)	$17.93^{d} \pm 0.03$	$14.76^{a} + 0.06$	$15.03^{b} \pm 0.09$	$17.10^{c} \pm 0.06$
Leaf width initial (cm)	$2.96^{a} \pm 0.15$	$3.10^{a} \pm 0.10$	$2.83^{a} \pm 0.17$	$2.93^{a} \pm 0.23$
Leaf width final (cm)	$9.50^{c} \pm 0.12$	$7.82^{a} \pm 0.08$	$8.03^{a} \pm 0.09$	$8.90^{b} \pm 0.20$
Percentage height gain	$417.44^{c} \pm 5.46$	$327.42^{a} \pm 4.09$	$332.18^{a} \pm 9.30$	$386.62^{b} \pm 5.46$
Yield (kg)	$1.666^{b} \pm 37.56$	$1.290^{a} \pm 30.55$	$1.351^{a} \pm 25.21$	$1.600^{b} \pm 28.16$

[1]Mean values with same superscript did not show any significant difference ($P > 0.05$).

At the end of the 60 d experiment, percentage weight gain also varied significantly ($P < 0.05$). T1 had the highest percentage of weight gain as compared to all treatments and control. Among the treatments and control, the highest percentage weight gain was observed in T1 (65.68 ± 1.2%) followed by C (61.20 ± 0.63%). Lowest weight gain was observed in S2 (19.57 ± 0.231). Fedlite and Milstein (1999) reported common carp were extremely sensitive to stocking density when food availability was not a problem, but increased stocking density reduced the space available for fish. Violating behavior requirements for space can affect the growth through endocrine responses or disruption to feeding efficiency (Pankhurst and Van der Kraak 1997; Schreck et al., 1997). The SGR of T1 (0.84 ± 0.01) and C (0.79 ± 0.01) were significantly higher as compared to all other treatments ($P \leq 0.05$).

Plant Growth Parameters

The overall height of spinach at harvest in all treatment groups varied significantly ($P \leq 0.05$); while, the height of spinach plant was highest in A (30.86 ± 0.29 cm) followed by T3 (29.66 ± 0.33 cm), T2 (26.33 ± 0.17 cm), and T1 (25.50 ± 0.25 cm). The leaf length of spinach at harvest in all groups was significantly different ($P \leq 0.05$) and was highest in A (17.93 ± 0.03 cm) followed by T3 (17.10 ± 0.05 cm), T2 (15.03 ± 0.09 cm), and T1 (14.76 ± 0.06 cm). The leaf width of spinach at the end of 60 d varied significantly in all the treatments and was observed highest in A (9.50 ± 0.12 cm) followed by T3 (8.90 ± 0.20 cm), T2 (8.03 ± 0.09 cm), and T1 (7.82 ± 0.08 cm).The percent of height gain of spinach at the end of 60 d varied significantly ($P \leq 0.05$). Further, A (417.44 ± 5.46) showed significantly higher percentage of length gain followed by T3; whereas, T1 and T2 did not show any significant difference. The yield of spinach at the end of 60 d varied significantly ($P \leq 0.05$). The highest yield was observed in A (1.666 ± 37.56 kg) followed by T3 (1.600 ± 28.16 kg); whereas, T2 (1.351 ± 25.21 kg) and T1 (1.290 ± 30.55 kg) did not show any significant difference (Table 4).

Lennard and Leonard (2006) compared three different types of hydroponic subsystems (gravel bed, floating raft, and Nutrient Film Technique [NFT]) and recommended gravel bed or floating raft hydroponic subsystem in an aquaponic system. Therefore, a gravel bed hydroponic subsystem was used in this study for growing spinach. Growth of spinach in terms of height gain, leaf width, and leaf length was higher in A, followed by T3, T1, and T2. But yield did not show any significant difference between A and T3. Watten and Busch (1984) reported yield of tomatoes was better in hydroponics as compared to field trial and supplemented inorganic nutrients (N, P, K, S, Fe, Mn, B, Zn, Cu, and Mo) in the aquaponic system. In this study, overall growth variables of spinach plants were highest in A (field) as compared to aquaponic treatments, because in the aquaponic system treatments were not supplemented with any inorganic nutrients throughout the experiment. From the results, it is clear overall growth variables of spinach were better in T3 as compared to other treatments, T2 and T1, as it had higher fish to plant ratio.

From the results, T1 had greater growth in fish biomass when compared with treatments T2 and T3. However, in terms of greater plant biomass production, T3 yielded highest. Although T1 showed lower plant yield, fish reached similar marketable size as compared to the other groups. Thus, the stocking density T1 ($1.4\,kg/m^3$) was recommended as ideal stocking density of Koi Carp using a plant density of 28 plants/m^2. Similarly, these findings are supported by Klanian and Adame (2013) who recommended stocking tilapia fingerlings at $0.84-1.04\,kg/m^3$ for recirculating aquaculture systems.

Conclusion

From this study, it is concluded $1.4\,kg/m^3$ was considered an optimum stocking density for the production of Koi Carp with spinach. Water quality and nutrient removal was found best in aquaponic treatments as compared to the treatments without plants. Spinach plants effectively utilized the nutrients and maintained water quality which was ideal for Koi Carp production. However, percentage of nutrient removal was most efficient in treatment T1. This experiment suggests aquaponic systems can effectively reduce the dissolved nutrients in aquaculture production systems, but the ratio of fish density to plant density is important to maintain a sustainable system.

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