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Standardization of the stocking density ratios of Koi carp (*Cyprinus carpio* var. *koi*): Goldfish (*Carassius auratus*) in Polyculture Aquaponic Recirculating System

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Abstract

A 60 days experiment was conducted for standardization of the stocking density proportions of Koi carp (*Cyprinus carpio* var. *koi*): Goldfish (*Carassius auratus*) along with water spinach (*Ipomoea aquatica*), and for evaluate the performance of recirculating aquaponic system. Different stocking density ratios of Koi carp: Goldfish (1:1=T1; 1:2=T2; 2:1=T3 with aquaponics and 1:1=C without aquaponics) were tested with three replicates, at an initial total fish density of 0.8 kg/m^3. Growth of Goldfish was not significant different ($P \geq 0.05$) among the treatments. However, the T2 group showed the highest growth for the Koi carp ($P<0.05$). Significantly higher plant growth (mean plant height 62.98±1.44 cm) was observed for the T2 fish density ratio. Nutrient removal (NO_3-N, PO_4-P and K) was significantly higher for the T2 density ratio. Thus, Koi carp: Goldfish of 1:2 can be suggested as the optimum stocking density ratio in polyculture aquaponic recirculating system with *I. aquatica*.

Key words: aquaponics, Goldfish, Koi carp, water spinach, stocking density ratio

Introduction

Aquaponic production, which combines aquaculture and hydroponics, is a suitable solution for the emerging problems as limited water and land resources. The treatment of waste water using aquaponic plants is one of the best, cost-effective and beneficial phytoremediation strategies. In aquaponic systems, the nutrient enriched water that results from raising fish provides a source of natural fertilizer for growing plants. Compared to conventional aquaculture methods, it creates a sustainable ecosystem, where both plants and fish can exists using less water and space, producing less pollutants. Aquaponics has several advantages over generalized recirculating aquaculture systems (RAS). Only the aquaponic systems have biofilter that generates income, which is obtained from the sale of hydroponic productions such as vegetables, herbs and flowers (Rakocy & Hargreaves, 1993). Polyculture refers to production of multiple species using same water body. Polyculture of aquatic species in aquaponic recirculating is a form of agro-biodiversity and key to sustainable agriculture which decreases vulnerability to insects, pests and diseases. Among ornamental fishes, Goldfish and Koi carp, which belong to the Cyprinidae family, had been produced in captivity for a long time period (Watson, Hill, & Pouder, 2004). Capability of producing huge amount of

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wastes and hardy nature identified these two species as ideal for aquaponics. Few plants have been tested and identified as economically viable in aquaponic recirculating systems. Vegetables are candidate plants for use in recirculating hydroponic systems as they grow rapidly in response to the high levels of nutrients in water. *Ipomoea aquatica*, a leaf vegetable, which belongs to the family Convolvulaceae, is supposed to be originated from China and native to tropics and subtropics. It can grow wild and sometimes cultivated in Southeast Asia, India and Southern China (Fu et al., 2011; Gothberg, Greger, & Bengtsson, 2002). Considering its habitat and the value of nutrient uptake, it can be used efficiently in aquaponic recirculating system.

Goldfish and Koi carp have previously been identified as suitable species in monoculture aquaponic recirculating systems (Shete et al., 2013; Shete, Verma, Kohli, Dash, & Tandel, 2014; Hussain et al., 2014, 2015). There are sufficient evidences in using these species in monoculture aquaponic recycling system; but, there is no published literature available with regard to their polyculture, neither density ratio in aquaponics. Therefore, the aim of this study is to standardize the stocking density ratio of Koi carp:Goldfish and determine the production potential of these species under polyculture aquaponic recirculating system using water spinach as a secondary crop.

Materials and Methods

The experiment on aquaponic recirculating system was designed and carried out in Central Institute of Fisheries Education, Mumbai, India. The system consisted of 9 individual and identical aquaponics units as described by Hussain et al. (2014). The experiment was conducted with Koi carp (*Cyprinus carpio* var. *koi*) and Goldfish (*Carassius auratus*) along with water spinach (*Ipomoea aquatica*) in the recirculating aquaponic units during 60 days. Three different stocking density ratios of Koi carp: Goldfish (1:1=T1; 1:2=T2; 2:1=T3) were assigned as experimental treatments and the control group (C) consisted of the 1:1 density ratio but rearing fishes without aquaponics. All treatments and control were stocked with 0.8 kg/m^3 total fish density and initial fish density was stocked according to the weight of fish. Different stocking density (kg/m^3) ratios of Koi carp: Goldfish (0.4:0.4=T1; 0.26:0.54=T2; 0.54:0.26=T3; 0.4:0.4=C) were used in treatments. Each treatment having three randomly assigned replicates against control in which fish were stocked in tank without aquaponics and were maintained with normal feeding and water exchange. In all treatments and control, the fingerlings were provided with artificial pelleted feed @ 2% of their body weight twice a day (10.00 am and 5.00 pm). Water spinach (28 plants/m^2) was grown in 18 hydroponic tanks (gravel bed system) and field condition (soil filled to hydroponic tanks instead of gravel without fish tank) as a control to compare with aquaponic system. Constant water flow rate of 2.4 L/min was maintained in all treatments throughout the experiment. Plant growth, fish growth, nutrient dynamics, nutrient removal and water quality parameters were measured and analyzed ascertaining the effect from different compositions for the polyculture aquaponic recirculating system.

Stocking

Fingerlings of Koi carp and Goldfish were stocked according to assigned stocking density ratios. The average weight and length of the Koi carp (*Cyprinus carpio* var. *koi*) were 0.30±0.001 g and 2.39±0.04 cm, respectively. The

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average weight and length of the Goldfish (*Carassius auratus*) were 1.55±0.01 g and 4.19±0.05 cm, respectively. Two different sizes of fishes were used in two species. Plantlets of water spinach (5.17±0.17 cm) were transplanted from seedling trays to hydroponic tanks.

Sampling

Sampling of fishes was carried out at 15 days interval for assessment of growth (length and weight). Sampling of plants was done by measuring the height of plants and length of leaves. Growth parameters: weight gain percentage, specific growth rate (SGR), feed conversion ratio (FCR), feed efficiency ratio (FER), and protein efficiency ratio (PER) were estimated.

$$\text{SGR (\% / Day)} = \frac{\text{Log}_e\ \text{Final Weight} - \text{Log}_e\ \text{Initial Weight}}{\text{No. of days}} \times 100$$

$$\text{FCR} = \frac{\text{Net Weight Gain (Wet Weight in g)}}{\text{Feed Given (Dry Weight in g)}}$$

$$\text{FER} = \frac{\text{Net Weight Gain (Wet Weight in g)}}{\text{Feed Given (Dry Weight in g)}}$$

$$\text{PER} = \frac{\text{Net Weight Gain (Wet Weight in g)}}{\text{Protein Feed (g)}}$$

Water quality parameters were measured at an interval of 10 days. Thermometer and pH meter (OAKTON water proof pH tester 30) were used to measure temperature and pH respectively. The standard methods outlined in APHA (2005) were used to measure the water quality parameters such as dissolved oxygen, free carbon dioxide, total hardness, alkalinity, total suspended solids, ammonia, nitrite, nitrate, and phosphorus. Sodium, potassium, and calcium were estimated by flame atomic emission spectrometry (FAES) using a flame photometer (Elico CL 378, India). Mg, Fe and Zn were analyzed by atomic absorption spectrophotometer (Analyst 800, Perkin Elmer, USA) using flame atomization.

Statistical Analysis

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The data were analyzed using statistical package SPSS version 16. Significance level of ($P<0.05$) at 95% confidence limit was used to know the significant difference between the treatments and control means for different parameters by one way ANOVA and Turkey multiple range test.

Results

Fish growth parameters

The body weight of Goldfish at the time of harvest did not show any significant difference ($p>0.05$) between the treatments. The body weight of Koi carp in T2 only showed significant difference ($p<0.05$) with the other treatments at the time of harvest. The highest growth of Koi carp was obtained in T2 (2.39±0.014 g) followed by T3 (2.35±0.023 g), T1 (2.27±0.008 g) and C (2.24±0.030 g). Percentage weight gain of Goldfish did not show any significant difference ($p>0.05$) between treatments. As far as percentage weight gain was concerned, Koi carp in T2 showed significant difference ($p<0.05$) with the control. The highest percentage weight gain was obtained in T2 (687.13±12.65%) followed by T3 (644.49±3.08%), T1 (641.58±3.59%) and C (635.51±6.18%). Specific growth rate of Goldfish did not show any significant difference ($p>0.05$) between treatments. Specific growth rate of Koi carp in T2 showed significant difference ($p<0.05$) with the other treatments and T2 (3.44±0.03%) showed the highest specific growth rate, followed by T3 (3.34±0.01%), T1 (3.33±0.01%) and C (3.32±0.03%). Mean length of Goldfish did not show significant difference ($p>0.05$) among all treatments. Considering the Koi carp mean length of T2 (4.96±0.02 cm) showed the highest length followed by T1 (4.85±0.03 cm), T3 (4.69± 0.05 cm) and C (4.4±0.05 cm). According to the results, Koi carp in T1 and T2 showed significantly different ($p<0.05$) with C and T3. FCR, PER and FER of Goldfish and Koi carp did not show significant difference ($p>0.05$) between treatments (Table 1).

Plant growth parameters

The overall height of water spinach at the time of harvest in all treatment groups varied significantly ($p<0.05$); while, the height of water spinach plants were the highest in T2 (Table 2). The leaf length of water spinach at the time of harvest was significantly different ($p<0.05$) only between T2 and T3. The highest leaf length was obtained in T2 (12.92±0.80 cm). The percentage height gain of water spinach in T2 at the end of 60 days experimental period was significantly different ($p<0.05$) with other treatments and the control. Percentage height gain of T1 also showed significant different ($p<0.05$) with other treatments including control. Further, T2 showed the highest percentage height gain (1130.10±40.51%). The yield of water spinach at the end of 60 days experimental period varied significantly ($p<0.05$) in all treatment groups; while, the highest yield was obtained in T2 (1766.66±18.56 g). Thus, considering all parameters, the highest and significant plant growth was observed in T2 as compared to T1, T3 and C (Table 2).

Water quality parameters and nutrient dynamics

The mean dissolved oxygen (DO) content in T2 significantly varied ($p<0.05$) with T1 and T3. The DO in control was also significantly different ($p<0.05$) with T1 and T3. The DO value was the highest both in T1 and T3, and the

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lowest value was observed in C (Table 3). However, concentrations of DO were decreasing with the time period in all treatments. The concentration of free CO_2 in T2 varied between 2.0 - 6.7 mg/L during the experimental period and it showed the highest variation among other treatments and the control. However, free CO_2 level was increasing with the time period in all treatments and the control. The total alkalinity varied between 100-235 mg/L during the experimental period. The lowest mean alkalinity was recorded in T2 and it was significantly different ($p<0.05$) with other treatments and the control. Mean hardness of the water varied between 317.57 – 322.57 mg/L in all treatment and the control. The highest mean ammonia nitrogen (NH^+_4- N) was observed in C, and it varied significantly ($p<0.05$) with other treatments, which followed by T3, T1 and T2. The mean nitrite –nitrogen (NO_2-N) concentration varied significantly ($p<0.05$) among all treatments, while highest value was observed in C followed by T3, T1 and T2. The mean nitrate – nitrogen (NO_3-N) in C varied significantly ($p<0.05$) with the other treatments and the lowest NO_3-N was obtained in T2 which was not significantly different ($p>0.05$) with T1 and T3. The mean phosphate (PO_4-P) concentration during the experiment varied significantly ($p<0.05$) with the C and it showed the highest PO_4-P value when compared to other treatments. The lowest PO_4-P value was observed in T2 and it was not significantly different ($p>0.05$) with T3 and T1. However, PO_4-P values got increased with the movement of experimental period when considering all treatments and the control. The mean potassium concentration of T2 varied significantly ($p<0.05$) with other treatments and it showed the lowest mean potassium concentration. The highest potassium concentration was observed in C and it also showed significant difference ($p<0.05$) with treatments. However, T1and T3 did not show any significant difference ($p>0.05$) between two treatments. Considering the experimental time period, potassium level decreased in all the treatment except control (Table 3).

The percentage nutrient removal was calculated when compared to the control and significance levels were analyzed among treatments. Percentage NO_2-N removal did not show significant difference ($p>0.05$) between treatments. However, the highest percentage NO_3-N removal, was seen in T2 (50.56±1.98%) followed by T1 (48.66±1.94%) and T3 (41.61±2.27%). The highest PO_4-P removal was observed in T2 (43.94±3.74%) followed by T3 (40.24±1.15%) and T1 (29.45±0.86%) while T1 significantly different with T2 and T3. The potassium removal significantly varied ($p<0.05$) among all treatments and the highest value was observed in T2 (41.86±0.39%) followed by T1 (26.79±0.45%) and T3 (19.75±0.10%) (Fig. 1).

Discussion

Fish growth parameters

Monoculture and polyculture systems of male Nile tilapia (*Oreochromis niloticus)* and Australian red claw crayfish (*Cherax quadricarinatus*) were studied by Rouse and Kahn (1998). According to them, polyculture system negatively affected the fish growth; while in monoculture, the average weight was 76 g and in polyculture 48 g for Australian red claw crayfish. In the same experiment, considering the average weight of Nile tilapia, it had an average weight of 403 g when stocked comparatively at high densities and 444 g when stocked comparatively at low densities. Author suggested that lack of aggressiveness is cause for decreased growth in polyculture with tilapia. Because these species belong to the same family, they did not show any incompatible behavior in aquaponic

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recirculating system. Two different life cycle stages in fish species reduced the competition between the two fish species. The higher survival rate was observed in both species throughout the experimental period. Thus, in the present experiment; polyculture system did not affect the growth of both fish species. Considerable growth of Goldfish and Koi carp were observed in this experiment. Growth performance of Goldfish in aquaponics recirculating system was carried out by Shete et al. (2013) and observed that weight gain and SGR were comparable with present result. While considering the Koi carp, comparison of growth, survival rate, and number of marketable Koi carp produced under different management regimes were done by Jha, Barat, and Nayak (2006) and comparable results were observed. According to the results, stocking density of Goldfish did not affect the fish growth. However, Koi carp in T2 showed the highest FER and PER compared with other treatments and control. The lowest FCR obtained in T2 indicates the best feed utilization among all treatments. Thus, considering fish growth parameters, the stocking density of Koi carp: Goldfish, 1:2 ratio can be taken as the optimum composition for aquaponic recirculating system.

Plant growth parameters

A comparative study of 3 different hydroponic sub-systems (gravel bed, floating and nutrient film technique) in an aquaponic test system was conducted by Lennard and Leonard (2006) and reported the highest yield and biomass of *Lactuca sativa* in gravel bed system when compared to floating and Nutrient Film Technique (NFT) systems. Thus, in the present experiment, *Ipomoea aquatica* was used, and succeeded in gravel bed system as Lennard and Leonard (2006). In the present experiment, water spinach plants grew actively in hydroponic system and did not show any nutrient imbalance or deficiency symptoms during the experimental period of 60 days. Endut, Jusoh, Ali, Nik, and Hassan (2009) also did not observe any nutrient imbalance or deficiency symptoms using water spinach plants in aquaponic system in 28 days of experimental period. Trang, Schierup, and Brix (2010) also reported that *I. aquatica* is the most promising because of its high growth and nutrient uptake capacity when compared to *Lactuca sativa*, *Brassica rapa* var. *chinensis* and *Brassica rapa* var. *parachinensis*. Trang et al. (2010) also revealed that *I. aquatica* grew best in completely water-saturated substrate. In the present experiment also, higher and significant growth of *I. aquatica* was observed. An integrated fish tilapia (*Sarotherodon aurea*) and hydroponic tomato (*Lycopersicon esculentum*) production system was evaluated by Watten and Busch (1984). According to the reports, yield and quality of fruit produced by hydroponically exceeded that produced under field trial conditions. Considering the overall results, plant growth in the present experiment, T2 and T1 showed considerable growth that exceeded the growth under field trial condition. All these evidences proved the suitability of Goldfish and Koi carp with water spinach in recirculating aquaponic polyculture system. Thus, considering the plant growth, T2 can be suggested as the best stocking density ratio for acquaponic recirculating system.

Water quality parameters and nutrient dynamics

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In the present experiment, very low values of toxic compound showed proper nitrification cycle in all treatments when compared to control. The mean values of ammonia-N, nitrite-N and nitrate-N varied between 0.2-0.35 mg/L, 0.02-0.07 mg/L and 1.6-3.4 mg/L in T1, T2 and T3 respectively.

Sikawa and Yakupitiyage (2010) showed comparable result in above mentioned N-nutrients. Lower mean ammonia-N, nitrite-N values showed that the regular nitrification process occurred in all the treatments other than the control. Ammonia-N is the major nitrogenous excretory product of fishes and also occurs from the decomposition of uneaten feed. It is toxic to fish if gets accumulated in higher levels in fish production systems. According to Hargreaves and Tucker (2004), daily exposure (2 to 3 hours) to 0.92 mg/L of ammonia-N did not affect the growth and feed conversion ratio. In the present experiment, control (0.35 mg/L) showed the highest mean ammonia-N concentration. All values were found to be in nontoxic range. Tilapia and vegetable production in the UVI aquaponic system developed by Rakocy, Bailey, Shultz, and Thoman (2004) showed that 0.4 mg/L nitrite-N value is appropriate. In the present experiment, control showed the highest value (0.071 mg/L and others were very less when compared with the results obtained by Rakocy et al. (2004). Comparatively high nitrate-N value was found, in control and the lowest value was obtained in T2 when comparing the treatments. High plant growth in T2 showed the best consumption of nitrate-N by plants; thus, the lowest nitrate-N value was noticed in the T2. When compared to Rakocy et al. (2004), low nitrate-N value was found in this experiment; however, nutrient deficiency symptoms were not observed in plants. Nitrate-N is relatively nontoxic to fish and has no health hazards, except above 90 mg/L (Stone & Thomforde, 2004). In this aquaponic system, plants utilized the nitrate-N; thus, low concentration was available in the water. Removal of nitrate- N from the system influences the nitrification process in aquaponic system. Phosphorus is one of the essential minerals which must be supplied via diet. Bussel, Mahlmann, Kroeckel,Schroeder, and Schulz (2013) found 26 mg/L of ortho-P concentration which showed higher specific growth rates in Turbots. They also revealed the accumulation of ortho-P in RAS, not negatively affected for health of Turbot. In this experiment, mean phosphate (PO_4-P) concentration during the experiment varied between 1.31-0.74 mg/L in all treatments and significantly higher values were observed in control than other treatments. Treatment 2 showed the lowest value compared to other treatments due to high consumption of phosphate by plants. Total phosphate value showed by Sikawa and Yakupitiyage (2010) also compared with present results. Rakocy et al. (2004) externally supplemented calcium, potassium and iron to fulfill plant needs. In this experiment, potassium level varied between 52.28-41.95 mg/L among all treatments including control. Also external deficiency symptoms of any nutrients were not visible. Thus, supplementation of any nutrient was not done throughout the experiment.

Rana et al. (2011) identified nutrient removal from domestic wastewater by aquaponics system with tomato plants and showed more than 75% nitrate-N removal in all treatments. When considering the phosphate removal, T2 showed the highest (43.94%) phosphate removal. T1 also showed 40.24% phosphate removal and T3 having less plant growth, and thus, showed the lowest value. Rana et al. (2011) revealed that PO_4-P was removed by 58.14–74.83% in aquaponic system. Shete et al. (2016); Shete et al. (2017) also revealed the high efficiency of nutrient removal in an aquaponic system. The potassium removal significantly varied ($P<0.05$) among all treatments and the highest value was observed in T2 (41.86%) followed by T1 (26.79%) and T3 (19.75%). Keeratiurai (2013) identified

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the efficiency of wastewater treatment with hydroponics and showed 44.44% of potassium removal from fish wastewater. This system also revealed that the potassium was the most eliminated nutrient comparing to nitrogen and phosphorus.

According to Shete et al. (2014), 0.75 kg/m^3 fish density of Gold fish found to be the optimum stocking density. However, Hussain et al. (2015) suggested 1.4 kg/m^3 as the optimum stocking density for Koi carp production with spinach (*Beta vulgaris* var. *bengalensis*) in aquaponic recirculating systems. Thus range of 0.75 to 1.4 kg/m^3 stocking densities were used considering both species which used in monoculture system. According to present study, Gold fish and Koi carp 0.8 kg/m^3 total stocking density was used with three different Koi: Goldfish stocking density ratios. Among these ratios, Koi carp: Goldfish 1:2 provides significant higher plant growth, fish growth and nutrient removal in water. Thus, considering overall results of the present experiment, proportion of 1:2 (T2) Koi carp: Goldfish can be suggest as the optimum stocking density ratio for polyculture aquaponic recirculating system when total fish density was 0.8 kg/m^3. Further, similar stocking density was used with those two fish species and tested effect of water flow rate in aquaponic recirculating system by Nuwansi et al. (2016).

Conclusion

Based on the results obtained in the present study, it can be concluded that the nutrients do not accumulate at toxic levels in this integrated polyculture aquaponic recirculating system. Considering water quality parameters and nutrient dynamics, stocking density ratio of 1:2 (T2) Koi carp: Goldfish can be suggested as the optimum stocking density ratio among three proportions of stocking densities ratios of Koi: Goldfish (1:1 =T1; 1:2 =T2; 2:1 =T3). The suggested stocking density ratio was capable for high nutrient removal and less nutrient persistence due to high plant growth in aquaponics recirculating system. Plant height, yield, and fish growth (Koi carp) in T2, also suggested that the composition of Koi: Gold fish1:2 is more suitable for polyculture of Koi carp and Gold fish with water spinach for a aquaponic recirculating system.

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Table 1. Fish growth parameters

Parameter		Stocking density ratio Koi: Goldfish			
		1:1(C)	1:1(T1)	1:2(T2)	2:1(T3)
Body weight (g)	Goldfish				
	initial	1.53±0.01^{a}	1.55±0.01^{a}	1.56±0.01^{a}	1.55±0.01^{a}
	final	5.06±0.09^{a}	5.04±0.07^{a}	5.02±0.03^{a}	4.90±0.07^{a}
	Koi carp				
	initial	0.30±0.001^{a}	0.30±0.001^{a}	0.30±0.003^{a}	0.30±0.002^{a}
	final	2.24±0.030^{a}	2.27±0.008^{a}	2.39±0.014^{b}	2.35±0.023^{a}
Body length (cm)	Goldfish				
	initial	4.17±0.03^{a}	4.25±0.02^{a}	4.14±0.07^{a}	4.20±0.06^{a}
	final	5.79±0.05^{a}	5.81±0.04^{a}	5.79±0.05^{a}	5.69±0.09^{a}
	Koi carp				
	initial	2.39±0.03^{a}	2.39±0.03^{a}	2.38±0.06^{a}	2.40±0.03^{a}
	final	4.40±0.05^{a}	4.85±0.03^{b}	4.96±0.02^{b}	4.69±0.05^{c}
Percentage weight gain (%)	Goldfish	229.67±3.66^{a}	224.25±7.43^{a}	222.19±5.96^{a}	216.19±5.96^{a}
	Koi carp	635.51±6.18^{a}	641.58±3.59^{ab}	687.13±12.65^{b}	644.49±3.08^{ab}
Specific growth rate (%/ day)	Goldfish	1.98±0.02^{a}	1.96±0.04^{a}	1.94±0.01^{a}	1.91±0.03^{a}
	Koi carp	3.32±0.03^{a}	3.33±0.01^{a}	3.44±0.03^{b}	3.34±0.01^{a}
Survival rate %	Goldfish	93.33±3.33^{a}	100^{b}	100^{b}	100^{b}
	Koi carp	91.66±2.40^{a}	99.12±0.88^{b}	100^{b}	98.88±0.56^{b}
Feed conversion ratio (FCR)	Goldfish	1.95±0.03^{a}	1.96±0.05^{a}	2.01±0.01^{a}	2.06±0.05^{a}
	Koi carp	1.32±0.03^{a}	1.33±0.01^{a}	1.26±0.02^{a}	1.28±0.02^{a}
Feed efficiency ratio (FER)	Goldfish	0.51±0.01^{a}	0.51±0.01^{a}	0.50±0.00^{a}	0.49±0.01^{a}
	Koi carp	0.76±0.02^{a}	0.75±0.00^{a}	0.79±0.01^{a}	0.77±0.01^{a}
Protein efficiency ratio (PER)	Goldfish	1.60±0.02^{a}	1.60±0.04^{a}	1.55±0.01^{a}	1.52±0.04^{a}
	Koi carp	2.36±0.06^{a}	2.34±0.01^{a}	2.47±0.04^{a}	2.43±0.03^{a}

Values with same superscript did not show significant difference (P≥0.05)

Table 2. Plant growth parameters

Parameter	Stocking density ratio Koi: Goldfish			
	1:1(C)	1:1(T1)	1:2(T2)	2:1(T3)
Mean plant height (cm)	5.00±0.06^{a}	5.33±0.09^{a}	5.13±0.23^{a}	5.20±0.32^{a}
	22.03±0.58^{a}	38.33±1.20^{b}	62.98±1.44^{c}	21.94±0.75^{a}
Mean leaf length (cm)	3.36±0.18^{a}	3.40±0.11^{a}	3.56±0.88^{a}	3.50±0.03^{a}
	11.43±0.58^{a}	10.75±1.27^{a}	12.92±0.80^{b}	7.55±1.13^{c}
Percentage of height gain (%)	340.90±14.75^{a}	618.98±20.89^{b}	1130.10±40.51^{c}	323.52±13.49^{a}
Yield (g)	808.66±35.61^{a}	1215.01±12.76^{b}	1766.66±18.55^{c}	694.66±32.99^{d}

Values with same superscript did not show significant difference (P≥0.05)

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Table 3. Water quality parameters and nutrient dynamics

Parameter	Stocking density ratio Koi : Goldfish			
	1:1(C)	1:1(T1)	1:2(T2)	2:1(T3)
Temperature (^{0}C)	24.04±0.06^a	23.46±0.03^b	23.45±0.10^b	23.5±0.04^b
pH	7.63±0.02^a	7.5±.0.01^b	7.4±0.02^b	7.54±0.01^c
DO (mg l^{-1})	6.58±0.02^a	6.93±0.08^b	6.60±0.03^a	6.93±0.05^b
Free CO_2 (mg l^{-1})	2.70±0.03^a	3.12±0.07^a	4.04±0.04^b	2.96±0.04^a
Hardness (mg l^{-1})	317.57±0.24^{ab}	320.00±0.97^a	315.00±0.20^b	322.57±0.24^c
Alkalinity (mg l^{-1})	202.42±1.03^{ab}	200.19±1.22^a	171.05±1.28^c	205.87±0.57^b
Ammonia (mg l^{-1})	0.35±0.01^a	0.264±0.01^b	0.219±0.01^c	0.279±0.00^b
Nitrite- N (mg l^{-1})	0.07±0.001^a	0.04±0.001^b	0.02±0.000^c	0.05±0.002^d
Nitrate- N (mg l^{-1})	3.36±0.12^a	1.72±0.06^b	1.66±0.07^b	1.96±0.08^b
Phosphate (mg l^{-1})	1.31±0.11^a	0.93±0.01^b	0.74±0.05^b	0.78±0.02^b
Potassium (mg l^{-1})	52.28±2.04^a	38.27±0.23^b	30.39±0.20^c	41.95±0.05^b
Calcium (mg l^{-1})	363.35±3.23^a	384.38±5.89^b	360.10±0.44^a	377.20±1.02^b
Magnesium (mg l^{-1})	351.76±0.66^a	372.66±2.52^b	329.35±2.03^c	357.33±5.20^a
Sodium (mg l^{-1})	383.43±5.82^a	400.48±4.71^a	401.54±4.94^a	399.87±4.60^a
Iron (mg l^{-1})	0.24±0.01^a	0.27±0.02^a	0.22±0.03^a	0.29±0.00^a
Zinc (mg l^{-1})	0.035±0.002^a	0.033±0.001^a	0.026±0.002^b	0.030±0.001^a

Values with same superscript did not show significant differ

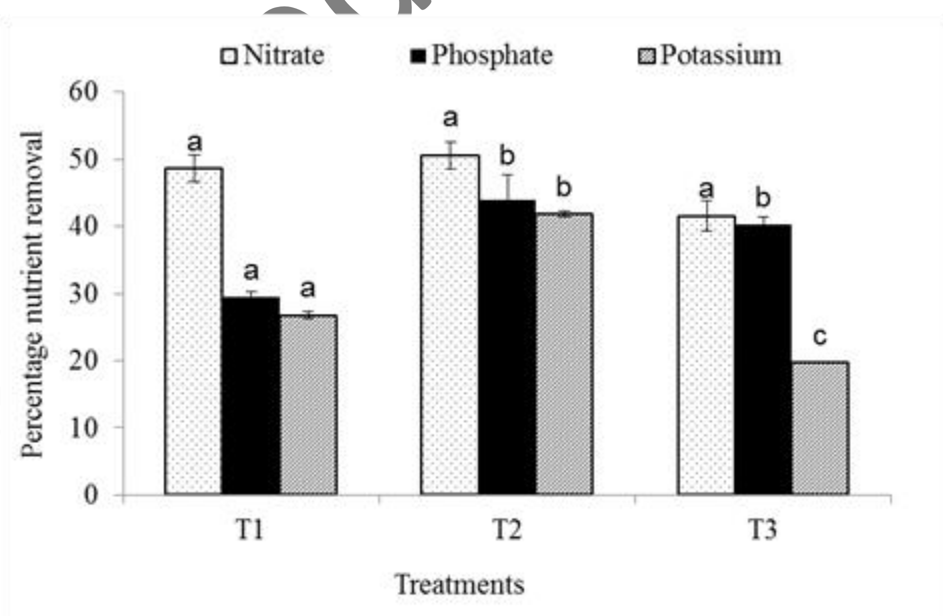

Figure 1. Percent nutrient removal at the end of 60 days in different treatments (Same superscript did not show any significant difference (P>0.05))

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