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Research Article

Effect of water hyacinth (*Eichhornia crassipes*) density on water quality, growth performance and survival of koi carp (*Cyprinus carpio carpio)* in an aquaponic system

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Abstract

The present study aimed to assess the effect of water hyacinth (*Eichhornia crassipes*) as a biofilter on water quality, growth performance and survival of koi carp (*Cyprinus carpio*) in an aquaponic system. Water hyacinth was used at three densities of 2 bushes, 4 bushes and 6 bushes. Each treatment contained 10 fish weighing 11.43±0.1 g (in 80 L aquarium) in 3 replicates, and the experiment was run at 22-24°C for 42 days. A control group was also included without the plant. The obtained results showed that there was no significant difference in dissolved oxygen (DO) level among the treatments while DO in all treatments were almost insignificantly higher than the control ($p>0.05$). Total dissolved solid in all treatments were significantly lower than control with the lowest level obtained in 4 bushes group ($p<0.05$). Total suspension solid level in all treatments were significantly higher than control ($p<0.05$). Also, PO_4 value was decreased with an increasing in the plant density. Both nitrite and nitrate in all treatments were lower than control group. Cadmium content of water was reduced with increasing in plant density. These values in all densities were lower than the control group. A better performance in final weight, weight gain, daily growth weight, specific growth rate, protein efficiency ratio and food conversion ratio were obtained in 4 bushes than the other treatments. Also, significantly lower mortality (about 12-34%) was observed in all treatments than the control fish ($p<0.05$). These data show that the combination of water hyacinth especially at 4 bushes density together with koi carp can improve water quality and increases fish production.

Key words: Aquaponic, *Eichhornia crassipes*, Growth, Survival, Water quality, Koi carp

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Introduction

Aquaponic is the combination of aquaculture (raising fish) and hydroponics (the soil-less growing of plants) that grows fish and plants together in an integrated system. The fish waste provides an organic food source for the growing plants and the plants provide a natural filter for the water that the fish live in. Aquaponic initiatives can be found throughout the world, from deserts to northern cities and tropical islands. The industry is dominated by technology and training suppliers, consultants, "backyard" systems and community/organic/local food initiatives. There are very few established commercial systems and most of those that have been cross subsidized by the other economic activities, at least in the start-up phase (Love *et al*., 2014). High capital, energy and labor costs , and lack of flexibility in meeting market demand along with constraints on pest management have been the major problems to date. Nowadays, aquaponic is well developed type of aquaculture activities particularly for producing both organic fish and vegetables (Love *et al*. 2014). Such system of growing fish and vegetables/plants synchrony-logically is becoming more popular in suburbs and even in some urban areas. Use of such system for producing of fish and vegetables is more important because of high limitations for both freshwater and land in many regions (Watten and Busch, 1984; Lennard and Leonard, 2006; Rakocy, 2012). However, using a suitable vegetable/plant to completely remove the toxic substances e.g., NH_3, NO_2, N_2 and phosphorus produced by fish in the system is crucial (Jones, 2005; Tyson *et al.,* 2008). Several vegetables/plants e.g., lettuce, (tomatoes, strawberries, peppers) have been used in aquaponic systems with some variables benefits (Vermeulen and Kamstra, 2013). However, use of a plant with a right density is important for a complete removal of toxic substances of the re-circulated water (Jones, 2005; Tyson *et al.*, 2008).

Water hyacinth, *E. crassipes* (leafy lettuce, Swiss chard) as one of the fastest growing plant (Rakocy, 2004; Sikawa and Yakupitiyage, 2010) has a high ability to absorb phosphorus and nitrate from the water column (Pramanick *et al*., 2015; Wenwei *et al*., 2016; Ayanda *et al*., 2020*;* Lima and Asencios, 2021; Datta *et al*., 2021). From the literature review available, there are few data concerning the effect of *E. crassipes* to remove toxic substances produced by fish in an aquaponic system (Placek *et al*., 2015; Sharma *et al*., 2016; Thapa *et al*., 2016. Priya and Selvan, 2017; Saha *et al*., 2017; Saha *et al*., 2018; Basu *et al*., 2021). Therefore, this study was aimed to assess the effect of density of *E. crassipes* on water quality parameters and growth performance of koi carp (*Cyprinus carpio carpio*) for a period of 42 days at water temperature 22-24ºC.

Materials and methods

Fish

One hundred and twenty koi fish (*C. carpio*) weighing 11.43±0.1 g was obtained from an ornamental fish farm and were transferred into the 80 L aquaria each containing 10 fish. A similar recirculating system was provided for each aquarium with water quality: temperature (22.1±2ºC), pH (7.22±0.18), and dissolved oxygen (4.79±1.14 mg/L). The fish were adapted to this new condition for two weeks before starting the experiment. Fish were fed 5% body weight daily (Rakocy, 2005) using commercial diet (Biomar, INICIO plus 801 No. 1.5, France; protein brute 43%, crude fats 18%, cellulose brute 3.7%, crude ash 7.1%, phosphorus 1.15%, calcium 1.4%, sodium 0.5%, digestible protein/digestible energy 25.4%, vitamin A 7500 I.U/Kg, vitamin D_3 1500 I.U/Kg, vitamin E 260 mg/kg, and vitamin C 500 mg/kg).

Plant

Water hyacinth (*Eichhornia crassipes*) was freshly obtained from north Iran and was kept under optimum conditions (water temperature 22-23ºC and pH 7) for a week **(**Rakocy and Hargreaves, 1993).

System design

The schematic of system design is shown in Figure 1. The platform holds the plant usually made of Styrofoam and floats directly on the water. An air pump supplied air to the air stone that provided bubbles and the nutrient solution and supplied oxygen to the roots of the plant (Fig. 1). The study was designed with three treatments consisting of culture tank containing 2 bushes (T_1), culture tank containing 4 bushes (T_2), culture tank containing 6 bushes (T_3), and culture tank without plant (control). Each treatment was used in three replicates. Routine maintenance of water quality was monitored daily.

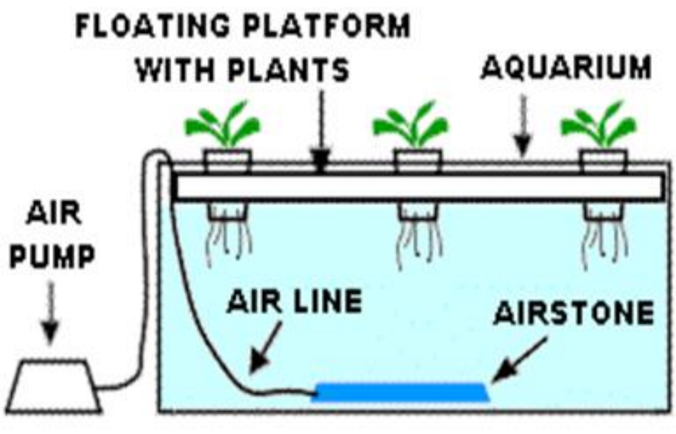

Figure 1: Schematic design (drawn by authors) of the system used in this study. The aquaponics system was set up in Zakaria Laboratory of Science and Research Branch of Islamic Azad University in Tehran

Daily activities

Air pumping, water exchanging, cleaning of water inlets and outlets, lightning, water flowing, removal of dead fish, feeding the fish and removal of uneaten feed were undertaken as daily routine activities.

Water quality analysis

The drinking fresh water was used in this study. Water samples were taken once a week from each aquarium. The samples analyzed for water quality parameters were also recorded each time. Weekly sampling was carried out between 8.30 AM and 9.30 AM in each sampling date and refrigerated at 4˚C in labeled polythene bottles for chemical analysis. Water quality parameters consisting of water temperature, dissolved oxygen (DO-meter model wtwoxi 330/SET, Germany), pH (pH meter 230 Senso Direct, Germany), total dissolved solid (standard method No. 2540), nitrite, nitrate, total and PO_4 (DR 5000™ UV-Vis Spectrophotometer) were analyzed. During the experiments 10% of water volume of each tank was replaced with fresh water daily (Vermeulen and Kamstra, 2013), and no heavy metal was artificially added.

and water volume was exchanged at 10% v/v daily. Dissolved oxygen (>5 mg/L) was provided using separated aeration; and light was provided 12 hours a day. Fish were weighted every 10 days interval, but the plants were weighed just at the beginning and at the end of the trail (Rakocy, 2012). A plastic mesh was used on the surface of each aquarium to separate the fish from the roots of the plants. The plants were also kept by providing a sheet of foam on the upper surface of each aquarium. Under such condition the roots of plant were completely protected from any possible damage by fish.

Detection of nutrients levels absorbable by the plant

Potassium (k), iron (Fe), magnesium (Mg), cadmium (Cd), zinc (Zn), and copper (Cu) were measured in the water column every four days intervals using atomic absorption method (GBC Avanta ver.1.33).

Growth performance of fish

Feed conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER), condition factor (CF), weight gain (WG), survival rate (SR) and daily growth rate (DGR) of each treatment was recorded daily (Rakocy *et al.*, 2006):

FCR = Feed intake/ weight gain

$SGR = [(LnW_1 - lnW_0)/T] \times 100$
W_0 = The average initial weight
W_1 = Average final weight
T=The number of test days
PER(g)=WM/EP
WM= Weight gain
EP= The amount of protein

$CF = W/L^3 \times 100$
W= Weight fish
L= length fish

$WG (g) = W_1 - W_0$
W_0 = The average initial weight
W_1 = Average final weight

$SR = (S-D)/S \times 100$
S= The number of samples tested
D= The number of casualties

$DGR (g/day) = (W_1 - W_0)/T$
W_0 = The average initial weight
W_1 = Average final weight
T=The number of test days

Statistical analysis

Statistical software of Statistical Package for the Social Sciences (SPSS) version 16 and Microsoft excel were used to calculate mean, standard deviation, and one-way ANOVA, and differences of means were evaluated for by the range tests of Tukey HSD (p<0.05) for homogeneous variances and by the range test of Dunnett T3 (p<0.05) for inhomogeneous variances, respectively (Schulz *et al*., 2003).

Results

Water quality parameters

The results of water quality parameters are given in Table 1. The water temperature in control group was 23.83±0.1°C throughout the experiment while in other treatments it was insignificantly increased to 24.5°C. There was no significant difference in dissolved oxygen (DO) levels among the treatments while DO in all treatments were almost insignificantly higher than the control (p>0.05). Also, pH value in control group was higher than the other treatments throughout the trail (p>0.05). The level of total dissolved solid (TDS) in all treatments was lower than the control, but this value in 4 bushes group was significantly lower than to control and other treatments (p<0.05). Also, total suspended solid (TSS) level in all treatments were significantly higher than the control (p<0.05). In addition, PO4 in 6 bushes group was significantly lower than the control and 2 bushes groups (p<0.05), but no significant difference was found between 6 bushes and 4 bushes groups (p>0.05). Furthermore, no significant difference was seen in level of NO_2 among the treatments (p<0. 05), while these values were significantly lower than control group (p<0.05). Similarly, NO_3 value in all treatments was significantly lower than the control (p<0.0.05) (Table 1).

Table 1: Water quality parameters in an aquaponic system using koi carp and *Eichhornia crassipes* at 22 – 24°C for 6 weeks.

Parameter	Treatment			
	Control	**2 bushes**	**4 bushes**	**6 bushes**
Dissolved oxygen (mg/L)	5.50 ± 2.63	5.52 ± 2.27	5.74 ± 2.32	5.62 ± 1.36
Temperature (°C)	23.83 ± 0. 1	24.8 ± 0.7	24.32 ± 0.5	24.5 ± 0.4
pH	7.47 ± 0.05^a	7.1 ± 0.59^b	7.2 ± 0.04^b	7.31 ± 0.07^b
Total dissolved solid (mg/L)	578.15 ±.012^a	568.90 ±0.16^a	548.43 ± 0.08^b	567.16 ± 0.2^a
Total suspended solid (mg/L)	1.27 ± 0.3^a	2.5 ± 0.7^b	3.1 ± 0.6^c	3.2 ± 0.4^c
PO_4 (mg/L)	2.5 ± 0.6^a	2.4 ± 0.8^a	2.1 ± 0.5^a	1.7 ± 0.4^b
Nitrite (mg/L)	0.61 ± 0.11^a	0.22 ± 0.29^b	0.16 ± 0.06^b	0.15 ± 0.07^b
Nitrate (mg/L)	37.8 ± 2.8^a	18.3 ± 2.51^b	13.2 ± 4.3^b	12.4 ± 1.4^b

Heavy metals

Results of heavy metals are presented in Table 2. Value of Fe in 4 bushes and 6 bushes groups were significantly lower than the control group (p<0.05), while no significant difference was seen between 2 bushes and control fish (p>0.05). Also, value of Cu in 2, 4 and 6 bushes were lower than the control (p<0.05). In addition, level of Zn in control was significantly higher than that of both 4 bushes and 6 bushes groups ($p<0.05$). No significant difference was observed in Zn value between 2 bushes and control groups ($p>0.05$). Also, level of Mg in 4 bushes and 6 bushes were significantly lower than that of both 2 bushes and control group ($p<0.05$), while no significant difference was obtained between 4 and 6 bushes treatments ($p>0.05$) Also, Mg value in control fish was higher than 2 bushes group ($p<0.05$). Furthermore, no significant difference was observed in Cd value between 4 bushes and 6 bushes groups ($p>0.05$). Cd level were insignificant between 2 bushes and control, but significantly higher than in 4 and 6 bushes trails ($p<0.05$) (Table 2).

Table 2: Heavy metals of water in the aquaponic system using koi carp and *Eichhornia crassipes* at 22 – 24˚C for 6 weeks.

Parameter	Treatment			
	Control	**2 bushes**	**4 bushes**	**6 bushes**
Fe (µg /L)	71 ± 5^{a}	60 ± 20^{a}	41 ± 10^{b}	12 ± 4^{b}
Cu (µg /L)	30 ± 5^{a}	20 ± 6^{a}	10 ± 0^{b}	$<10^{b}$
Zn (µg /L)	3661 ± 570^{a}	1660 ± 52^{b}	665 ± 125^{c}	$<10^{d}$
Mg (µg /L)	3430 ± 551^{a}	2030 ± 20^{a}	860 ± 53^{b}	605 ± 11^{b}
Cd (µg /L)	22 ± 5^{a}	11 ± 0^{b}	$<10^{c}$	$<10^{c}$

Growth performance

The results of growth factors are shown in Table 3.

Table 3: Growth factors in koi carp under aquaponic system using *Eichhornia crassipes* at 22 – 24˚C for 6 weeks.

Parameter	Treatment			
	Control	**2 bushes**	**4 bushes**	**6 bushes**
Final weight (g)	13.12 ± 0.01	14.89 ± 0.01	15.39 ± 0.01	13.39 ± 0.01
Daily growth rate (g/day)	0.05 ± 0.005	0.06 ± 0.005	0.12 ± 0.008	0.11 ± 0.04
Weight gain (%)	2.23 ± 0.27	3.44 ± 1.23	4.9 ± 0.51	2.36 ± 0.03
Feed conversion ratio	1.71±0.08	1.62±0.05	1.08±0.04	1.11±0.03
Specific growth rate (%)	0.05 ± 0.01	0.06 ± 0.01	0.12 ± 0.08	0.11 ± 0.04
Protein efficiency rate (g)	4.49±0.05	5.72±0.05	8.13±0.05	7.37±0.05
Condition factor	0.64 ± 0.07	0.76 ± 0.01	0.89 ± 0.05	0.71 ± 0.02
Survival rate (%)	62.77 ± 53.46^{a}	74.68 ± 0.15^{b}	96.90 ± 0.16^{c}	89.80 ± 0.15^{d}

Final weight

Final weight in 4 bushes group was significantly higher than 6 bushes and control treatments ($p<0.05$), while no significant difference was observed in final weight between 2 bushes and 4 bushes ($p>0.05$).

Weight gain

The weight gain in 2 bushes and 4 bushes groups were significantly higher than the control, while no significant difference was found between 6 bushes and control fish ($p>0.05$).

Daily growth weight

The daily growth weight in 2 bushes, 4 bushes and 6 bushes groups were significantly higher than the control fish ($p<0.05$).

Specific growth rate

A higher specific growth rate was obtained in 2 bushes and 4 bushes trails than 6 bushes treatment and control fish ($p<0.05$).

Condition factor

Significantly higher levels of condition factor were obtained in 2 bushes, 4 bushes and 6 bushes groups than the control group ($p<0.05$).

Food conversion ratio (FCR)

Significantly lower level of FCR was obtained in 2 bushes, 4 bushes and 6 bushes groups compared to control group (($p<0.05$). Also, FCR in 4 bushes group was lower than the other treatments ($p<0.05$).

Protein efficiency ratio (PER)

The PER value in 4 bushes and 6 bushes groups was significantly higher than that of both 2 bushes and control groups ($p<0.05$). Also, no significant difference was seen between 4 and 6 bushes groups ($p>0.05$).

Survival rate

At the end of trail, the survival rate in 2 bushes, 4 bushes and 6 bushes groups were significantly higher than the control group ($p<0.05$).

Discussion

In this study, the influence of water hyacinth density in an aquaponic system was assessed on the water quality, growth performance and survival of koi carp. In an aquaponic system with increasing in nitrogen level and accelerating the plant growth, the need for other nutrients such as potassium (K), iron (Fe), zinc (Zn), copper (Cu), calcium (Ca) and magnesium (Mg) will increase (Graber and Junge, 2009). Spoilage of organic materials is the major sources of nitrogen due to releasing of nitrogen in forms of ammonium and nitrate ions that are simply absorbable by the plants. Decomposition of organic matters can occur faster when carbon to nitrogen ratio is low (Rakocy, 2004). Thus, in an aquaponic system, the organic materials (NO^3, NO_2, PO_4, uneaten food, feces) released by fish are well food sources for the plants resulting in removal of waste products in the water column. Under such condition the water can be recycled and frequently used for fish production (Rakocy, 2006).

Results of water quality parameters here in this study showed that with an increasing in the plant density, the water temperature was slightly increased. This was due to increasing in the plant roots covering the tank water surface. Moreover, DO in all treatments was higher than compared to control fish indicating a higher photosynthesis by the plant in the system. However, no significant difference was observed in DO levels among the treatments. Conversely, the pH value in the treatments was slightly decreased compared to control. Interestingly, the levels of TDS in all treatments were lower than the control with the lowest TDS measured in 4 bushes group (548.43±0.08 mg/L). In contrast, the TSS levels in all treatments were significantly higher than the control. Also, the values of PO_4, NO_2 and NO_3 were decreased with increasing in the density of the plant, with a lower levels of these parameters were detected in all treatments compared to control fish. In addition, with increasing in the density of plant more phosphate and nitrite were absorbed by the plant roots resulting in a better water quality condition for fish life. This was supported by significantly higher mortality (>30%) in control fish compared with the other treatments. The reason of such differences in the mortality is due to significantly higher level of NO_2 level measured in water column of control treatment than the other treatments. Such increasing in NO_2 level may produce methaemoglobinemia condition in fish causing morbidity and mortality.

The results of heavy metals (Fe, Cu, Mg, Zn and Cd) showed that hyacinth water can decrease the measured heavy metals significantly compared to control. Particularly with an increasing in the plant density the values of these heavy metals were reduced in the water column. With an increasing in nitrogen (NO_3) levels in the water column, hyacinth plant was able to absorb higher levels of these heavy metals, particularly Fe and Zn (Sipauba-Tavares *et al*., 2002).
Therefore, with an improving in water quality a better growth performance is expected as the obtained data here in this study insignificantly a better growth factors i.e., FW, SG, DGR, SGR and PER were seen in the treated experiments compared to control. However, the best result of growth was obtained in 4 bushes group. The reason why a better growth performance occurred in 4 bushes is not clearly understood. This may in part due to a more suitable water quality condition provided in 4 bushes group e.g., availability of some trace elements in water column. However, further studies are required to optimize the density of water Hyacinth in such aquaponic system. Rakocy (2012) indicates that the aquaculture medium has a marked effect on the production of ornamental fish, catfish, perch and some plant species (Watercress, Lettuce, Chard, Kohlrabi and Broccoli). This means that harmful and waste substances of aquatics are practically used as fertilizers and nutrients for producing plants. Nevertheless, the exact mechanism of nutrient absorption from water by the root is not well recognized.

In this study, FCR in all groups was lower than the control fish. Such difference in FCR is remarkably considerable and can be considered as well cost-effective criterion in case of a mass production condition. Therefore, improvements of water quality, a reduction in usage of water volume, saving in using of chemical fertilizers as well as an increase in fish production were obtained in this experiment. The positive effects of water Hyacinth on fish growth and health status have been demonstrated by some other researchers.

For instance, Sikawa and Yakupitiyage (2010) and Somerville *et al*. (2014) showed that hybrid culturing in aquaponic system, production of lettuce (*Lactuca sativa*) by using hybrid catfish (*Clarias macrocephalus* x *C. gariepinus*), resulted in an increase in fish production with a better efficiency in income and operational costs. Sikawa and Yakupitiyage (2010) reported that the biomass and average weight of catfish and aquatics plants (*Lactuca sativa*) in the aquaponics system increased compared to control fish. Common carp fry fed up to 40% water Hyacinth for 70 days showed a significant increase in fish weight when it was replaced with fish meal in diet formulation (Mohapatra, 2015). In study of Sarker and Aziz (2017), application of nutrients in fish by addition of water Hyacinth to fish feed reduced the cost of feed formulation, thereby raising profit margins, and addition of 15% water Hyacinth meal in a diet was the best for mirror carp. Water hyacinth could be a food strategy in angelfish *Pterophyllum scalare* diets and may be implemented in the fish diet up to 32% level of crude protein as an enhancement in growth and survival of angelfish, which was seen after the inclusion of water hyacinth biomass in fish diet (Sipaúba-Tavares *et al*., 2019). There is also a great opportunity of utilizing water hyacinth to reduce livestock feed shortage especially in areas where there is scarcity as Wimalarathne and Perera (2019) exhibited that other control methods notwithstanding, using water hyacinth as a livestock feed, can be a better approach to controlling the invasiveness of the plant. Indian spotted snakehead (*Channa punctata*) fed with 2.5% and 5% water hyacinth leaves supplementary feed enhanced alkaline phosphatase and immunoglobulin, phagocytosis and phagocytic index compared to control fish (Verma *et al*., 2021) that could be due to presence of phenol and flavonoids in methanol and ethanol extracts measured in the plant extract. In a study conducted by Safitri *et al*. (2021**)**, use of sludge worms cultured with recirculation system in combination treatment with water hyacinth (15%) and fermented corncob (5 or each at 10 % improved water quality.

Use of water hyacinth plant in an aquaponic system together with koi carp could improve some water quality criteria, reduced levels of some heavy metals, and enhanced the growth factors and survival of the fish. However, different results were obtained at different densities of the plant with the best results observed in 4 bushes group. Therefore, use of hyacinth plant in an aquaponic system is a useful way for either maintaining the fish in aquarium or growing it for production. However, determination of both plant density and fish crowding are important for optimizing the aquaponic system.

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