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BIOTECHNOLOGY

Integration of Nile tilapia (*Oreochromis niloticus L.*) production *Origanum majorana* L. and *Ocimum basilicum* L. using aquaponics technology

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ABSTRACT. This study aims to evaluate the effect of different initial densities of Nile tilapia fingerlings on water quality, fish growth, and production of marjoram (*Origanum majorana* L.) and sweet basil (*Ocimum basilicum* L.) in a recirculating aquaponic set up. The experiment was carried out on randomized block design and comprised four fish densities (0, 150, 250 and 500 fish m^{-3}) and two crops, in which each treatment was continued for 45 days and replicated three times. Except for pH and total alkalinity, all water physicochemical parameters were increased significantly at high fish densities. In contrast, most of the biological parameters for fish were unaffected by culture density, although at the highest density crude protein percentage was significantly increased and ether extract percentage was significantly reduced. Plant productivities were highest when fish were cultured at density of 500 fish m^{-3}, a density that was found to be appropriate for maintaining water quality and promoting fish growth while preventing toxicity and mortality. The present study has demonstrated the technical viability of coupling Nile tilapia culture coup with aquaponic production of marjoram and sweet basil.

Keywords: integrated aquaculture; Nile tilapia; vegetable crops; sustainability.

Integração da produção de tilapia do Nilo (*Oreochromis niloticus L.*), *Origanum majorana* L. e *Ocimum basilicum* L. utilização da tecnologia aquapónica

RESUMO. O objetivo do presente estudo foi avaliar o efeito de diferentes densidades iniciais de alevinos de tilápia do Nilo na qualidade da água, no crescimento de peixes e na produção de manjerona (*Origanum majorana* L.) e de manjericão (*Ocimumbasilicum* L.) em um sistema de recirculação em aquaponia. O experimento foi de delineamento em blocos ao acaso e compreendeu quatro densidades de peixes (0, 150, 250 e 500 peixes m^{-3}) e duas culturas, nas quais cada tratamento foi continuado por 45 dias e repetido três vezes. Com exceção do pH e da alcalinidade total, todos os parâmetros físico-químicos da água aumentaram significativamente em densidades de peixes altas. A maioria dos parâmetros biológicos dos peixes não foi afetada pela densidade da cultura, embora na maior densidade a percentagem de proteína bruta tenha aumentado significativamente e a percentagem de gordura tenha sido significativamente reduzida. As produtividades vegetais foram mais elevadas quando os peixes foram cultivados na densidade de 500 peixes m^{-3}, densidade que se mostrou adequada para manter a qualidade da água e promover o crescimento dos peixes, prevenindo simultaneamente toxicidade e mortalidade. A pesquisa demonstrou a viabilidade técnica da aquaponia para a cultura da tilápia do Nilo associada à produção da manjerona e do manjericão.

Palavras-chave: aquicultura integrada; tilápia do Nilo; culturas de vegetais; sustentabilidade.

Introduction

The escalating growth in the world population, plus increasing the demand for freshwater, has imposed enormous pressure on the agricultural and industrial sectors that are responsible for food production. Consequently, the sustainable food production, with minimal water and nutrient loss, has become a necessity rather than an ethical matter or political debate. In this context, aquaponic technique could allow the sustainable food production by integrating vegetables culture and fish farming (Nelson, 2007; Villarroel, Rodriguez Alvariño, & Duran Altisent, 2011). The water volume required in aquaponics is much lower than that employed in traditional olericulture and aquaculture methods (non circulated aquaculture) that involve irrigation and constant water replacement, respectively (Cortez, Araújo, Bellingieri, & Dalri, 2009; Roosta & Mhsenian, 2012; Hundley & Navarro, 2013; Van Rijn, 2013; Wongkiew, Chandran, Lee, & Khanal, 2017).

Present study found that the fish species had a high growth rate, a high feed conversion rate and a high market value (Fülber et al., 2010).

Vegetable species such as basil and marjoram are important in terms of adaptation to the tropical climate and can also be used in temperate climates. The basil has presented great economic potential through its essential oils, besides being used "in natura" in the cuisine. Linalool extracted from basil has also been directed to the pharmaceutical and cosmetics industry (Palaretti et. al. 2015).

In contrast to traditional agriculture, organic aquaponics is far less demanding regarding the use of water and land resources, it employs no chemical fertilizers and it eliminates the need for deforestation and the consequent increase in CO_2 emission to the atmosphere. Thus, aquaponics represents a strategy for the production of healthy food that is environmentally friendly, practicable and sustainable not only from a commercial standpoint (Hundley et al., 2013) but also for domestic scale systems, so-called "backyard aquaponics" (Hundley & Navarro, 2013; Hundley et al., 2013). According to Hundley et al. 2013, where the effluent effect of tilapia cultivation on the growth of basil and marjoram was observed, over the 28-day experiment period, the average growth of 20.50 cm of marjoram was observed, buying with hydroponic system this growth was observed only after 72 days, it has reached 21 cm (Haber, Luz, Dóro, Duarte, & Santos, 2004).

Considering that the continued success of fish-farming depends on the development of new technical and management methods, we set out to evaluate the effect of different initial Nile tilapia (*Oreochromisniloticus* L.) fingerlings densities on water quality, fish performance, and the growth of marjoram (*Origanum majorana* L.) and sweet basil (*Ocimum basilicum* L.) in a recirculating aquaponic system.

Material and methods

Site of study and experimental design

The experiment was carried out at Pine Tree farm (Brasília, DF, Brazil; 15°52'31.36"S, 47°48'01.28"W; altitude 1023 m) between November 2012 and April 2013. The experiment was held on a randomized block design with a 4 x 2 factorial scheme, comprising four different densities of tilapia fingerlings with average weight of 6.96 ± 1.19 g (represented by total weight of the fingerlings) and length 1.58 ± 0.38 cm: (T1(0): 0 fish m^{-3}, T2(150): 150 fish m^{-3}, T3(250): 250 fish m^{-3} and T4(500): 500 fish m^{-3}) and two crops: (marjoram and sweet basil),in which each treatment was continued for 45 days and replicated three times, in a recirculating aquaponic system. The study was approved by the ethical committee of the University of Brasília (CEUA/UnB protocol number 43058/2012). The feed amount was updated every 15 days.

Recirculating aquaponic system

The replicate treatments were carried out simultaneously in the same green house using three identical systems (Figure 1A), each consisting on five containers interconnected by polyvinyl chloride (PVC) tubing. Water from a 3000 L fish tank (1721 cm Ø x 1124 cm deep) flowed via a 100 mm Ø tube to a 240 L decantation tank, which was sequentially connected by a 60 mm Ø tube to a 500 L biological aerated flooded filter (BAFF) in which papyrus sedge (*Cyperus papyrus*) and white ginger lily (*Hedychium coronarium*) had been planted. The outflow from the BAFF passed through a 32 mm tube into a reservoir equipped with a pump (Boyu Group, Raoping, Guangdong, China) of maximum horizontal flow rate of 2400 L h^{-1} and actual flow rate of 1600 L h^{-1} at the lift height of 1.5 m used in the experimental setup. Water from the reservoir was delivered to the hydroponics table through a 25 mm Ø tube and distributed, by holes in the tubing, to 12 PVC gutters (each 6 m in length) spaced 15 cm apart. The gutters were filled with gravel consisting on 4-to-64-mm-diameter mineral and rock fragments (i.e. pebbles according to the Wentworth scale) in order to maintain the availability of nutrients to plants. Aeration was made constant by a 0.5-hp pump (Boyu Group). Water sampling was performed through a tap located at collection point A in the fish tank (Figure 1B).

A total of 2700 Nile tilapia fingerlings, from the Aquabel Company with an average weight of 6.96 ± 1.19 g were employed in the experiment. The fish were fed with isoprotein rations containing 42% raw protein (3400 kcal kg^{-1}) at a daily rate of 5% of body weight. Biometric measurements were performed after 45 days in order to adjust the diet using a Toledo digital scale (São Bernardo do Campo, Paraná State, Brazil).One-hundred-and-fifty standardized seedlings (12 cm long; 2-gram average weight) of marjoram and sweet basil were planted at 10 cm spacings in the hydroponic gutters such that a 4 cm portion of each seedling remained exposed.

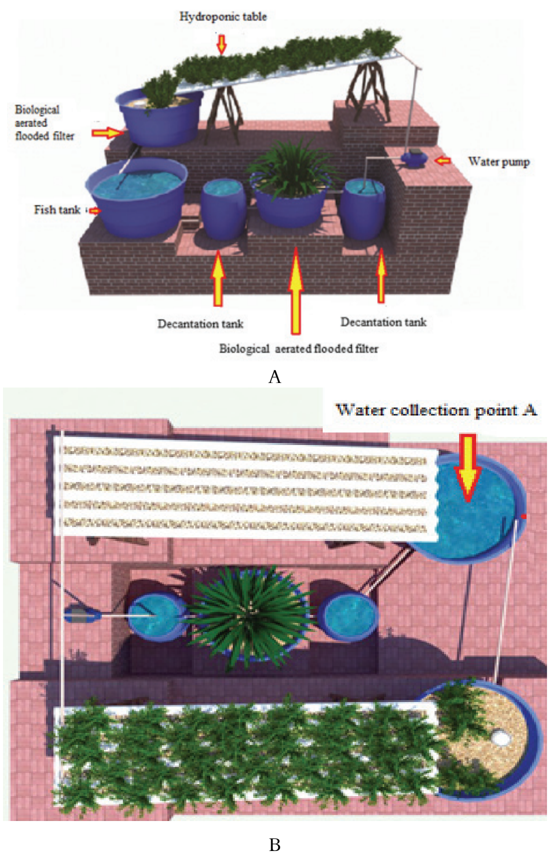

Figure 1. Schematic representation of the aquaponic system used in the study showing: (A) lateral view of the interconnecting units; and (B) top view indicating the point of water sample collection.

Data collection

Water and air temperatures were recorded daily at 09:00h, and the oxygen level in the water was monitored weekly with the aid of an oximeter. Water quality was assessed after 45 days at the Geochemistry Laboratory at Universidade de Brasília through measurement of pH, electrical conductivity (), mineral concentrations (Na+, K+, Ca2+, Mg2+, NH3, Cl-, NO3-, PO42- and SO42-) and total alkalinity (CaCO3) using standard methods. The efficiency of marjoram and sweet basil production was evaluated by measuring plant height samples after 45 days.

At the end of the experiment, after 24-hour fasting, the fish were collected, desensitized in 250 mg L Eugenol^{-1}, slaughtered then measured the total length and standard, final weight stored in plastic bags to be frozen for further analyzes of the whole fish bromatological. The fish were ground and dried and then used from each replicate for dry matter analysis, ethereal extract and crude protein according to (Navarro et al., 2010).

Statistical analysis

Normally distributed data were analyzed using one-way variance analysis (ANOVA) coupled with Student t and Duncan tests. Analyses were carried out with the aid of PAST and SAS/STAT (SAS Institute, Cary, NC, USA) softwares. The level of statistical significance was set at 5%.

Results

Air and water temperatures recorded during the experimental period were 24 ± 1.95 and 20.4 ± 1.44°C, respectively, while the mean concentration of dissolved oxygen in the fish tank water was 6.1 ± 0.36 mg L^{-1}. The water pH and total alkalinity were not influenced by fish density, while all of the other physicochemical parameters increased significantly in treatments T3 (250) and T4 (500) compared with T1 (0) and T2 (150) (Table 1). Most of the biological parameters of Nile tilapia fingerlings were unaffected by fish density (Table 2), although percentage crude protein was significantly higher in T4 (500) compared to T2 (150) and T3 (250), and percentage ether extract was significantly lower in T4 (500) compared with T2 (150) and T3 (250).

Table 1. Physicochemical characteristics of water collected in fish tanks (at point A in Figure 1) after 45 days in aquaponics integrating Nile tilapia fingerlings, marjoram and sweet basil.

Treatment (fishm^{-3})	pH	σ^a μSiemens cm^{-1}	Na^+ ppm	K^+ ppm	Ca^{2+} ppm	Mg^{2+} ppm	NH_3 ppm	Cl^- ppm	NO_3^- ppm	PO_4^{2-} ppm	SO_4^{2-} ppm	$CaCO_3^b$ mg L^{-1}
T1 (0)	6.80±0.15^a	61.8±4.5^b	5.5±0.63^b	0.10±0.18^b	7.10±0.96^b	0.00±0.0^c	0.10±0.11^b	0.10±0.08^b	1.30±0.34^b	2.70±0.36^b	0.30±0.37^b	34.20±4.89^a
T2 (150)	6.06±0.20^a	52.46±13.34^b	2.88±0.35^b	0.06±0.03^b	7.71±3.16^b	0.27±0.46^b	0.15±0.10^b	0.28±0.11^b	11.61±5.72^b	2.77±0.36^b	0.82±0.74^b	14.41±1.45^a
T3 (250)	6.50±0.23^a	160.10±60.40^a	6.60±0.76^a	0.30±0.07^a	22.00±9.24^a	1.60±0.76^a	0.40±0.11^a	3.40±2.26^a	35.90±29.02^a	8.20±2.78^a	6.20±4.09^a	32.60±9.27^a
T4 (500)	5.60±1.38^a	142.70±63.45^a	9.80±0.65^a	0.50±0.19^a	15.1±7.03^a	1.20±0.65^a	1.30±1.05^a	2.80±1.94^a	27.50±35.06^a	17.20±5.8^a	6.80±4.76^a	17.10±17.66^a

[a]Electrical conductivity; [b] Total alkalinity; Within each column, mean values (± standard errors) followed by dissimilar superscript lowercase letters are significantly different (Duncan test; $p < 5\%$).

Table 2. Performance of Nile tilapia fingerlings after 45 days in aquaponics integrating marjoram and sweet basil.

Treatment (fish m^{-3})	Final weight g	Total length cm	Survival rate %	Weight gain g	Daily weight gain g	Protein efficiency ratio	Dry matter %	Crude protein %	Ether extract %
T2 (150)	52.00±18.0^a	12.60±2.47^a	100±0.0^a	45.08±18^a	1.00±0.4^a	1.25±0.52^a	27.37±0.09^a	50.88±0.31^a	28.41±0.60^a
T3 (250)	57.79±20.5^a	13.14±2.43^a	100±0.0^a	50.83±20^a	1.12±0.4^a	1.41±0.57^a	26.77±8.0^a	50.64±0.31^a	29.22±0.67^a
T4 (500)	61.00±24.6^a	13.23±2.83^a	100±0.0^a	54.11±22^a	1.20±0.5^a	1.50±0.68^a	27.00±0.03^a	54.80±0.31^b	27.30±0.76^b

Within each column, mean values (± standard errors) followed by dissimilar superscript lowercase letters are significantly different (Duncan test; $p < 5\%$).

Regarding marjoram productivity, mean plant heights were significantly greater in T4 (500) compared to T1 (0) (Figure 2A), while mean weights were significantly higher in T4 (500) compared to T1 (0), T2 (150) and T3 (250) (Figure 2B). In the case of sweet basil, mean plant heights were significantly greater in T4 (500) compared to T1 (0) and T3 (250) (Figure 2A), while mean weights were significantly higher in T4 (500) compared to T1 (0) (Figure 2B). Thus, fingerling high densities in the aquaponic system were clearly associated with increased growth and development of marjoram and sweet basil in the hydroponic table.

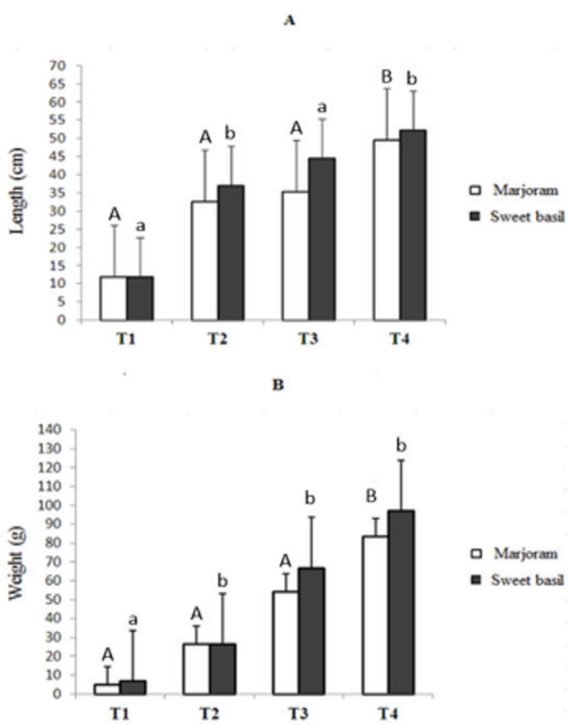

Figure 2. Efficiency on marjoram and sweet basil production after 45 days in aquaponic systems containing 0: T1 (0), 150: T2 (150), 250: T3 (250) or 500: T4 (500) g m^{-3} of Nile tilapia fingerlings as determined by plant height (A) and weight (B). Uppercase letters refers to the basil and lowercase letters to marjoram. Different letters are significantly different according to one-way ANOVA.

Discussion

The results presented herein demonstrate that nutrient-rich process water generated in aquaculture can be employed in the hydroponic production of marjoram and sweet basil. When Nile tilapia fingerlings were cultured at high densities (i.e. 250 or 500 g m^{-3} in T3 (250) and T4 (500), respectively), plant growth was significantly improved probably because of the increased NH_3 concentration in the water and, subsequently, of NO_3^-generated by bacterial nitrification. Palm, Bissa, and Knaus (2014) also observed an improvement in the growth (biomass weight gain) of lettuce cultured in an aquaponic system comprising Nile tilapia, with 55.89 g (± 49.77) for lettuce and 168.27 g (± 350.88) for cucumber fruits. Liang & Chien (2013) observed a 43% weight gain for tilapia *Oreochromis* sp using aquaponic system. However, other researchers did not observe effect of nitrogen fertilization on the growth of basil (*Ocimum basiicum* L.), but a significant effect over the growing season (Ferreira et al., 2015)

The presence of papyrus sedge in the BAFF unit is likely to may have enhanced the nitrification process since aerenchymaein the thin, bright green, thread-like stems that rise up from the rhizomes favor the formation of micro-aerobiczones, thereby promoting the nitrifying bacteria proliferation and survival (Abe, Ozaki, & KihOU, 1997).

The two-step nitrification mediated by naturally occurring nitrifying bacteria involves the oxidation of ammonia to nitrite followed by the oxidation of nitrite to nitrate. Nitrogen plays a fundamental role in the growth and development of leafy vegetables because it enhances the photosynthetic activity that is essential for vigorous vegetative growth (Hundley et al., 2013). Thus, the nitrification process performs a dual function by increasing the nitrate concentration, which is the main form of nitrogen incorporated by plants, and by eliminating those nitrogen compounds that are harmful to aquatic vertebrates. Filep et al. (2016) observed that nitrate reduction using the aquaponic system between basil and carp (*Cuprinius carpio*)

Together with intensive nitrification and easy access to nitrate, the oxygen availability and the presence of various nutrients and minerals, including phosphate, magnesium, calcium, sulfur and chlorine, are important factors in the aquaponic vegetable production (Palm et al., 2014). According to Rakocy Masser, and Losordo (2006), however, a nutrient balance is essential for optimal plant growth in an aquaponic system, and an excess of a particular component can be as detrimental as its insufficiency. For example, studies have shown that excess of potassium, magnesium or chlorine can negatively influence the sweet basil growth (Araujo, Matsumoto, Santos, César, & Bonfim, 2011).

Considering the above, the greater efficiencies of treatments T3(250) and T4(500) (in which fish densities were 250 or 500 g m^{-3}, respectively) in improving marjoram and sweet basil growth observed in the present study can be explained by the nutrients optimal proportions provided by the higher fish densities employed in the aquaponic systems.

Regarding the water quality parameters, all treatments preserved fish welfare and 100% survival was achieved under the different aquaponic conditions employed, a finding that is in agreement with that of Palm et al. (2014). However, treatments T3(250) and T4(500) produced higher ionic concentrations, and were responsible for the observed increase in electrical conductivity, as predicted by Tyson, Simonne, Treadwell, White, & Simonne (2008). Electrical conductivity is correlated to salt content and, on this basis, can be taken as a rough estimate of the total amount of dissolved solids in water. Although a minimum salt level is essential for osmotic balance, freshwater fish can thrive in a wide range of electrical conductivities, the upper and lower limits of which vary according to species. In general terms, the desirable range is 100 to 2000 μSiemens cm^{-1}, whereas the acceptable range is 30 to 5000 μSiemens cm^{-1} (Stone, Shelton, Haggard, & Thomforde 2013). In this context, the electrical conductivity levels recorded in T3 (250) and T4 (500) were well within the desirable range.

The water pH in the fish tank in T4 (500) was slightly lower in comparison to the other treatments, although the difference was not statistically significant. This lower value may be associated with increased concentrations of H^+ generated during bacterial nitrification (Rakocy, 2007; Tyson et al., 2008). According to Rackocy, Shultz, Bailey, and Thoman (2004), nitrification is favored by neutral pH of 7.0, but the more acidic pH observed in T4 (500) did not appear to have affected this process in comparison with the control T1. While most fish species can tolerate pH 6.5 to 9.5 (desirable range), values in the range 5.15 to 10.0 are acceptable according to Stone et al. (2013). Other studies such as de Zou et al. (2016) observed that the plants obtained better development in aquaponics system with pH 6 of water. Tang and Chen (2015) found that acid pH inhibited the proliferation of nitrifying bacteria. On this basis, pH values were within the acceptable levels in all of the treatments employed in the present study.

The nitrate increased concentrations observed in T3 (250) and T4 (500) derived from the higher ammonia amounts excreted by the fish. Ammonia is protein catabolism byproduct of most aquatic organisms (Kinne, 1976). Ammonia in water is present in non-ionized (NH_3) and ionized forms (NH_4^+), which together represent the total ammonia (Fromm & Gillette, 1968). Many researchers agree that the most toxic chemical form is ammonia unionized, due to its capability to expand the cell membranes (Fromm & Gillette, 1968). At higher pH values, the concentration of NH_3 increases and this may be toxic to fish, giving rise to reduced growth and, ultimately, mortality (Brinkman, 2009). For Nile tilapia, the maximum safe concentration of NH_3 is in the region of 0.42 mg L^{-1} (Karasu Benli & Koksal, 2005), although some researchers claim that the lethal level of total ammonia is 7.1 mg L^{-1} and that fish start dying at 2 mg L^{-1} (Rakocy, 1989). According to Stone et al. (2013), the desirable level of total ammonia is 0 to 2 mg L^{-1}, although levels < 4 mg L^{-1} are considered acceptable. In contrast, nitrate, the end product of nitrification, is relatively non-toxic to fish and levels up to 300/400 mg L^{-1} can be tolerated (Delong, Losordo, & Rackocy, 2009), although exceptionally high concentrations are reportedly toxic (Tyson et al., 2008). Based on the above, it is possible to state that nitrate and total ammonia levels in all treatments were within desirable ranges, and that the lower pH and intense nitrification reactions in these treatments contributed to this situation.

For many fish species, increasing population density results in reduced growth and performance (Saillant, Chatain, Fostier, Przybyla, & Fauvel, 2001). However, the present study has shown that increasing fish density in an aquaponic system is accompanied by the nitrification reaction intensification, thereby reducing ammonia toxicity risk. Although the growth parameters (final weight, total length, weight gain and protein efficiency ratio) of Nile tilapia in T4 (500) were not significantly enhanced compared with T2 (150) and T3 (250), the crude protein percentage was increased significantly. The mean gain in weight record during T4 (500) (54.11 ± 22) was higher (although not significantly so) than the values recorded for T2(150) and T3(250), but much lower than the gain of 164.01 ± 55.25 g reported by Palm et al. (2014) for Nile tilapia cultured in an aquaponic system. Such discrepancy may be associated with the original fish size and the diet employed.

Conclusion

The present study demonstrated the technical viability of aquaponics for the Nile tilapia culture coupled with marjoram and sweet basil production. The highest plant productivities were attained when fish were cultured at 500 fish m^{-3}, a density that was found to be appropriate for maintaining water quality and promoting fish growth while preventing toxicity and mortality.

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