ea engenharia agrícola

Journal of the Brazilian Association of Agricultural Engineering

ISSN: 1809-4430 (on-line)

EFFECT OF DIFFERENT SUBSTRATES IN AQUAPONIC LETTUCE PRODUCTION ASSOCIATED WITH INTENSIVE TILAPIA FARMING WITH WATER RECIRCULATION SYSTEMS

Doi:http://dx.doi.org/10.1590/1809-4430-Eng.Agric.v36n2p291-299/2016

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ABSTRACT: The integration of fish farming in intensive system and plant production, called "aquaponics" is practiced successfully in countries like the USA, Australia and Europe. In Brazil, this integration has attracted the attention of researchers and producers. In this context, the aim of this study was to evaluate the effect of two substrates (crushed stone number 3, CS III and flexible polyurethane foam, FPF) on the production of aquaponic lettuce, moreover, to show that the residual water from intensive tilapia production provides sufficient qualitative characteristics for competitive production of lettuce without adding commercial fertilizers. The treatment in which FPF was used provided higher concentrations of macro and micronutrients in the shoots, higher production of fresh matter of shoots (95.48 g plant^{-1}) and a larger number of leaves (14.90) relative to CS III. These results were attributed to the lower post-transplanting stress and the higher water retention time provided by the FPF. The residual water from tilapia intensive farming can provide sufficient nutrients for the production of lettuce, making the supplementary fertilization with commercial products unnecessary. Thus, the FPF presents the most suitable conditions to be used as substrate in aquaponics system with recirculation of the residual water from the intensive tilapia farming.

KEYWORDS: soilless cultivation, water recirculation and aquaponics system

EFEITO DE DIFERENTES SUBSTRATOS NA PRODUÇÃO DE ALFACE AQUAPÔNICA ASSOCIADA À CRIAÇÃO INTENSIVA DE TILÁPIA COM RECIRCULAÇÃO DE ÁGUA

RESUMO: A integração entre a criação de peixes em sistema intensivo e a produção vegetal, denominada “aquaponia”, é praticada com sucesso em países como Estados Unidos, Austrália e também, na Europa. No Brasil, essa integração vem atraindo a atenção de pesquisadores e produtores. Nesse contexto, o objetivo deste trabalho foi avaliar o efeito de dois substratos (pedra brita número 3, PB3 e espuma flexível de poliuretano, EFP) sobre a produção de alface aquapônica, além disso, demonstrar que a água residuária proveniente da produção intensiva de tilápia proporciona características qualitativas suficientes para produção competitiva de alface sem adição de fertilizantes comerciais. O tratamento onde foi utilizada EFP proporcionou maiores concentrações de macro e micronutrientes na parte aérea das plantas, maior produção de massa fresca da parte aérea (95,48 g planta^{-1}) e maior número de folhas (14,90) em relação à PB3. Esses resultados foram atribuídos ao menor estresse pós-transplantio e ao maior tempo de retenção de água proporcionado pela EFP. A água residuária proveniente da criação intensiva de tilápia pode fornecer nutrientes suficientes para produção de alface, não sendo necessário complementação da fertilização com produtos comerciais. Assim, a EFP apresenta condições mais adequadas para ser utilizada como substrato, em sistema aquapônico, com recirculação de água residuária proveniente da criação intensiva de tilápia.

PALAVRAS-CHAVE: cultivo sem solo, recirculação de água e sistema aquapônico

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Recebido pelo Conselho Editorial em: 22-8-2013

Aprovado pelo Conselho Editorial em: 6-1-2016

INTRODUCTION

Aquaponics is the science that integrates the production of fish and plants in a symbiotic environment where the fish waste is used as fertilizer (ROOSTA & AFSHARIPOOR, 2012). Generally, intensive fish farming is performed in this system, for it provides greater operational ease for performing water recirculation between plant growing and fish farming. As the plant cultivation is conducted in a hydroponic system, generally the vegetable product shows higher standard of commercial quality compared with the traditional vegetable cultivation (DEDIU et al., 2012).

The main obstacle for a wide spread of integrated fish and vegetables production is the need for high initial investment, in view of this, a measure that can be taken to alleviate this problem is the use of high fish densities in breeding tanks. However, this requires great attention and expertise, because the water quality must be maintained, in order not to reduce animal welfare (ROQUE D'ORBCASTEL et al., 2009).

Another important measure is the use of solid waste from fish farming, as the system can be integrated with a biodigester, which allows the production of biogas and biofertilizer, wherein biogas can be applied to meet part of the energy needs of the farming system (HOQUE et al., 2012). While the bio-fertilizer can be applied in vegetable growing (IHEJIRIKA et al., 2012), and even in the cultivation of microalgae for fish feed (MARTINS, et al., 2010).

Aquaculture has been widespread in developed countries, as it contributes to food production in two categories, fish farming and vegetable production. It also presents an environmental character, because the association of production systems enables the reuse of water, resulting in less water needed to drive the systems (DEDIU et al., 2012; GRABER & JUNGE, 2009).

By involving the operation of two production systems, it generates an alternative income source for the producer, however due to a high initial investment, the two activities, fish production and vegetable growing, should be at full production capacity. The choice of fish species is another strategy to maximize profits. Currently, the main species used commercially is tilapia (DEDIU et al., 2012).

The development of new lettuce cultivation methods, especially under protected cultivation, has provided a continuous increase in quality and productivity of this vegetable, one of the innovations is the soilless cultivation, characterized by hydroponics (PÔRTO et al., 2012), which offers some advantages, such as nutritional control, ability to use higher planting densities, reduction of pests, disease and weeds. Organic and inorganic substrates may be used in this method (AFSHARIPOOR & ROOSTA, 2010).

The characteristics of the various materials used as substrate directly and indirectly affect plant development and production. Aeration is an important factor affecting crop yield, since oxygen is a necessary element for cellular activity, involved in the breathing process, and when absent from the root system causes damage to the plant metabolic development. Therefore, substrate structure influences crop development, mainly for soilless crops. In brief, the choice of substrate in an aquaponic system must meet the appropriate water and air proportions that meet the plant needs (ROOSTA & AFSHARIPOOR, 2012).

In this sense, this work has the objective of evaluating the effect of two substrates (crushed stone number 3, CS III and flexible polyurethane foam, FPF) on the production of aquaponic lettuce. In addition, it aims to show that the residual water from intensive tilapia production provides sufficient quality characteristics for lettuce production without adding commercial fertilizer.

MATERIAL AND METHODS

The study was conducted at the experimental area of the College of Agricultural Sciences which belongs to the *Universidade Federal da Grande Dourados – UFGD* (Federal University of

Great Dourados), located in Dourados, Mato Grosso do Sul, in the second half of 2012. The average altitude is 446 m, and the coordinates are 22° 11' 45" south latitude and 54° 55' 18" north longitude.

Aquaponic System

Initially a greenhouse with 100 m^2 was built, under which the intensive farming system with recirculation was assembled, consisting of 10 fiber tanks of 500 L each. The fish breeding tanks were connected to a filter system for removing solids and ammonia, where water circulates in a closed system and then returns to the breeding tanks.

The fish species used in the system was tilapia of the Gift strain (*Oreochromis niloticus*). Its choice was mainly due to the knowledge of the production cycle, feeding management and availability of fingerlings in all seasons.

Filters were filled with crushed stone, gravel, shade cloth and broken tiles and bricks. The oven keeps the water at an appropriate temperature range for tilapia (between 26 and 28 °C) on most days. On colder days, a heat pump was used to keep the water temperature above 26 °C.

The water outlet of tanks is siphoned, having a valve for bottom discharge, through which the removal of the heavier material is done (feces and leftover feed), which is channeled to a buried reservoir. Subsequently the pumping of the material to a second tank is done, where the settling and separation of residual water from waste occurs (most concentrated part), which are at the bottom and later directed to a biodigester, producing biogas and biofertilizers.

Residual water is sent to two 1000 L tanks where it is mixed with the biofertilizer exiting the biodigester, and then used in aquaponic system for lettuce cultivation. Two fiber cement tiles were used as a channel for cultivation, with waves of 5 cm spaced at 18 cm, assembled on a bench with 3% inclination.

The substrates used were crushed stone number 3 (CS III) and flexible polyurethane foam (FPF) with a density of 0.021 g cm^{-3}. For each plant a piece of foam with a volume of about 63 cm^3 was used, whose dimensions were 3 cm high, 3 cm wide and 7 cm long, with storage capacity of up to 17.12 cm^3 of water, according to laboratory test.

Residual water remained recirculating between the tanks of 1000 L and the lettuce cultivation. Measurement of ammonia in water was made at intervals of 2 days. When the measured concentration reached zero, the water was then pumped into another tank, of 1000 L, being used for replacement in the fish farming system.

Figure 1 shows the general scheme of the system operation with each system component, involving the breeding tank with recirculating systems, waste collection, biodigester and hydroponics.

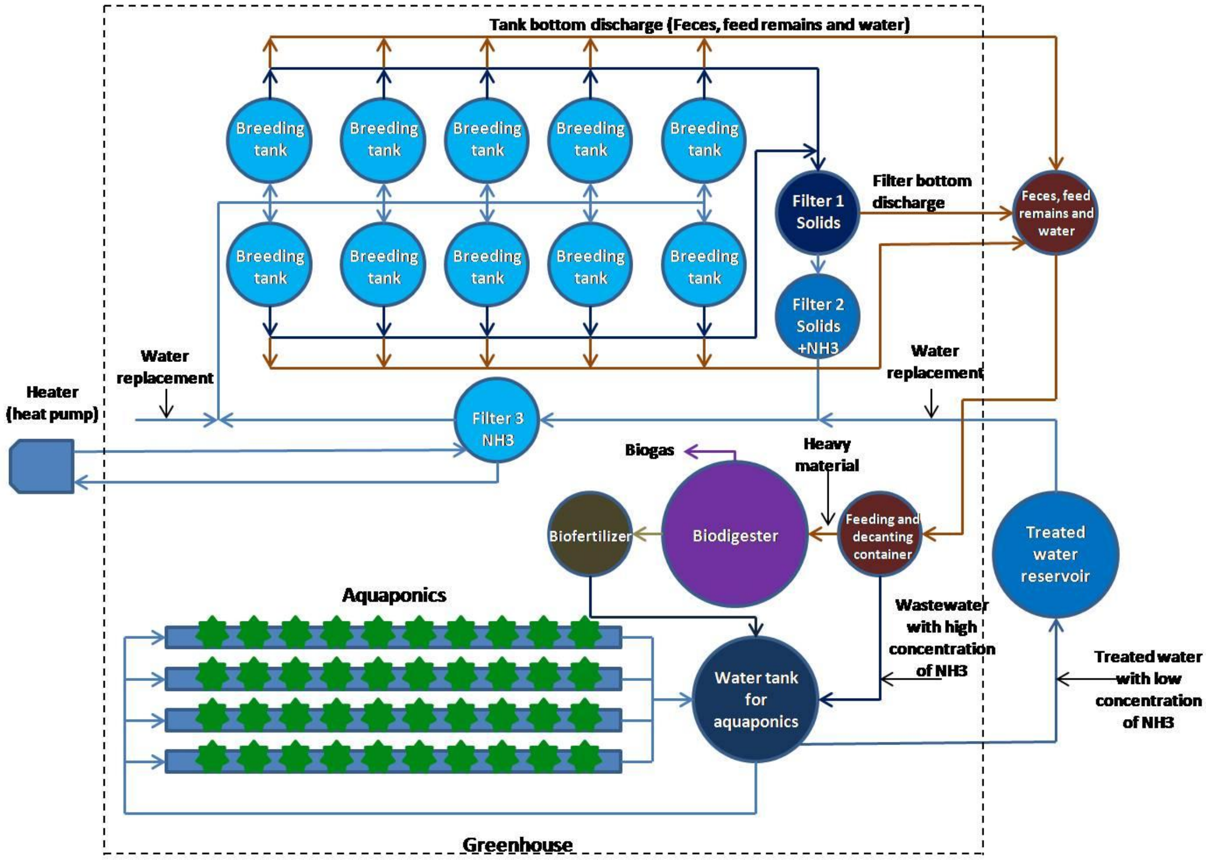

FIGURE 1. Basic scheme of general working system.

Experimental design

Completely randomized design with two treatments that corresponded to the substrates was used, with eight replications. The substrates tested were crushed stone number 3 (CS III) and flexible polyurethane foam (FPF).

Each plot consisted of 11 plants, where fiber cement tiles (18 cm) delimited the spacing between rows and between plants, it was 20 cm. Six plants in each plot represented the experimental unit.

The lettuce *Lactuca sativa* cv. Verônica was used, of the curly type; the seedlings were grown in trays of 200 cells filled with commercial substrate. The transplanting of seedlings to fiber cement tiles occurred on the 22nd day after sowing, when plants had an average of five leaves.

Performed Evaluations

At 33 days after transplanting, there was the harvesting of six useful plants in each plot, and then they were taken to the university irrigation lab to follow the appropriate evaluations. To evaluate the fresh weight of shoots and leaves, roots were removed and shoots were weighed (stem and leaves).

Subsequently, leaves were weighed and counted separately. Shoots were taken to a forced circulation oven, where they remained for 72 hours at 65 °C, and were then weighed to determine the dry mass of shoots.

Based on plot size and considering the space between them, the population of plants per hectare was estimated and, based on the data on the average shoot fresh mass, the productivity in each treatment. The percentage of stem per plant was obtained by the ratio between stem mass and the shoot mass. Results were submitted to variance analysis with the completion of the F test and subsequent comparison of means by applying the Bonferroni test at 5% probability.

RESULTS AND DISCUSSION

Shoot fresh mass (SFM) and dry matter (SDM) showed significant difference between the two substrates used (Table 1), with averages of 95.48 g plant^{-1} and 4.10 g plant^{-1} respectively, obtained with the use of flexible polyurethane foam (FPF). While using the crushed stone number 3 (CS III) the values of SFM and SDM were 86.20 g plant^{-1} and 3.50 g plant^{-1}, respectively. Regarding productivity, there was no significant difference, despite the superiority of 2.32 t ha^{-1} when using FPF.

It is observed that plants cultivated with FPF showed better productive characteristics (SFM and SDM); however, this trend is not reflected in crop productivity. Possibly, the plants cultivated with CS III showed higher stress after transplantation, so that when using the FPF the commercial substrate from the seedling production remains added to the roots longer than when compared to plants cultivated with CS III, in that substrate, the compact formation of the substrate with the root is broken easily, providing greater stress after transplantation. Over the plant cycle, they recover from the transplanting process and this period provides conditions so that in the harvest plants grown with CS III show no significant difference in crop productivity (TAPIA & CARO, 2009).

Several results for shoot SFM, SDM and crop yield are reported in literature, but we found no studies evaluating the type of substrate more fitted for growing lettuce using aquaponic system, even considering the conventional hydroponic system (with the use of commercial fertilizers), there is little research related to assessing growth substrate. Using the cultivar Veronica in conventional system with soil, SFM and SDM of 166 g plant^{-1} and 11 g plant^{-1} were found, respectively (KANO et al., 2012), better results than the ones reported in this study. However, values similar to those seen in this study are also reported in conventional cultivation with soil SFM and SDM of 90.30 g plant^{-1} and 4.86 g plant^{-1} were found, respectively (DUARTE et al., 2012).

Generally, the conventional hydroponic cultivation results in higher productivity when compared to the conventional soil systems, using cultivar Veronica the maximum yield of 51.12 t ha^{-1} was obtained (MARTINS et al., 2009). However, using conventional soil cultivation system, average yields ranging from 5.75 to 35.8 t ha^{-1} were obtained along five cultivation cycles (PEIXOTO FILHO et al., 2013). There is a variation between the given parameters, but it is observed that the values obtained for the culture SFM, SDM and yield present an alternative for growing lettuce with competitiveness in aquaponic system, where plant production can be considered a complement to the income from fish farming (ROOSTA & AFSHARIPOOR, 2012).

TABLE 1. Fresh mass of shoots, SFM (g plant^{-1}), dry mass of shoots, SDM (g plant^{-1}) and productivity (t ha^{-1}) in relation to the substrates assessed (Crushed Stone Number 3, CS III and Flexible Polyurethane Foam, FPF).

Substrates	Analyzed variables		
	SFM (g plant^{-1})	SDM (g plant^{-1})	Productivity
CS III	86.20 a	3.50 a	21.55 a
FPF	95.48 b	4.10 b	23.87 a
CV (%)	6.90	1.28	6.90

Values followed by the same letter in rows do no differ among themselves by the Bonferroni test at 5% probability.

Leaf fresh mass (LFM) in the treatment using FPF was 10.06 g plant^{-1} higher when compared to the treatment with CS III. The number of leaves per plant (NL) and the percentage of stem per plant (PSP) was also higher in the treatment using the FPF. Values of 12.20 and 14.90 were obtained per plant when using PP3 and FPF, respectively. Analyzing PSP using when FPF, it is observed that the higher NL provided by this substrate resulted in increase of 2.22% stem per plant (Table 2).

These results can be attributed to the higher retention time of water and, therefore, of nutrients near the roots when using FPF as substrate, so the water flow used in an aquaponic system directly affects the usability of nutrients present in residual water (DEDIU et al., 2012).

TABLE 2. Fresh mass of leaves, LFM (g plant^{-1}), number of leaves per plant, NL and percentage of stem per plant, PSP (%) in relation to the substrates assessed (crushed stone number 3, CS III and flexible foam polyurethane, FPF).

Substrate	Analyzed variables		
	LFM (g plant^{-1})	NL	PSP (%)
CS III	73.79 a	12.20 a	12.16 a
FPF	83.85 b	14.90 b	14.38 b
CV (%)	6.54	2.51	6.04

Values followed by the same letter in rows do no differ among themselves by the Bonferroni test at 5% probability.

For macronutrient, concentrations present in the lettuce shoots (g kg^{-1}) cultivated in FPF and CS III, an increase in concentration of almost all macronutrients was observed when using FPF as substrate (Table 3).

Phosphorus concentrations were appropriate for the two treatments with FPF and CS III values of 5.81 and 5.99 g kg^{-1}, respectively. It can be seen that the P concentration presented was increased when CS III was used as substrate. When the plant nears the reproductive stage, usually, P concentration tends to be higher; the accumulated P is used in bolting, flowering and seed formation (KANO, et al., 2011). Possibly the lowest concentration of nutrients in plants with CS III provided an adverse situation to the vegetative development, resulting in premature phosphorus accumulation.

TABLE 3. Concentration of macronutrients (g kg^{-1}) present in the shoots of lettuce grown in Crushed Stone Number 3, CS III and Flexible Polyurethane Foam, FPF.

Macronutrient	Concentration in FPF	Concentration in CS III
	(g kg^{-1})	
Phosphorus, P	5.81	5.99
Nitrogen, N	34.38	32.46
Potassium, K	54.00	52.00
Calcium, Ca	18.05	15.40
Magnesium, Mg	2.90	2.65
Sulphur, S	4.28	3.58

The P concentration recommended by RAIJ et al. (1996) for the shoot, is in the range from 4 to 7 g kg^{-1}. MARTINS et al. (2009), who making a nutrient absorption curve in hydroponic lettuce obtained P concentration of 5.8 g kg^{-1}, being similar to our results. Moreover, KANO et al. (2012) who in conventional soil cultivation found values ranging from 2.1 to 5.0 g kg^{-1}.

According to RAIJ et al. (1996), foliar nitrogen and potassium levels were adequate for both treatments, as they were within the range between 30 and 50 g kg^{-1} of N and 50 to 80 g kg^{-1} of K. KANO et al. (2012) obtained similar results, using conventional soil system these authors reported concentrations ranging from 32 to 37 g kg^{-1} of N and 57 to 62 g kg^{-1} of K. However, ALMEIDA et al. (2011) obtained reduced concentrations of N in hydroponics compared to this work, with N concentrations ranging from 9.5 to 23.2 g kg^{-1} and K concentrations ranging from 3.2 to 58.9 g kg^{-1}.

Calcium concentrations were appropriate, as they were in the range from 15 to 25 g kg^{-1} (RAIJ et al., 1996), with values of 18.05 g kg^{-1} using FPF and 15.40 g kg^{-1} using CS III. KANO et al. (2012) found similar results with conventional soil system with Ca concentrations ranging from

13 to 16 g kg^{-1}. However, ALMEIDA et al. (2011) reported lower Ca concentrations in hydroponics compared to this work, with concentrations ranging from 3.6 to 12,1g kg^{-1}.

As for magnesium, the two substrates (FPF and CS III) showed values below the desired range (4 to 6 g kg^{-1}) by RAIJ et al. (1996). However, they showed no visible deficiency symptoms. KANO et al. (2012) found similar results, in conventional soil system, with Mg concentrations between 3.1 to 3.6 g kg^{-1}, below the recommended by RAIJ et al. (1996). In hydroponic culture, ALMEIDA et al. (2011) also found concentrations below the appropriate, with Mg concentrations between 0.7 and 5.5 g kg^{-1}.

The S concentrations were appropriate for both substrates (4.24 and 3.58 g kg^{-1}) for they are above the recommended concentrations, 1.5 to 2.5 g kg^{-1} (RAIJ et al., 1996). However, KANO et al. (2012) had lower results for S concentrations in conventional soil system, with values ranging from 1.3 to 1.9 g kg^{-1}. In hydroponic culture, ALMEIDA et al. (2011) also found concentrations below the appropriate, with S concentrations of 1.5 g kg^{-1}.

Regarding micronutrient concentrations present in lettuce shoot (mg kg^{-1}) cultivated in FPF and CS III, it was observed that there was an increase in the micronutrient concentration when FPF was used compared to CS III (Table 4).

The copper concentration obtained when using FPF was adequate (7.0 mg kg^{-1}) for lettuce cultivation, as it remained within the recommended range, 7 to 20 mg kg^{-1} (RAIJ et al., 1996), whereas for CS III the concentration was below the adequate (6.0 mg kg^{-1}). KANO et al. (2012) reported similar results with conventional soil system with Cu concentration ranging from 1.5 to 11.2 mg kg^{-1}.

For RAIJ et al. (1996) Zn concentration should be between 30 and 100 mg kg^{-1}. Thus, for treatment with FPF the concentration obtained (42.5 mg kg^{-1}) was adequate. However, for the substrate with CS III, Zn concentration was below the recommended range (28.5 mg kg^{-1}). By cultivating lettuce in conventional soil system, KANO et al. (2012) found Zn concentrations ranging from 23 to 24 mg kg^{-1}, values below what is recommended in literature.

The manganese concentrations found (200.0 and 141.0 mg kg^{-1}) for the two substrates (FPF and CS III) was above what is considered appropriate, 30 to 150 mg kg^{-1} (RAIJ et al., 1996). KANO et al. (2012) have also reported Mn levels below the recommended, with Mn values ranging from 59 to 76 mg kg^{-1}. The boron concentration remained below the range considered appropriate, 30 to 60 mg kg^{-1} (RAIJ et al., 1996). KANO et al. (2012) reported similar results, obtaining B levels ranging from 23 to 25 mg kg^{-1}.

With regard to iron, the concentrations found (580.0 and 230.0 mg kg^{-1}) were above the recommended, 50 to 150 mg kg^{-1} (RAIJ et al., 1996). KANO et al. (2012) also reported values above the recommended with conventional soil cultivation system, with concentrations between 122 to 167 mg kg^{-1}. The high iron concentrations can be the result of ion precipitation of the metal parts present in the water recirculation system.

The decreasing order of nutrient accumulation in plants cultivated in aquaponic system was K > N > Ca > P > S > Mg > Fe > Mn > Zn > B > Cu. Different results were found by KANO et al. (2012), who obtained the following decreasing order of accumulation with soil cultivation: K > N > Ca > P > Mg > S > Fe > Mn > Zn > B > Cu. KANO et al. (2011), evaluating nutrients accumulation for seed production found the following nutrient accumulation order: K > N > Ca > Mg > P > S > Fe > Mn > Zn > B > Cu. Different results with respect to lettuce nutrient accumulation can be attributed to the fact that the surveys were carried out under different conditions, both environmental and agronomic (KANO et al., 2012).

TABLE 4. Minimum micronutrients concentration (mg kg^{-1}) present in shoots of lettuce cultivated in Crushed Stone Number 3, CS III and Flexible Polyurethane Foam, FPF.

Micronutrient	Concentration in FPF	Concentration in CS III
	(mg kg^{-1})	
Copper, Cu	7.0	6.0
Zinc, Zn	42.5	28.5
Manganese, Mn	200.0	141.0
Boron, B	35.3	34.4
Iron, Fe	580.0	230.0

Among all nutrients analyzed, only Mg and B showed concentrations below the appropriate for both substrates evaluated (FPF and CS III). However, features that could jeopardize the marketing of vegetables were not observed. Therefore, it is observed that the quality of residual water provided by tilapia intensive farming provides sufficient nutrients for the competitive lettuce production with conventional hydroponic systems and conventional soil systems.

CONCLUSIONS

The treatment in which flexible polyurethane foam (FPF) was used provided higher concentrations of macro and micronutrients in the shoot, higher production of fresh shoot mass (95.48 g $plant^{-1}$) and larger number of leaves (14.90) compared to the substrate with crushed stone number 3 (CS III). These results were attributed to less post-transplanting stress and to the increased water retention time provided by the FPF. Thus, the FPF presents the most suitable conditions to be used as substrate in aquaponics system with residual water recirculation from intensive tilapia farming.

The residual water from the tilapia intensive farming can provide sufficient nutrients for lettuce production, making supplementary fertilization with commercial products unnecessary. This enables competitive lettuce production with other production systems, such as conventional hydroponics and the conventional soil system.

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