doi: 10.15174/au.2018.1387 | ISSN online: 2007-9621

Acta Universitaria
Multidisciplinary Scientific Journal

Growth and development of herbaceous plants in aquaponic systems

Crecimiento y desarrollo de plantas herbáceas en sistemas acuapónicos

Recibido: 23 de mayo del 2016
Aceptado: 26 de octubre del 2017
Publicado: 23 de mayo del 2018

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Cómo citar:

Espinosa-Moya, A., Álvarez-González, A., Albertos-Alpuche, P., Guzmán-Mendoza, R., & Martínez-Yáñez, R. (2018). Growth and development of herbaceous plants in aquaponic systems. *Acta Universitaria, 28*(2), 1-8. doi: 10.15174/au.2018.1387

Keywords:
Aquaponics; basil; peppermint; spearmint; biological filters.

Palabras Clave:
Acuaponía; albahaca; hierbabuena; menta; filtros biológicos.

ABSTRACT

Aquaponics integrates aquaculture and hydroponic production using fish waste as nutrients for various vegetable crops. Herbaceous plants such as basil (*Ocimum basilicum* L.), peppermint (*Mentha piperita* L.) and spearmint (*Mentha spicata* L.) are in great demand due to their properties; however, there is very little information about their behavior in aquaponics. The objective of this study was to evaluate the growth and development of these species under aquaponic conditions. According to the results, the evaluated herbaceous plants suit crop conditionsand they can be used as part of the biological filters in aquaponic systems with tilapia (*Oreochromis niloticus* L. var. Stirling) production. Water quality could be maintained within appropriate ranges for both fish and plant production. Spearmint was the plant where the highest productivity was observed, suggesting that it assimilates the nutrients produced in this type of system more efficiently.

RESUMEN

La acuaponía integra la producción acuícola e hidropónica utilizando los desechos de los peces como nutrientes para diversos cultivos vegetales. Las herbáceas como la albahaca (*Ocimum basilicum* L.), la menta (*Mentha piperita* L.) y la hierbabuena (*Mentha spicata* L.), tienen gran demanda por sus propiedades, sin embargo, existe poca información sobre su comportamiento en acuaponía. El objetivo del presente estudio fue evaluar el crecimiento y desarrollo de estas especies cultivadas en acuaponía. De acuerdo a los resultados, las herbáceas evaluadas se adaptan a las condiciones del cultivo y pueden ser utilizadas como parte de los filtros biológicos de sistemas acuapónicos para la producción de tilapia (*Oreochromis niloticus* L. var. Stirling). La calidad del agua se mantuvo en rangos adecuados tanto para la producción de tilapia como herbáceas. La hierbabuena fue la que tuvo mayor productividad, sugiriendo que esta planta asimila de forma más eficiente los nutrientes producidos en este tipo de sistema.

INTRODUCTION

Aromatic herbs are plants whose fresh, dried or dehydrated leaves are used as essence, spice, for medicinal purposes, processed or as infusions that are in high demand in developed countries; therefore, they have a high potential for the export market. Globally, aromatic herbs in culinary use are becoming more important because consumers have a desire for healthier foods, with a significant increase in the choice of organic foods and the rise in ethnic foods (Makri & Kintzios, 2007). Among the herbs with strong demand in the market are basil (Ba; *Ocimum basilicum* L.) (Pérez-Rostro, Hernández-Vergara & Ronzón-Ortega, 2012), spearmint (Sp; *Mentha spicata* L.) and peppermint (Pe; *Mentha piperita* L.) (Dzida, 2010; Nelson, 2005). Some of these species have also been used as medicine due to their antiseptic properties, eg *M. spicata* is a natural repellent for *Anopheles stephensi*, the malaria vector and it acts as antimutagenic with effects on reproductive biology in humans (Iannacone & Alvariño, 2007). With these features, aromatic plants create a business opportunity for producers in developed countries who are able to produce them with high quality and the ability to meet demanding markets (Sanchez & Lucero, 2012) under organic production systems such as aquaponics (Blidariu & Grozea, 2011).

Aquaponics is the integration of hydroponics with recirculating aquaculture system (Rakocy & Hargreaves, 1993). This technique has been proposed as a sustainable alternative for an efficient use of water and to reduce the environmental impact associated with agricultural production (Adler, Harper, Wade, Takeda & Summerfelt, 2000; Ramirez, Sabogal, Jimenez & Hurtado, 2008). Overall, aquaponics is a comprehensive production system in which waste, synthesized by an aquatic organism (usually fish), is converted by bacterial action into nitrates, which serve as a nutrient source for plants (Rakocy, 2010; Zhang *et al.*, 2011). The biological principle is based on the required mineral nutrients for plant growth and development such as nitrogen and phosphorus, which are produced as waste by fish. Uneaten food and animal excretion free ammonia in water, which is converted by bacterial action in these nitrites and nitrates. Subsequently, plants work as biological water filters and take what they need from water, and so, absorbing these nitrates, they clean the liquid which returns to fish, allowing the latter to live in a suitable environment for growth and development (Rakocy, 2010). In this sense, there has been an increase in crop production in response to high concentrations of nutrients derived from microbial decomposition observed in aquaponic systems (Pérez-Rostro *et al.*, 2012). This is due to the ability of plants to absorb and store nitrates, which are a product of fish waste (Blidariu & Grozea, 2011). All these lead to the production of food without the external addition of chemicals, fertilizers and, therefore, aquaponics can be considered as organic farming (Al-Hafedh, Alam & Salaheldin, 2008).

Basil, spearmint, and peppermint may be very useful as biological filters in aquaponic systems to absorb and prevent the accumulation of nutrients produced by fish excreta (Rakocy, 2010). On the one hand, production of herbaceous plants in aquaponic systems can be considered as an alternative sustainable production that increases incomes and mitigate the conventional aquaculture production systems environmental impact. On the other hand, they can produce different fish species for human consumption coupled with the production of commercially used plants, which increases its production value (Graber & Junge, 2009). However, it is remarkable the shortage of information on the herbaceous production in aquaponic systems, particularly peppermint and mint, which are plants with productive potential in such systems (Bakiu & Shehu 2014; Campos-Pulido, Alonso-López, Avalos-de la Cruz, Asiain-Hoyos & Reta-Mendiola, 2013; Espinosa *et al.*, 2016). Therefore, the aim of this study was to evaluate the growth and development of basil, spearmint and peppermint as part of the biological filter in aquaponics with tilapia.

MATERIALS AND METHODS

Systems and Water

The experiment lasted 50 days and was carried out at the Experimental Aquaculture Unit, at the Life Sciences Division (Diciva, for its acronym in spanish), Campus Irapuato-Salamanca of the University of Guanajuato, Mexico (20°44'34.42''N 101°19'50.7"W; 1,745 MASL). One macro tunnel with film and plastic mesh (70/30) for the safeguard of the experimental aquaponic systems was used.

Moreover, three single and equal aquaponic systems were used, each consisting of a circular pond (V_{EF} = 1.5 m^3), a clarifier (V_{EF} = 0.25 m^3) and three biofilters formed by two subunits each: a Tower type filter (wet/dry), 1.5 m high and 0.10 m in diameter, completely filled with a plastic substrate (spheres Ø5 cm, V_{EF}= 0.006 m^3) and a hydroponic bed (HB) (0.27 m^3 total volume and 0.9 m^2 of crop area) (figure 1).

Each HB substrate contained a volume of 0.076 m^3, and a volume of water of 0.104 m^3, giving a total of $V_{EF\ (Total)}$ = 0.18 m^3. The substrate used was river stone with grain size 1", washed and disinfected (NaClO 5%). These elements are interconnected with PVC pipes, and a submersible pump (BOYU Mod. PQF 6000) was used. Each pond was injected with air through aerator stones (53.5 L min^{-1} compressor BOYU ACQ-009). The whole system was integrated so that the nutrient-rich water from the pond went into the clarifier, hence biological filters (filter type hydroponic tower + bed) and returned to the pond. The hydraulic retention time (HRT) in HB was 40 min.

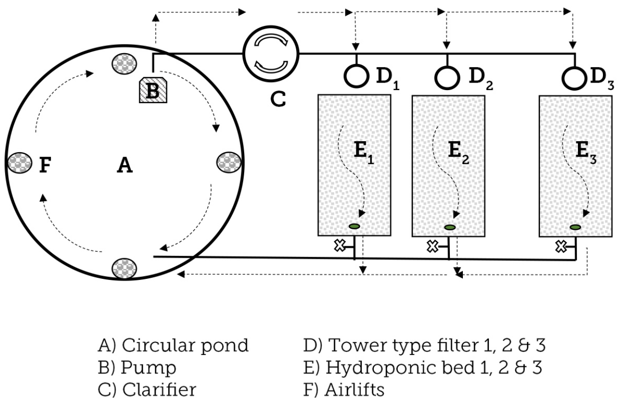

Figure 1

Items in experimental aquaponic system used. Not drawn to scale.
Source: Aurhor's own elaboration.

Systems were filled on the same day with water from a single source and launched the circulation and oxygenation. Subsequently, the protocol reported by Espinosa *et al.* (2016) was followed by the biological systems start. After 15 d recirculating and aerating, fish and herbaceous were introduced to the systems (day 0). In a daily basis, from 9:00 am to 6:00 pm, parameters were determined in ponds and HB: pH. Also, electrical conductivity (EC, mS / cm^3) and temperature (°C) with a multiparametric Hanna model HI9813-5, and dissolved oxygen (DO, mg L^{-1}) was evaluated with a Hanna equipment HI9146-O4N.

Herbaceous and Fish

The plants used in this experiment were basil (Ba) (O. *basilicum*, 20.1 g ± 8.6 g (fresh weight FW), 15.8 cm ± 2.2 cm, 12.0 true leaves ± 5.1 true leaves), peppermint (Pe) (*M. piperita*, 15.8 g FW ± 3.1 g FW, 10.4 cm ± 4.3 cm, 92.1 true leaves ± 12.2 true leaves) and spearmint (Sp) (*M. spicata*, 33.4 g FW ± 20.8 g FW, 17.1 cm ± 1.2 cm, 4.7 true leaves ± 4.0 true leaves), obtained from a commercial nursery (about 90 days old). 10 plants were randomly placed in each HB and one species per system was used. At the beginning and end of the experiment, all plants were weighed (including root, using a digital ESNOVA analytical scale, model ES-4000H). Every week, height was measured (cm, from the plant stem to the tip using a measuring tape) as well as the specific leaf area by counting the number of true leaves. The latter was determined as: SLA (cm^2) = Length × width × 0.08 (Cifuentes-Sánchez & Navarro-Cerrillo, 1999; Martinez, 1984). To determine this variable, four out of every 10 plants in each HB were randomly selected and measured every week, where the length (midrib) and the width of the middle part of 6 randomly selected leaves were measured without cutting the plant. The absolute growth rate (AGR) was also calculated on a wet base (WB) as: AGR (g plant day^{-1}) = (Final Weight (WB) - Initial Weight (WB))/day experimental period (Aristizábal, 2008). At the end of the experiment, the plant biomass production in HB in dry matter (DM) was determined by randomly selecting two plants from each HB, which were cut into small pieces (leaves and tender stems) and placed in an oven at 60 °C until a constant weight (n = 6 per species) was obtained.

240 tilapia specimens (*Oreochromis niloticus* L. var. Stirling) were used at 80 fish per pond (126.19 g wet ± 30.37 g wet weight and 19.90 cm length ± 1.6 cm length). The food used was a commercial feed (Nutripec, Purina) for specific species (dry matter: 88%, crude protein 32%, fat 6%, ash 8%, fiber 6% and nitrogen-free extract: 39%) which was supplied at a rate of 3% in relation to body weight and divided in three doses during the day (9:00 h, 13:00 h and 17:00 h). At the beginning and end of the experiment, the fish were weighed on a Labtronic Scientific digital scale, model 21-2544-09. Survival percentage (taking into account the starting number and ending number of fish) and biomass gain per pond were calculated (g harvested tilapia - g planted tilapia). Food consumption per pond was also calculated by the sum of the food supplied during the experimental period. Finally, the feed conversion ratio was determined as: FCR = feed consumed kg/kg of tilapia produced.

Statistic Analyses

A Canonical Discriminant Analysis (CDA) was applied to the variable height, number of true leaves, SLA and AGR of herbaceous, followed by calculation of Pearson correlation coefficients to estimate the relationship between vegetative variables (height and number of leaves) with productivity (Johnson & Wichern, 2007). Because the variables analyzed have different units, prior to the CDA, numerical data was normalized [standardized values = (x-mean)/standard deviation] (Dawson, 2008; Prieto & Herranz, 2010; Statgraphics, 2009). The homogeneity of variance-covariance matrix was verified by Box's M test. Other data were analyzed using one-way ANOVA followed by Tukey test ($p < 0.05$) with the Statgraphics Centurion program (Statgraphics, 2009).

RESULTS

Systems and Water

Temperature values in ponds varied from 20.4 °C to 25.6 °C. The pH range appeared to be between 7.88 and 8.30. As for the dissolved oxygen concentration (OD) in the pond water, this value was observed ranging from 4.6 mg L^{-1} to

7.4 mg L^{-1}. Electric conductivity (EC) values were low during the first 10 d of the experiment (0.579 mS/cm^3 - 0.631 mS/cm^3) and increased at the end (0.720 mS/cm^3 - 0.775 mS/cm^3). Temperature values in the hydroponic beds (HB) ranged from 20.2 °C to 25.2 °C. The pH range appeared between 7.80 and 8.30. The minimum OD values obtained in the HB water were 2.4 mg L^{-1}, reaching maximum concentrations off 7.4 mg L^{-1}.

Herbaceous and Fish

The three herbaceous crops differed considerably in their development (table 1). Sp exhibited a greater number of leaves and a larger absolute growth rate (AGR), whereas Pe were the tallest and Ba had the largest specific leaf area (SLA) (figure 2, table 1).

Box's M test showed equality in the variance-covariance matrix of the independent variables of all groups. The Wilks Lamba test (95% confidence level) showed that the discriminant functions 1 and 2 were statistically significant. According to canonical discriminant analysis (CDA), the variables obtaining a coefficient near 1 were the height and the number of leaves, which provided the greater effect of discrimination to be evaluated with respect to plant growth (table 2), accounting for 44.6% and 55.4% variance, respectively.

Table 1 Vegetative growth of peppermint, mint, and basil cultivated as biological filters under an aquaponic system for Nile tilapia production (Var. Stirling) (50 days after transplant)

	Spearmint	Peppermint	Basil	*P* =
Height (cm)	46.87 ± 1.67 b	60.17 ± 2.39 a	43.03 ± 1.52 b	0.0001
Leaves number	621.63 ± 7.34 a	305.36 ± 7.81 b	139.23 ± 6.91 c	0.0001
SLA (cm^2)	22.53 ± 0.57 b	18.07 ± 1.42 c	35.53 ± 1.55 a	0.0001
AGR (g plant day-1)	6.55 ± 0.48 a	1.49 ± 0.07 b	1.29 ± 0.16 b	0.0001

Means ± EE; SLA: Specific Leaf Area; AGR: Absolute Growth Rate.
Source: Aurhor's own elaboration.

Figure 2

A. *Ocimum basilicum* L.; B. *Mentha piperita* L.; C. *Mentha spicata* L.
Source: Aurhor's own elaboration.

Table 2 Standardized coefficients of canonical variables

Variables	1	2
Height	0.99	-0.043
Leaves number	-0.33	0.51
SLA	-0.31	-0.681
AGR	-0.59	0.281
% explained variance	79.9	20.1
Canonic Correlation	0.97	0.897

SLA: Specific Leaf Area; AGR: Absolute Growth Rate
Source: Aurhor's own elaboration.

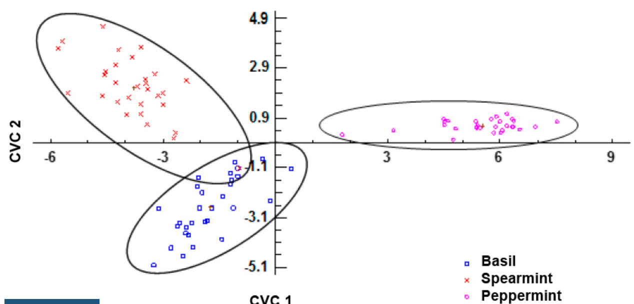

Figure 3

Discriminant functions chart among the three herbaceous plant species grown in biological filters in aquaponic systems. CVC: Canonical Variation Coefficient
Source: Aurhor's own elaboration.

98.89% of the observations were correctly classified in the CDA from the number of leaves and height, and species grouped by the evaluated variables were correctly classified 100%, 96.67% and 100% for Ba, Sp, and Pe, respectively. Moreover, the scatter plot of herbaceous showed the relationship between the first two discriminant functions (figure 3), where three groups were clearly formed; one per species, and where Sp and Ba were relatively similar in development terms.

Pearson correlation coefficients showed a negative relationship between height (r^2 = -0.36, F1, 88 = 13.3, p = 0.0004) and cumulative production (g $plant^{-1}$ in DM) and a positive relationship between the leaves number (r^2 = 0.88, F1, 88 = 311.99, p < 0.0001) with g $plant^{-1}$ in DM. Significantly higher values of DM (F2, 87 = 157.79, p < 0.0001) were observed in Sp (58.01 ± 19.5), followed by Ba and Pe (13.32 ± 7.27 and 7.22 ± 2.26, respectively). Furthermore, no significant differences among the herbaceous in the percentage content of DM were found, but there was a statistical difference regarding the DM production g per m^2 (table 3), where it was confirmed that Sp is the most productive plant species compared to Ba and Pe which, despite having the greatest height and specific leaf area, foliage was lower, affecting production.

Table 3 Evaluated variables in dry matter, survival and final production of herbaceous grown in integrated aquaponics with Nile tilapia Var. Stirling

	Spearmint	Peppermint	Basil
	g DM / 100 g FW		
DM	10.78 ± 0.67	9.67 ± 2.07	10.19 ± 0.32
Survival	100%	100%	100%
	Production g per m^2		
Wet Base	5980.14 ± 329.05 a	830.61 ± 72.09 b	1452.56 ± 332.92 b
DM	644.65 ± 35.47 a	80.32 ± 6.97 b	148.01 ± 32.92 b

Means ± EE, DM: Dry Matter.
Source: Aurhor's own elaboration.

A 98.6% ± 0.6% survival rate, 2.5 kg ± 0.08 kg fresh fish production per pond during the experimental period and feed conversion ratio (FCR) of 1.41 kg ± 0.16 kg (mean ± SD) were presented. During the experimental period, no signs of illness or injury in fish were observed.

DISCUSSION

According to the results, the pond's water physicochemical parameters were observed within the optimal range for tilapia (*Diario Oficial de la Federación* [DOF], 2012), showing the system's biological maturity. This suggests that it is possible to integrate this freshwater species culture with the production of the evaluated herbaceous. Meanwhile, the temperature recorded at the HB values were slightly outside the optimum range considering that water temperature in the aquaponic system must be maintained between 21.1 °C and 23.8 °C, suitable for plant growth (Nelson, 2008; Rakocy, 2010) because higher temperatures can cause reduced growth and susceptibility to pathogens (Nelson, 2005; 2008). However, during the experimental period no signs of disease were observed in plants. The pH range (7.80 - 8.30) was considered high. The pH is a key factor for plant growth because it affects the nutrients absorption capacity available in water and, thus, their development (Karimaei, Massiha & Mogaddam, 2004; Rakocy, Shultz, Bailey & Thoman, 2004). As reported by Caló (2011), ideally pH should be maintained between 5.5 - 6.5, so that plants achieve greater nutrients absorption. Literature suggests that the temperature out of range coupled with a high pH can cause growth problems in plants (Rakocy, 2010). However, no abnormalities were detected in any herbaceous that may have been related to lack of nutrients. This shows that the assessed species are able to adapt to the physicochemical conditions that may occur in aquaponic systems. OD values observed in the HB water are considered optimal for the plants. According to Nelson (2008) and Rakocy (2010), it is extremely important to maintain high OD level (close to 80% saturation) due to various factors such as fish respiration, the oxidation process of nitrifying bacteria, and oxygen absorption by the root region of plants which, added to the high organic loads produced, consume considerable amounts of oxygen in the chemical transformation processes in these systems (Rakocy, 2010).

EC was recorded in multiple levels recommended for aquaponic systems: 0.3 dS/m^3 - 0.8 dS/m^3 (0300 mS/m^3 - 0800 mS/cm^3) (Nelson, 2008), 0.3 dS/m^3 - 0.6 dS/m^3 (0300 dS/m^3 - 0600 mS/cm^3) (Rakocy *et al.*, 2004, Rakocy, 2010) 0.51 dS/m^3 to 0.54 dS/m^3 (0510 mS/m^3 - 0540 mS/cm^3) (Roosta & Hamidpour, 2011); although Nelson (2008) considers these values to be lower than the recommended range for optimal plant growth and development if it were a hydroponic system. Such EC difference recommended for hydroponics and aquaponics is attributed to two factors: a) the nutrients in aquaponics are generated constantly (Rakocy, 2010), and b) the organic nature of the nutrients low concentrations of salts is generated, hence low conductivity values (Nelson, 2008). Based on the above, it is considered that the EC values observed indicate the good concentration of nutrients available in water, which were efficiently utilized by plants even when roots were in a medium with slightly alkaline pH, showing that used herbaceous adapt to such systems. A key point to consider is that aquaponic systems require some time to balance their physicochemical parameters and this stability is directly associated with the maturity of their biological filters (Caló, 2011) and the load of beneficial nitrifying bacteria.

The recorded height of the evaluated plants in this study is compared with observed data in hydroponics (Pérez-Rostro *et al.*, 2012) and even with greater heights as in the case of Pe. In the case of Ba, the recorded height and SLA values were lower compared to hydroponic plantations under experimental conditions (González, Rodríguez, Sánchez & Gaytán, 2009) and higher compared with the reported production by Carrasco, Ramírez & Vogel (2007) in hydroponic systems with Nutrient Film Technique (NFT). The lower values of height and specific leaf area observed in the present study may indicate a deficit of nutrients in the aquaculture effluents whose concentration is controlled in hydroponics by managing nutrient solutions. The highest values are possible due to the increased colonization of biological filters by bacteria that constantly transform ammonium and nitrite into nitrates, essential for plant nutrition (Pérez-Rostro *et al.*, 2012) due to the presence of river gravel in HB, compared to using NFT. Spearmint showed the best adaptive response, at the end of the experimental period with greater leaves number developed and a greater weight gain per day (AGR), similar to that observed by Salam, Prodhan, Sayem & Islam (2014) who considers such plants to be highly profitable for aquaponic raft systems. Furthermore, the results suggest that plants can achieve greater development in aquaponic systems if the culture period extends (Campos-Pulido *et al.*, 2013), which would result in greater crop production compared with conventional crop systems. In this sense, it is necessary to analyze how growth factors (temperature and nutrient concentrations) in aquaponic systems affect plants in order to assess their effect on economically important properties as it has been observed that the aquaponic influences the amount of fruit and flavor (Graber & Junge, 2009) that are not always positive, so there must have a control of the water physicochemical parameters according to the plant culture needs (Campos-Pulido *et al.*, 2013).

Such observed productivity in Sp, suggests that this herb growth is favored by the aquaponic systems, compared to traditional cultivation (Campos-Pulido *et al.*, 2013). However, Ba production obtained in experimental systems is considered high compared with the reported by Carrasco *et al.* (2007) and for the production of Pe. According to the literature, this study would be the first report for this species grown in aquaponics.

Growth and FCR recorded data suggest that fish that consumed and used the supplied food presented a rapid and remarkable growth and development, resulting in a proper final biomass production and a harvest of healthy organisms. Based on the fish, growth is directly related to metabolism, environment, the offered conditions and that ratio may vary within the same area, with time and from one specimen to another (Enberg, Dunlop & Jørgensen, 2008). Given the above, it was considered that the planted fish density in each pond was right because, as noted, two basic conditions were met: 1) fish were healthy and therefore a high survival rate was achieved (almost 100% of organisms); moreover, during the experimental period fish showed no disease in the skin, eyes or gills and at the end of the experiment the growth of each organism was considerable, and 2) by maintaining full tilapia culture is possible to obtain sufficient amount of metabolic waste that then go through the process of bacterial decomposition with the consequent production of nutritive elements for plants in this way, cleaning the recirculating water.

If these elements are not removed, they may reach toxic levels for fish, but in aquaponic systems they fucntion as liquid fertilizer (mainly nitrates) for plants which, in turn, act as a natural pond water filters and hydroponic beds (Mateus, 2009). Thereby, this allows the survival of fish and shows that it is possible to integrate the production of this freshwater species with the evaluated herbaceous crops.

The plants studied here act as biological filters (Espinosa *et al.*, 2016), being particularly important the role played by Sp since the data suggest an increased production in biologically mature aquaponic systems compared to the traditional production systems (Caló, 2011; Campos-Pulido *et al.*, 2013). In aquaponics, some productivity aspects in herbaceous are unknown, as the essential oils content are known to be strongly influenced by the environment (Tabatabaie & Nazari, 2007) and which are economically important (Telci *et al.*, 2011). Therefore, information on management aquaponic system and the results presented here are important bases that can be used to establish future studies on the evaluated herbaceous. Although tilapia did not present diseases caused by poor water quality, it is considered important to continue these studies, particularly on those factors affecting the welfare and animal health and on the quality of plants obtained in aquaponic systems.

CONCLUSIONS

The use of evaluated herbaceous in biological filters in aquaponic systems for intensive production of tilapia is feasible; as basil, mint, and spearmint suit aquaponic farming conditions. Nutrients from excreta and uneaten food decomposition are found in adequate concentrations and assimilable chemical form, which resulted in plant growth, biomass production and 100% survival of organisms. Water quality was kept in acceptable ranges for both tilapia and herbaceous production. It was considered important to conduct studies on hydraulic retention rate in HB, biomass balance (plant and animal) as well as vegetables nutritional and nitrates values obtained in aquaponic systems to establish whether or not they meet the commercial standards and innocuousness for organic farming.

ACKNOWLEDGEMENTS

This experiment was part of Espinosa Moya Master's Thesis in Biosciences. The authors thank the Direction of Support for Research and Postgraduate studies (DAIP, for its acronym in spanish) at the University of Guanajuato for funding this project.

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