

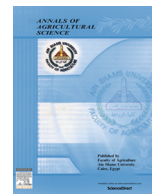
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Growth, yield, plant quality and nutrition of basil (*Ocimum basilicum* L.) under soilless agricultural systems



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ABSTRACT

Traditional agricultural systems are challenged by globally declining resources resulting from climate change and growing population. Alternative agricultural practices such as aquaponics (includes crop plant and aquatic species) and hydroponics (includes crop plant only) have the potential to generate high yield per unit area using limited land, water, and no soil. A soilless agricultural study was conducted at the Georgia Southern University, Statesboro, GA, USA from August to November, 2015. The growth, yield, quality, and nutrition of basil (*Ocimum basilicum* L.) cultivar *Aroma 2*, were compared between aquaponic and hydroponic systems using crayfish (*Procambarus* spp.) as the aquatic species. Non-circulating floating raft systems were designed using 95 L polyethylene tanks. Equal amounts of start-up fertilizer dose were applied to both systems. The objective was to understand how the additional nutritional dynamics associated with crayfish influence the basil crop. Both fresh and dry basil plant weights were collected after harvest, followed by leaf nutrient analysis. Leaf chlorophyll content, water pH, nitrogen and temperature were measured periodically. Aquaponic basil (AqB) showed 14%, 56%, and 65% more height, fresh weight, and dry weight, respectively, compared to hydroponic basil (HyB). It is logical to assume that crayfish waste (excreta and unconsumed feed) has supplied the additional nutrients to AqB, resulting in greater growth and yield. The chlorophyll content (plant quality) or leaf nutrients, however, did not differ between AqB and HyB. Further research is needed to investigate aquaponic crayfish yield, overall nutritional dynamics, cost-benefit ratio, and other plant characteristics under soilless systems.

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Introduction

The human population worldwide currently exceeds 7 billion, and it is projected to reach 8.5 billion by 2030, and 9.7 billion by 2050 (UN, 2016). With a fast growing global population, the demand for soil and land for crop production is likely to increase, and more urban area development is projected to take place. Earth's arable land is finite and challenges such as soil degradation, water scarcity, and urban area development need to be addressed by developing new and modified agricultural systems (Lehman et al., 1993; Lal, 2013). Alternative food production systems that require limited land, soil, and water, and which can be developed in urban areas may play a major role in future agriculture.

Hydroponics and aquaponics are soilless agricultural systems that are highly productive, suitable for urban areas, and can

address the shortage of land in relation to growing demand for food production (Medina et al., 2016). Hydroponics is the culture of plant crops in soilless water-based systems, where nutrients come only from formulated fertilizer (Liang and Chien, 2013). Aquaponics, is an integration of hydroponics and aquaculture, where crop plants and aquatic species can be grown together in a soilless water-based system (Seawright et al., 1998; Rakocy et al., 2006). Aquaculture is growing of aquatic animals/organisms in a designated water body (Boyd and Tucker, 1998). Large amounts of polluted water are produced in aquaculture systems with a potential for environmental pollution (Piedrahita, 2003), which can be reduced by techniques such as aquaponics (Schneider et al., 2005). Aquaponics, a combined culture of fish and plants has been proposed as a means to decrease waste accumulation from aquatic monoculture and to increase the productivity and profitability of the system (Rakocy and Hargreaves, 1993).

Soilless water-based systems are commonly set-up in vertical integration systems in indoor urban settings, which addresses the limitations of soil quality and space availability (Lal, 2013; Orsini et al., 2013). Being indoor practices, hydroponics and

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aquaponics are not directly affected by the changing climatic patterns and abrupt weather conditions, and hence could be effective adaptive strategies, as well. Aquaponics has several advantages over aquaculture and hydroponics. This system reduces the need for formulated fertilizers, eliminates the possibility of agricultural run-off, and cleanses the water through biofilter treatments (Rakocy et al., 2006). The nutrients released from fish excreta and microbial breakdown of organic wastes are used by plants in aquaponic systems (Roosta and Hamidpour, 2011). This way the plant component serves as a biofilter, and therefore a separate biofilter is not needed unlike aquacultural systems. In addition, this biofilter also generates income through the sale of the economic plant products (Rakocy and Hargreaves, 1993). Therefore, the aquaponic systems develop an economically advantageous symbiotic system, where aquatic species and the plant component benefit each other and the grower receives two marketable products. In contrast, the crop plant is the only marketable product in hydroponics and it is devoid of the commercial aquatic species and associated nutrient supply. Aquaponics can also be a strategy to combat water scarcity, as it has been shown to lower overall water consumption (McMurty et al., 1997) and prolong the useful life of water by reducing turnover rates and subsequently the environmental pollution, with improved economic return (Rakocy et al., 2006).

Primarily, fishes are used as the aquatic species in aquaponic studies and the potential for other commercial species such as crayfish (*Procambarus* spp.) are little known. Crayfishes have high economic importance globally, including southern United States and Southeast Asia (FAO, 2014). However, very few aquaponic studies have incorporated crayfishes and observed plant animal interaction effects. Effendi et al. (2015) observed that spinach-aquaponic systems resulted 5% higher crayfish survival rates than crayfish monoculture. They concluded that plant biofilters such as spinach, is very effective in cleansing the water resulting better crayfish survival. Crayfish can also be grown together with common fishes under aquaponic systems. In Louisiana, cultivation of crayfish (*Procambarus clarkii*) and rice (*Oryza sativa*) together increased yield for both (Chiena and Avault, 1980). Gallardo-Collí et al. (2014) reported that tilapia cohabited with crayfish (*Procambarus acanthophorus*) in an aquaponic system that produced 9.4% higher green corn fodder (*Zea mays*) compared to hydroponics.

Selection of plants for soilless systems is critical. Basil (*Ocimum basilicum* L.) is an annual herb that is commercially important and both fresh and dried leaves are used for culinary purposes (Chalchat and Ozcan, 2008). Basil is considered a medicinal herb (Ahmed et al., 2014) for its diuretic and stimulating properties and also used in perfume compositions (Nguyen et al., 2010). Basil is suitable for soilless production, and several studies have used basil as aquaponic or hydroponic crop (Rakocy et al., 2004; Roosta, 2014; Mangmang et al. (2016)). Basil responds with better yield under soilless systems than conventional systems. Rakocy et al. (2004) reported that aquaponic basil produced higher yield (1.8 kg m^{-2}) than field basil (0.6 kg m^{-2}). However, no studies have compared aquaponics and hydroponics systems for basil production.

Hydroponics and aquaponics are emerging fields of alternative agriculture with the potential to address the contemporary challenges faced by traditional agriculture. However, of the few studies that have compared these two systems, mostly focused on the commercial aquaponic species and little information is available on the plant production dynamics. In addition, little is known about the potential for non-fish aquatic species such as crayfish. We conducted a greenhouse study comparing crayfish-based aquaponic systems to hydroponic systems with a focus on the basil plant. The parameters under study included basil plant growth, yield, quality, and nutrition.

Materials and methods

Study location and components

A greenhouse experiment on soilless crop production was conducted from August to November, 2015 at the biology department of Georgia Southern University, Statesboro, GA, USA ($32^{\circ}26'43''\text{N}$, $81^{\circ}46'45''\text{W}$). The growth, yield, plant quality, and nutrition of basil (*Ocimum basilicum* L.) cultivar *Aroma 2* were evaluated under hydroponic and crayfish-aquaponic systems. Red crayfish (*Procambarus clarkii*) and White River crayfish (*Procambarus zonangulus*) were used as the commercial aquatic species. The basil seedlings were collected from a local nursery in Newington, GA and the crayfishes were purchased from Carolina Biological Supply Company, Burlington, NC and Duluth, GA.

Study set-up

Dark polyethylene containers ($16.88 \times 18.75 \times 27 \text{ cm}^3$ – $h \times w \times d$) of 95 L (25 Gal) capacity were used as study tanks. Four tanks each for aquaponics and hydroponics were allotted. Tanks were filled with 83 L (22 Gal) of tap water with a neutral pH. Two air stones (30 mm round) and double air pump (75–225 L capacity) were placed in all tanks. Four mature crayfishes were released in each aquaponic tank. Crayfishes are territorial, so to prevent aggression and increase their survival, assorted pieces of polyvinyl chloride (PVC) pipe shelters were placed in each tank. A similar strategy of using PVC shelters was adopted by Gallardo-Collí et al. (2014) that resulted better crayfish survival. The surface area of each container lid was 506.25 cm^2 ($18.75 \text{ cm} \times 27 \text{ cm}$). The lids were given five circular cuts, one in each corner and one in the center, for inserting the net pots holding the seedlings. The planting density was 5 basil seedlings per 506.25 cm^2 ($100 \text{ plants m}^{-2}$). Slotted net pots with 12.7 cm (5 in) diameter were used in this study. Pots were prepared with coconut coir lining and vermiculite (60:40) to hold the basil seedlings. Three week old seedlings were planted in the net pots and were transplanted to tanks on August 14, 2015. The net pots containing seedlings were set down into the circular holes of the lids, so that the lower half of each pot containing the roots remained submerged in the water. Each lid holding five submerged basil seedlings in each tank developed a non-circulating floating raft system, which is a common design used in soilless studies (Lennard and Leonard, 2006; Roosta and Hamidpour, 2011). Water loss due to evaporation and transpiration was replenished with tap water.

Chemical treatments and applications

Low potassium (K), sulfur (S), iron (Fe), and manganese (Mn) have been reported in aquaponic plants that received nutrition only from fish waste (Adler et al., 1996; Seawright et al., 1998). Thus, to provide a complete plant nutrition, supply of external nutrients is necessary (Rakocy et al., 2006). Both aquaponic and hydroponic tanks received a one-time basic start-up nutritional dose. Hydroponic liquid fertilizer Floranova Grow (7:4:10) (General Hydroponics Inc., Sebastopol, CA) was applied at the rate of 1.25 ml L^{-1} and each tank received 104 ml of fertilizer. Floranova Grow had 7% N, 4% P_2O_5 , 10% K_2O , 4% Ca, 2% S, 1.5% Mg, and 0.1% Fe and less than 0.1% of each micronutrient (B, Cl, Co, Mn, Mo, Zn) (General Hydroponics Inc., Sebastopol, CA). A dechlorinator (AquaSafe) was applied to all tanks at the rate of 10 ml per 37.85 L (10 Gal) with each tank receiving 22 ml. To promote the nitrification process, 260 ml of Zym-Bac (Home Grown Ponics, Wilbraham, MA), a source of nitrifying bacteria, was applied to each

tank. AquaSafe and Zym-Bac were only applied once at the beginning to all tanks. Pelletized feed was given to crayfishes, which included Spirulina flakes (Ocean Nutrition, Newark, CA) and algal wafers (Tetra Holding, Inc., Blacksburg, VA). In each aquaponic tank, 3 g of algal wafers and 1.5 g of Spirulina flakes were applied weekly, and these eventually provided an additional source of nutrients for aquaponic basil plants.

Data collection

Water pH and water temperatures were measured twice weekly and daytime greenhouse temperature was collected daily. Water nitrogen (nitrate, nitrite, and ammonium) content was measured weekly using freshwater aquarium master test kit. Weekly basil leaf chlorophyll content was measured using SPAD 502P chlorophyll meter (Spectrum Technologies Inc., Aurora, IL). In each plant a lower leaf and an upper leaf were measured in full sunlight and then averaged. Leaf chlorophyll content is a reflection plant health/quality (Ristic et al., 2007) and hence, the SPAD readings are commonly used to determine the health quality of plant. Plant height (from base to growing tip) was measured every week using a standard ruler. Harvesting of basil plants took place on November 19, 2015. All plants were cut from the base at the intersection of the shoot and root. Roots were removed and the fresh weight of the individual shoot vegetation was measured. Basil is commercially important both as fresh and dry and hence both weights were measured and presented. After weighing, cut portions were dried in a Fisher Scientific Isotemp Standard Lab Oven at 60 °C for 96 h. Fresh and dry weights of individual basil plants (without roots) were used to calculate the fresh and dry crop yield.

Leaf nutrient analysis

Leaf samples were analyzed for macro- and micronutrients at the Soil, Plant, and Water Laboratory of the University of Georgia, Athens, Georgia. Nutrients analyzed included nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), sulfur (S), boron (B), copper (Cu), manganese (Mn), iron (Fe), sodium (Na), and zinc (Zn). The dried leaf samples were digested (EPA Method 3052; USEPA, 1995) and analyzed for nutrients (EPA Method 200.8; Creed et al., 1994) using Inductively Coupled Plasma - Optical Emission Spectroscopy (ICP-OES) (Spectro Arcos FHS16, Germany).

Experimental design and analysis

We consider the proc glm procedure to fit a general linear model (GLM) with two factors, namely treatment (aquaponics, hydroponics) and four locations (L1, L2, L3, L4). The locations (or blocks) were situated in four different portions of the greenhouse with exposure to different degrees of sunlight and airflow. The full model including the interaction term can be presented as,

$$y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk},$$

$$i = 1, 2; j = 1, 2, 3, 4; k = n_{ij}; \sum_{ij} n_{ij} = 40$$

where y_{ijk} is the k th observation for the i th Treatment and j th location.

μ is the overall mean effect,

α_i is the effect of the Treatment i ,

β_j is the effect of the location j ,

$(\alpha\beta)_{ij}$ is the interaction effect of Treatment i and location j ,

ε_{ijk} is a normal and identically distributed random error term.

Note that when the experiment was designed as randomized block design, two treatments were randomly assigned after considering a blocking variable, location. At a given location we had two plots; each consisted of 5 plants. Each individual container was considered as a plot. Two treatments had been randomly assigned between two plots. As a result, at each location, there were five plants receiving either treatment 'aquaponics' or 'hydroponics'. Hence there were five replicates at location j receiving treatment i . An analysis of variance (ANOVA) has been carried out using SAS program (SAS, 2012) to determine if the effect of each factor was statistically significant. A significance level of 0.05 has been used throughout the analysis.

Results and discussion

Study environment

The mean water pH in aquaponic ($6.9 \pm 0.24^*$) and hydroponic (7 ± 0.15) systems were not significantly different. This neutral pH is well within the desirable range, as the optimum growth of freshwater fishes and aquatic organisms occurs at pH 6–9 (Boyd and Tucker, 1998). The mean daytime water temperature in the tanks was 23.4 °C (± 0.41) and the greenhouse mean daytime air temperature was 25.1 °C (± 0.72). Both the water and air temperature were congenial for crayfish and basil survival and growth.

Plant growth and yield

Plant growth and yield were higher in aquaponic basil (AqB) than in hydroponic basil (HyB). The basil mean plant height at harvest was 14% higher in AqB ($89.9 \text{ cm} \pm 4.5$) than in HyB ($78.7 \text{ cm} \pm 3.9$) (Fig. 1). Weekly height measurements also indicated a consistently higher growth pattern for AqB compared to HyB (Fig. 2). Both fresh and dry biomass yields were higher in AqB than in HyB. AqB ($150.2 \text{ g} \pm 18$) generated 56% more fresh mean harvest weight than HyB ($96.6 \text{ g} \pm 10.4$) (Fig. 3). Corresponding mean dry weight for AqB ($15.9 \text{ g} \pm 2$) was 65% higher than HyB ($9.6 \text{ g} \pm 1$) (Fig. 4).

The same basic starting fertilizer dose (Floranova Grow) was applied to both hydroponic and aquaponic tanks. In addition, aquaponic systems received nutrients from crayfish waste (excreta and unconsumed feed). Pelletized commercial food Spirulina flakes and algal wafers were applied as crayfish feed, which has 55% and 30% of crude protein, respectively, which is a source of nitrogen. Schneider et al. (2005) reported that aquatic species such as fishes consume only 20–30% of N from the applied feed and about 70–80% of the N are released as in the water waste (Krom et al., 1995). Kristiansen and Hessen (1992) reported that when crayfishes (*Astacus astacus* L.) were fed with commercial fish pellets they produced ($\mu\text{g/g}$ dry weight per day) 254 (N) and 3.4 (P) in urine and 34 (N) and 29 (P) in feces. Our study crayfishes were also fed with commercial pelletized food and the amount of N and P released from their urine and feces (excreta) and unconsumed feed may have provided additional nutrients to the aquaponic plants. This is further supported by the weekly water analysis that showed higher nitrogen in aquaponic tanks than in hydroponic tanks. The mean content of nitrate, nitrite, and ammonium during last 8 weeks before harvest were (in ppm), 95 ± 19.2 and 80 ± 12.5 ; 1.26 ± 0.4 and 0.3 ± 0.3 ; and, 0.34 ± 0.1 and 0.03 ± 0.01 ; for aquaponic and hydroponic tanks, respectively. It is likely that the available additional nitrogen was taken up by the AqB. For plant vegetative growth, nitrogen is a key element (Evert and Eichhorn, 2013), and it is the most important nutrient for crop yield (Wagger, 1989;

* The numbers presented with \pm are standard error (SE) values.

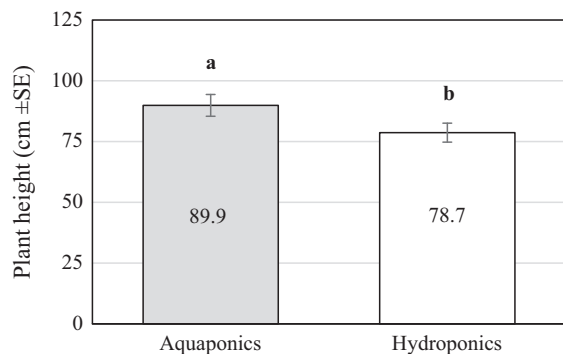


Fig. 1. Basil plant mean height (cm) at harvest under aquaponic and hydroponic systems. Lower case letters indicate significant difference at $P < 0.05$ level between soilless systems. \pm SE represents standard error.

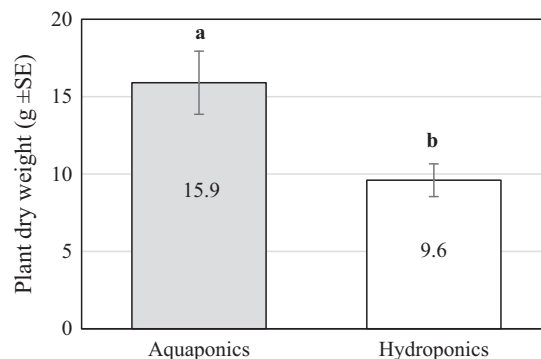


Fig. 4. Basil plant mean dry weight (g) at harvest under aquaponic and hydroponic systems. Lower case letters indicate significant difference at $P < 0.05$ level between soilless systems. \pm SE represents standard error.

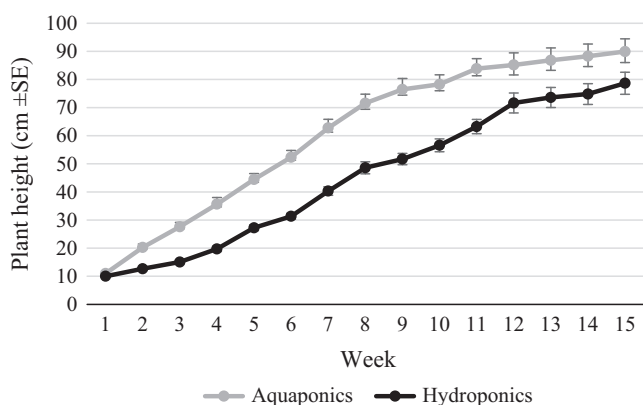


Fig. 2. Basil plant mean weekly height (cm) under aquaponic and hydroponic systems. \pm SE represents standard error.

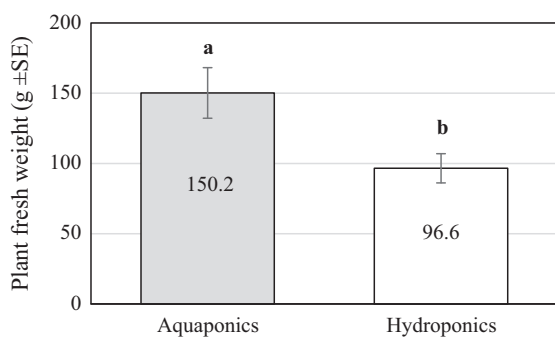


Fig. 3. Basil plant mean fresh weight (g) at harvest under aquaponic and hydroponic systems. Lower case letters indicate significant difference at $P < 0.05$ level between soilless systems. \pm SE represents standard error.

Stockle et al., 1994). Therefore, it is logical to assume that additional nutrients, especially N that were available to aquaponic basil, resulted in their 14% greater plant height and 56% more fresh weight compared to HyB. Similar results were reported by Savidov (2005) and Lennard and Leonard (2006), where greater production was observed in tomato, cucumber, and lettuce grown under aquaponic systems compared to hydroponic systems. Five plants were grown in each container that resulted total mean dry plant weight of 79.5 g (AqB) and 48 g (HyB) per lid surface area (506.25 cm²). This translates to a dry basil yield of 15,900 kg ha⁻¹ in aquaponics and 9600 kg ha⁻¹ in hydroponics. Zheljzakov et al. (2008) compared production of 38 basil cultivars grown on soil and the mean

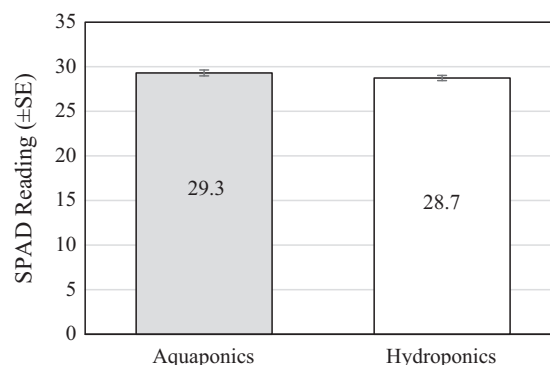


Fig. 5. Basil mean leaf chlorophyll content (SPAD) under aquaponic and hydroponic systems. \pm SE represents standard error.

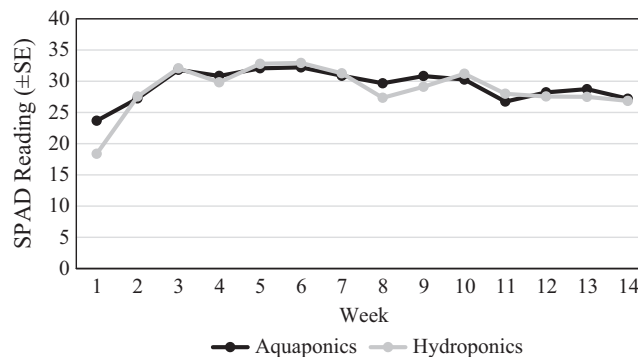


Fig. 6. Basil mean weekly leaf chlorophyll content (SPAD) under aquaponic and hydroponic systems. \pm SE represents standard error.

yield was 4363 kg ha⁻¹ (highest 6166 kg ha⁻¹ and lowest 1812 kg ha⁻¹). This indicates that the soilless basil cultivation yielded considerably higher plant biomass. Biomass production was noted higher in location 3 compared to all other locations, which was probably due to the differences in availability of sunlight, air flow, and proximity to wall.

Plant quality and nutrition

The basil plant quality was measured by the leaf chlorophyll content. The mean leaf chlorophyll content between AqB (29.3 SPAD \pm 0.3) and HyB (28.7 SPAD \pm 0.3) was not significantly different (Fig. 5). Similar results were observed by Rakocy and

Table 1
Basil leaf mean macronutrients (%) ($\pm SE$) under soilless systems.

	N	P	K	Mg	Ca	S
Aquaponics	5.45 (± 0.08)	1.71 (± 0.05)	0.78 (± 0.02)	0.54 (± 0.01)	2.93 (± 0.11)	0.32 (± 0.01)
Hydroponics	5.21 (± 0.11)	1.63 (± 0.05)	0.69 (± 0.02)	0.45 (± 0.02)	2.92 (± 0.13)	0.30 (± 0.01)

Means that are not followed by different letters were not significantly different at $P < 0.05$ level. $\pm SE$ represents standard error.

Table 2
Basil leaf mean micronutrients (mg kg^{-1}) ($\pm SE$) under soilless systems.

	B	Cu	Fe	Mn	Na	Zn
Aquaponics	42.5 (± 1.7)	14.1 (± 0.86)	96.1 (± 4.1)	100.3 (± 10.5)	89.7 (± 13.5)	62.2 (± 5.3)
Hydroponics	37.6 (± 1.6)	15.9 (± 0.74)	99.1 (± 3.8)	92.7 (± 3.6)	86 (± 8.2)	65.4 (± 2.2)

Means that are not followed by different letters were not significantly different at $P < 0.05$ level. $\pm SE$ represents standard error.

Hargreaves (1993), who did not find any differences in chlorophyll content in lettuce crop grown under aquaponic and hydroponic systems. It is likely that the applied basic fertilizer was sufficient for the HyB to develop a healthy chlorophyll content in relation to its growth, and no significant difference was observed. The weekly mean chlorophyll readings displayed a similar trend, as no significant differences between AqB and HyB plant quality were observed throughout the study (Fig. 6).

Leaf nutrient analysis is important to understand the effects of treatments on the basil nutritional value. Leaf macronutrient analysis was done for nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), and sulfur (S) (Table 1). None of the macronutrients differ between aquaponics and hydroponics. Although greater amounts of N and P were available in the aquaponic tanks and it is likely plants have taken them up resulting additional growth and yield, but it did not affect the nutrient content in the leaves. Basil leaf micronutrient analysis was done for boron (B), copper (Cu), iron (Fe), manganese (Mn), sodium (Na), and zinc (Zn) (Table 2). None of the micronutrient contents were shown to be different between aquaponic and hydroponic basil. Additional nutrients supplied by crayfish waste did not have any effect on leaf nutrient content. Investigation of leaf macro- and micronutrient content for basil grown under soilless systems has not been done before, and thus the findings provided in this paper are novel and of value for future research.

Conclusions

Both hydroponic and aquaponic systems can produce basil crop with limited water and without soil, which has the potential to address the issues of land and water scarcity. Aquaponic basil produced 14% more height and 56% more fresh weight compared to the hydroponic basil. We conclude that this additional growth and yield in AqB has resulted from the additional nutrients supplied by the crayfish excreta and unconsumed feed. Thus, crayfish can be a potential aquatic species for aquaponic crop production. We, however, did not observe any differences in plant quality (chlorophyll) or leaf nutrient contents between the two soilless systems. Additional nutrients in the aquaponic systems did not affect these two parameters. Future research may investigate the aquaponic crayfish yield, nutrient cycling, isolation and quantification of nutrient sources, specific patterns of nutrient availability and uptake, cost-benefit analysis of productivity, and different aspects of basil and other crops under soilless systems.

Research facility

The study was conducted at a climate-controlled greenhouse of Georgia Southern University located on campus, Statesboro, GA 30460, USA.

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