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Efficiency of pumpkin (*Cucurbita pepo*), sweet wormwood (*Artemisia annua*) and amaranth (*Amaranthus dubius*) in removing nutrients from a smallscale recirculating aquaponic system

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Abstract

In aquaponic systems, plants absorb dissolved nutrients from aquaculture wastewater for their growth. The removal of nutrients allows reuse of water and minimises wastewater discharge to the surrounding environment. This study evaluated the relative nutrient removal efficiencies of three plant species and the performance of Nile tilapia (*Oreochromis niloticus*) in a small-scale aquaponic system. Three aquaponic systems were assigned as treatments PU (pumpkin), SW (sweet wormwood) and AM (amaranth). Physical-chemical water quality parameters were measured in the fish tanks as well as hydroponic inlets and outlets. Fish sampling was done every 2 weeks for 60 days. Results showed significantly ($p < 0.05$) lower nutrient concentrations in the hydroponic outlets than the inlets. There was no significant difference in nutrient removal efficiency of the plants ($p > 0.05$) for all the tested nutrients except for phosphorus. Phosphorus removal was substantially high in PU ($75.5 \pm 16.8\%$) than SW ($47.36 \pm 14.5\%$) and AM ($40.72 \pm 13.2\%$). In addition, the SW system had better ($p < 0.05$) relative growth rate (0.06 ± 0.00) than that of PU (0.05 ± 0.00) and AM (0.05 ± 0.00). Nitrogen and phosphorus content in plant tissues was significantly high ($p < 0.05$) in SW and PU plants respectively. Water quality parameters had a significant influence on the growth of fish ($p < 0.05$). The growth of fish was not different ($p > 0.05$) in the three treatments. The findings indicate that the tested plants can reduce nutrients in aquaculture discharge water.

Keywords Aquaponic system · Biofilter · Nutrients · Recirculating aquaculture system

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Introduction

Aquaculture has been proposed as the only way of ensuring that the increasing global demand for fish is met (FAO 2016). However, there are many environmental concerns with conventional aquaculture including the frequent exchange of nutrient-rich water with freshwater from adjacent water bodies to provide optimum conditions for fish production (Konnerup et al. 2011). The frequency of water exchange can be daily or weekly and increases during the harvesting season with renewal volumes of between 30 and 100% (Konnerup et al. 2011). Discharge of untreated or partially treated aquaculture effluents can cause eutrophication and degradation of the recipient water bodies (Godfray et al. 2010). Besides, the expansion of conventional aquaculture systems depends on freshwater and land resources. Therefore, aquaculture development may be constrained by land and freshwater availability (Troell et al. 2014; Waite et al. 2014). Further development in aquaculture will require the application of improved technologies that can increase fish production, conserve water and reduce environmental impact (Konnerup et al. 2011; Trang and Brix 2014; Zou et al. 2016).

Moreover, increasing food insecurity, water scarcity, coupled with climate change challenges and resource degradation in Sub-Saharan Africa requires development and application of sustainable food production systems such as aquaponics (Hu et al. 2015; Zou et al. 2016; Gichana et al. 2018). Aquaponic systems integrate recirculating aquaculture and soilless crop production (hydroponics) in a single- or double-recirculating system (Wongkiew et al. 2017). In these systems, the wastes produced from the aquaculture system serve as nutrients for plant production. Microbial activities, as well as nutrient absorption by plants in the hydroponic subsystem, reduce nutrient concentration in the culture water, which in turn improves the environmental conditions for fish production (Boxman et al. 2017; Effendi et al. 2017). The integration of fish and vegetable production increases the diversity and yield of multiple products and water is reused through the process (Somerville et al. 2014). Therefore, aquaponic systems can meet the increasing demand for food and at the same time reduce negative impacts on the environment (Tyson et al. 2011), hence the need to embrace aquaponic systems to get the most out of the available land and water resources. Besides, there is potential for aquaponic development in the tropics because of the favourable climatic conditions (Trang et al. 2010). However, aquaponic initiatives are still at relatively early stages of research and development but have great potential to improve the sustainability of aquaculture (Gichana et al. 2018; Monsees et al. 2017).

Several aquaponic studies evaluating the production performance, nutrient removal capacity and economic sustainability established that aquaponics can be sustainable food production systems (Endut et al. 2011; Hu et al. 2015; Love et al. 2015; Endut et al. 2016; Estim et al. 2018). The studies demonstrated the potential of plants to utilize nutrients from aquaculture wastewater and maintain water quality for fish production. Endut et al. (2011) reported that water spinach was able to reduce 79.17–87.1%, 75.36–84.94% and 78.32–85.48% of nitrate, phosphorus and total ammonia nitrogen respectively in catfish culture wastewater. Besides, daily water loss can be reduced to 2% of the total water volume of the system. The integration of hydroponics and aquaculture increase sources of food, profitability and reduce operating costs when considering each system individually (Love et al. 2015). Cost-benefit analysis studies of aquaponics recommended proper selection of plant species for the target market to generate income at the end of the production cycle (Love et al. 2015). The study showed that the gross sales revenue and profitability were higher for operations that diversified their revenue streams by selling non-food products such as educational training (Love et al. 2015).

More than 150 different herbs, small trees and vegetables have been cultivated successfully in experimental, domestic and commercial aquaponic systems. Most operations prefer leafy greens and herbs such as lettuce, salad rocket, basil, mint, parsley, coriander, chives, watercress, pak choi and chard because they are well adapted to aquaponics, they grow faster and they can be used for culinary and aromatic purposes that have low to medium nutritional requirements (Wahyuningsih et al. 2015; Somerville et al. 2014; Rakocy and Masser 2006), whereas fruiting plants including tomatoes, pepper and cucumbers have a higher nutritional demand and perform better in well-established systems with adequate fish stocks (Somerville et al. 2014). However, the selection of plant species depends on the stocking density of fish tanks and the subsequent nutrient concentrations of aquaculture wastewater (Diver 2006). The three plant species namely sweet wormwood, pumpkin and amaranth were selected because they can grow well in aquaponic systems with low to medium nutrient concentrations due to the low-stocking density of fish. Besides, in selecting plant species for aquaponic systems, the use of species that can grow for a short period of time as well as species that can withstand hydroponic and local climatic conditions and have economic value is recommended.

Sweet wormwood is a medicinal plant indigenous to China but can grow in subtropical environments. It is used in modern pharmaceuticals to produce artemisinin, a well-established and widely used ingredient in artemisinin-based combination therapy (ACT), the current best medicine for treating malaria that is prevalent in Sub-Saharan Africa (Pulice et al. 2016). Artemisia can be used in aromatherapy, preservation and flavouring and has antioxidant as well as anti-inflammatory properties and antimicrobial capabilities against diverse pathogens (Kim et al. 2015). Pumpkin and amaranth are popular indigenous vegetables in Kenya. Pumpkin is famous for its fruits and vegetables and amaranth is grown for its edible and tasty leaves and grains. The indigenous vegetables have high micronutrient content, medicinal properties and economic values (Abukutsaonyango 2007). Although previous aquaponic trials have grown herbal and indigenous plants in aquaponic systems (Henze and Ulrichs 2015; Oladimeji et al. 2018; Gichana et al. 2019), there is still not enough information about growth and efficacy of the plants in aquaponic systems. Hence, the use of these plants as part of the biofilters in aquaponic systems should be further explored. This study was therefore designed to evaluate the effectiveness of *A. annua*, *C. pepo* and *A. dubius* to treat wastewater from a recirculating aquaculture system for Nile tilapia (*Oreochromis niloticus*). The corresponding effect on the growth of *O. niloticus* was also investigated.

Materials and methods

Experimental design

The study was conducted in Aqualife fish farm in Machakos county, Kenya for 60 days from May to June 2017. Three identical coupled (continuous recirculation between aquaculture and hydroponic subsystems) aquaponic systems with gravel bed hydroponic subsystems were constructed under a greenhouse. One system consisted of nine plastic fish tanks measuring (500 L containing 400 L of water volume) and nine hydroponic subsystems (Fig. 1). Each hydroponic subsystem with 0.1125 m³ volume and 0.75 m² crop area was made of timber and pond liner. In each hydroponic unit, 0.06 m³ of gravel, thoroughly rinsed and sundried, were used a substrate for plant growth. Three treatments, PK, SW and AM, representing pumpkin, sweet wormwood and amaranth, were replicated 3× in 1.5 m × 0.5 m × 0.8 m hydroponic

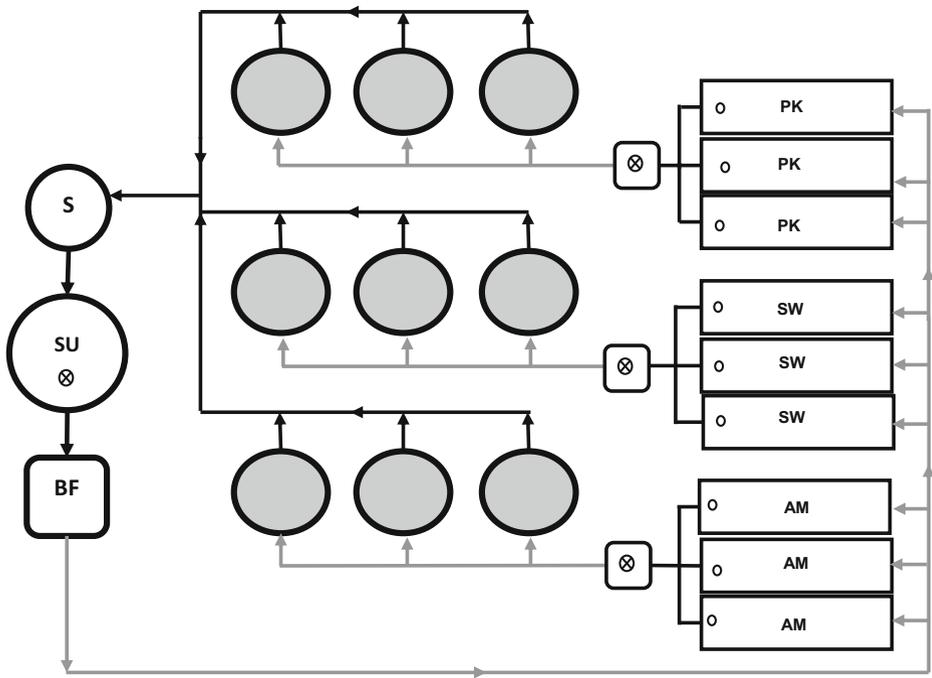


Fig. 1 Schematic presentation of the experimental aquaponic system (not drawn to scale). PK, SW and AM: indicate hydroponic subsystems, whereby PK, were cultivated with pumpkinkin; SW, sweet wormwood and AM; amaranth. Small circles at the outlet of grow beds represent auto-siphons. Represent water pump; grey circles represent the fish rearing tanks, SF; sand filter, SU; sump, BF; biofilter; grey and black lines with arrows indicate water flow direction with grey representing inlet and black the outlet

subsystems. The outflow from three replicates for each treatment was combined before being directed into groups of fish in triplicate. The discharge from each hydroponic subsystem was constructed as a bell siphon with an auto-mechanical water out-movement initiating the ebb underwater pressure modified from (Bruno et al. 2011).

The recirculating aquaculture system was connected to a sand filter (210 L volume tank, 0.93 m height) and a biological filter. The biological filter consisted of a 210-L plastic barrel with a 0.73-m water-filled height, 0.59-m diameter and 0.25-m false bottom to allow water to settle and be distributed evenly. Thoroughly rinsed and sundried pumice stones (0.18 m³) were used as biofilter media. A porous disc diffuser was used to aerate the biofilter. The biofilter and air diffusers were subjected to biweekly cleaning to reduce accumulation of fine solids. We used a cold start method to activate the biofilter. This is a passive biofilter activation method which involves stocking of fish without an activated biofilter to encourage the growth of bacteria in the biofilter substrates (DeLong and Losordo 2012). Fish were, therefore, stocked 4 weeks before the start of the experiment to allow bacteria to naturally colonize the media substrate. Polyvinyl chloride (PVC) pipelines with ball valves were installed to circulate water between the aquaculture and hydroponic subsystems. A constant water flow rate of $1.42 \pm 0.23 \text{ L min}^{-1}$ was maintained in all the hydroponic subsystems, and no additional fertilisers or pesticides were used. The flow rate was controlled by ball valves. The flow rate was then used to determine the hydraulic loading rate (HLR) which was calculated as flow rate was divided by the total surface area of each hydroponic unit (Endut et al. 2010). The hydroponic

subsystems were subjected to the same HLR of 2.72 m/day during the experimental period. Water from the fish tanks flowed through the sand filter by gravity to the sump, where a centrifugal pump (DPP 60, 0.5 HP, 2500 L/h, 0.37 kW, Davis and Shirtliff) was used to deliver water (6 ± 0.24 L/min) to the biological filter. The filtered water flowed by gravity to the hydroponic subsystems and was then pumped back to the fish tanks. An air pump (> 0.03 Mpa, 60 L/min, Aqua Forte, V-60) was used to aerate the fish tanks and biofiltration unit. Approximately 50% of the culture water in each fish tank was exchanged with clean water once in 2 weeks during fish sampling.

Fish and plant species

Fifty Nile tilapias with an initial weight of 54.5 ± 4.8 g and 13.7 ± 0.4 cm in length were stocked in each tank. The fish were hand-fed to satiation twice a day (09:00 h and 16:00 h), with a 30% crude protein commercial pelleted feed. The total feed input was recorded in each treatment. Fish sampling was done biweekly to monitor the length and weight. Fish performance was evaluated using growth performance indices including percentage weight gain, maximum growth rate, feed conversion ratio (FCR) and survival rate (SR). The performance indices were calculated using standard formulae as follows:

$$\text{Percentage weight gain (PWG)} = (\text{Final weight} - \text{initial weight}) \times 100$$

$$\text{FCR} = \frac{\text{Total weight of dry feed given(g)}}{\text{Total wet weight gain(g)}}$$

$$\text{SR} = \frac{N_0 - N_t}{N_0}$$

where N_0 and N_t are fish number at time 0 and at time t .

The von Bertalanffy growth function (VBGF) was used to estimate the maximum growth rate of fish. The VBGF have length and weight equations in the form as follows:

$$L_t = L_\infty \left(1 - e^{-k(t-t_0)} \right)$$

$$W_t = W_\infty \left(1 - e^{-k(t-t_0)} \right)^b$$

where L_t and W_t are the length and weight at time t , L_∞ and W_∞ are the maximum mean length and weight a fish of a given population is expected to reach if they live and grow indefinitely, K is a growth coefficient, t_0 is a scaling constant and b is the exponent of length-weight relationship in the form as follows (Hopkins 1992):

$$W = aL^b$$

where W and L are weight (g) and total length (cm), a is the intercept of the regression line and b is the slope of the regression line.

Pumpkin and amaranth seeds were obtained from a local seed supplier (Simlaw seeds, Kenya) and sweet wormwood seeds were obtained from Anamed International e.V (Schafweide, Germany). The seeds were sown in three seedling trays (filled with loam soil) to seedling size under a greenhouse. During this period, the plantlets were irrigated with freshwater daily for 3 weeks. The plantlets were then transplanted into six hydroponic beds at a density of 24 plants/m². Plant parameters such as the height of the plant (cm) were measured every 2 weeks and the weight (g) measured on day 1 and after 60 days. The final wet weight of the plants consisting of stems, leaves and roots were measured after uprooting the whole plant from the hydroponic subsystem. The fresh weight was then used to determine the relative growth rate (RGR) of the plant species which was calculated as follows:

$$RGR = (\ln W_2 - \ln W_1) / (t_2 - t_1)$$

where W_2 and W_1 are weights at time t_2 and t_1 , t_2 and t_1 are initial and final periods and \ln is natural logarithm (Tylova-Munzarova et al. 2005). At the end of the experiment, three plants from each hydroponic unit were randomly selected to determine nitrogen and phosphorus content. The plants were cut into small pieces and dried to constant weight in an oven at 80 °C. The nitrogen content in the dry samples was determined using the Kjeldahl method and phosphorus content (digestion with nitric-perchloric acid) according to (Motsara and Roy 2008).

Water quality parameters

Water quality parameters including dissolved oxygen (DO), temperature, pH and conductivity were measured twice daily (10:00 h and 16:00 h) using a multiparameter probe (HACH HQ40d Portable meter, Loveland, Colorado, USA). Water samples were collected in triplicate from the fish tanks, sump, biofilter and the biofilter inlets and outlets of each hydroponic subsystem. The samples were then analysed for ammonia (NH₃), nitrates (NO₃), nitrites (NO₂) and orthophosphate phosphorus (PO₄) according to standard methods (APHA 2005). Nutrient (NH₃, NO₃, NO₂ and PO₄) removal efficiencies of the experimental plants were calculated as the change in nutrient concentration in the hydroponic subsystem inlets (water coming from the biofilter) and outlets (water leaving the hydroponic subsystems) (Boxman et al. 2017) were as follows:

$$\text{Nutrient removal efficiency (\%)} = \frac{C_i - C_e}{C_i} \times 100$$

where C_i = concentration of inlet and C_e = concentration of outlet.

Data analysis

Data were presented as means and standard deviation (SD) of three replicates. Percentage data were arcsine transformed before statistical analyses. Normality and homogeneity of means were evaluated by Shapiro-Wilks and Levene tests respectively. Length and weight fish data were log-transformed and analysed using a simple linear regression. One-way analysis of variance (ANOVA) was used to test the difference in nutrient removal efficiency, plant growth parameters and fish growth between plant species treatments. A two-way analysis of variance (ANOVA) was used to determine differences in physical-chemical parameters. Post hoc multiple comparisons of means were performed when necessary using the Tukey's HSD

procedure. The relationship between fish growth and water quality variables was determined using multiple regressions. Differences between means were considered significant at $\alpha = 0.05$. Statistical analyses were performed using the Statgraphics XVI[®] centurion version 16.1.11 (StatPoint Technologies, Inc., USA).

Results

Physical-chemical water quality parameters

Water quality parameters in the sump, biofilter (inlet to the hydroponic unit) and across different hydroponic outlets (PK, SW, and AM) are shown in Fig. 2. Water temperature was relatively high at the start of the experiment but decreased gradually with time in all the treatments. Mean values of water temperature ranged from 22.9 to 32.4 °C and 20.4 and 29.7 °C at the start and end of the experiment respectively. Dissolved oxygen (DO) values fluctuated between 1.7 and 4.5 mg/L during the study period. The concentration of DO was significantly high ($p < 0.05$) in the hydroponic inlet (3.68 ± 0.35 mg/L) but decreased steadily in other systems, with lowest values in SW system (1.77 ± 2.4 mg/L). But there were no significant differences ($p > 0.05$) in temperature, conductivity and pH between systems. Conductivity was maintained between 1285.7 and 1419.45 μS/cm during the study period and pH values ranged between 7.67 and 8.09 in all the systems. Significant ($p < 0.05$) interactions between parameters and sampling days were observed in dissolved oxygen and

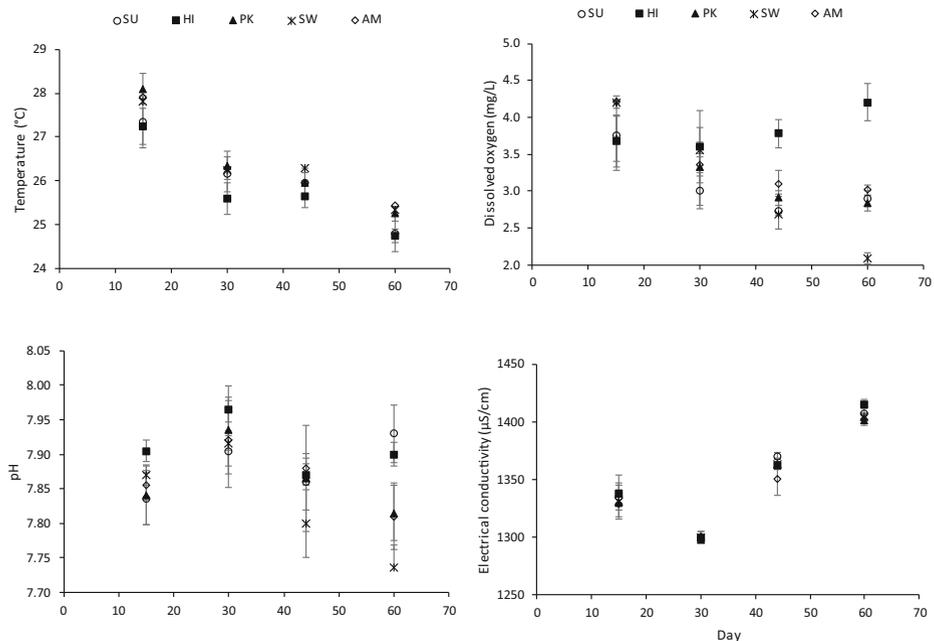


Fig. 2 Physical-chemical water quality measurements. All values are means ± standard deviation obtained from different recirculating aquaculture subsystems. SU, sump; HI, hydroponic inlet; PK, pumpkin, SW, sweet wormwood, AM, amaranth (two-way ANOVA)

conductivity. Multiple regression analysis showed a significant ($p < 0.05$) relationship between fish weight and water quality parameters.

Nutrient removal by plant species

The concentrations of ammonia and nitrate were generally high at day 15 but gradually decreased with time in the hydroponic subsystems (Fig. 3). The highest ammonia concentration was found in SU (3.82 ± 0.20 mg/L) and lower values were recorded in PK (0.58 ± 0.10 mg/L), SW (0.57 ± 0.10 mg/L) and AM (0.53 ± 0.10 mg/L) hydroponic subsystems. Nitrate and nitrite concentrations were lower in the SW system, though not significantly different from AM to PK. There were significant interactions between the sampling period and nutrient concentrations in different subsystems. Relatively high nutrient concentrations were observed in the sump and hydroponic inlets. However, nitrite and phosphorus levels were inconsistent during the study period. All the nutrient concentrations were significantly high ($p < 0.05$) in the sump than in the hydroponic inlet. Furthermore, significantly lower ($p < 0.05$) concentrations of all nutrients except nitrite were observed in the hydroponic subsystems (PK, SW and AM). But there was no significant difference in ammonia, nitrate and nitrite concentrations between the plant species treatments in the hydroponic subsystem. However, phosphorus was significantly lower in the pumpkin hydroponic subsystem than in sweet wormwood and amaranth subsystems. The ammonia removal efficiency was comparable between the plant species and ranged between $48.8 \pm 4.6\%$ and $50 \pm 3.13\%$, whereas nitrate removal was slightly higher ($76.03 \pm 15.9\%$) in sweet wormwood, though not significantly different from pumpkin ($67.0 \pm 21.9\%$) to amaranth ($68.1 \pm 13.3\%$) treatments (Fig. 4).

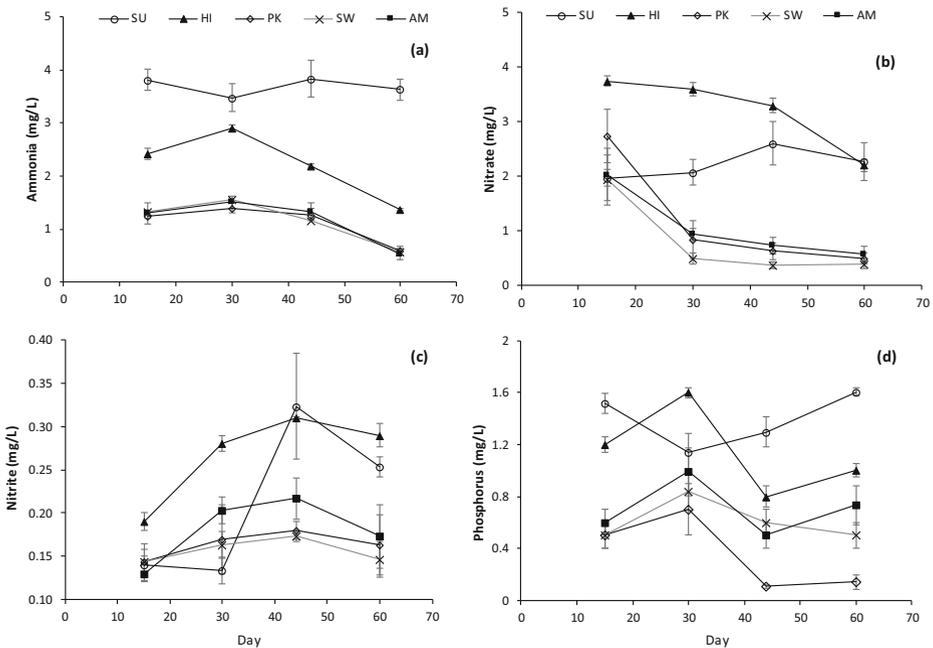


Fig. 3 Concentrations of **a** ammonia, **b** nitrate, **c** nitrite and **d** phosphorus measured during the experiment. Points are means \pm standard deviation. SU, sump; HI, hydroponic inlet; PK, pumpkin, SW, sweet wormwood and AM, amaranth (two-way ANOVA)

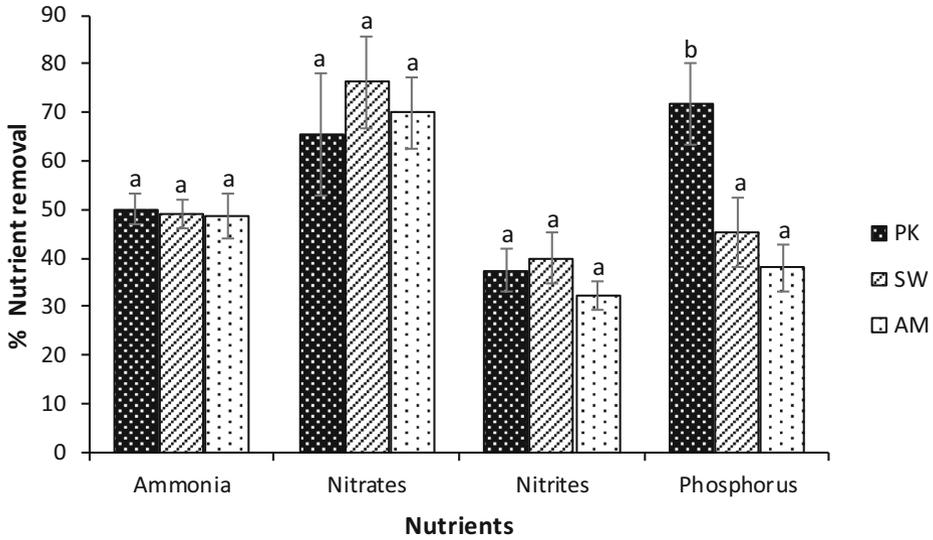


Fig. 4 Mean nutrient removal efficiency (%) from each hydroponic subsystem treatment. Error bars show standard deviation. Different letters above error bars indicate significant difference ($p < 0.05$) between treatments. PK, pumpkin; SW, sweet wormwood and AM, amaranth (one-way ANOVA)

Statistical analysis indicated that pumpkin was more effective ($p < 0.05$) in absorbing phosphorus ($75.6 \pm 16.8\%$) than sweet wormwood and amaranth.

Fish and plant growth

Table 1 presents the growth performance of *O. niloticus* in the aquaponic system. There were no significant differences ($p > 0.05$) in initial weight, final weight, weight gain, SGR, FCR and survival rate of *O. niloticus* between the three different aquaponic systems. The final weight gain in SW (99.5 ± 9.1 g) was slightly high though not significantly different from PU to AM. The survival of fish was more than 90% under the investigated conditions. The SW subsystem had significantly ($p < 0.05$) high relative growth rate and nitrogen content in the plant tissues

Table 1 Growth performance (means \pm SD, $n = 3$) of Nile tilapia in an aquaponic system with different plants (PU, pumpkin; SW, sweet wormwood and AM, amaranth). FCR, feed conversion ratio; SGR, specific growth rate

Parameters	Treatments		
	PU	SW	AM
Initial weight (g)	67.5 \pm 8.2	74.2 \pm 4.9	72.9 \pm 7.1
Final weight (g)	90.0 \pm 3.8	99.5 \pm 9.1	97.5 \pm 12.4
Weight gain (%)	34.5 \pm 14.9	33.9 \pm 4.2	33.6 \pm 4.6
SGR (% d ⁻¹)	0.49 \pm 0.18	0.49 \pm 0.05	0.48 \pm 0.06
FCR	2.4 \pm 0.3	2.3 \pm 1.2	2.3 \pm 1.1
Survival rate (%)	90.0 \pm 4.3	95.8 \pm 7.2	97.5 \pm 4.3

No significant differences between the treatments

Table 2 Relative growth rate, nitrogen and phosphorus content in PU, pumpkin; SW, sweet wormwood and AM, amaranth (mean \pm SD, $n = 3$)

Parameters	PU	SW	AM
Relative growth rate (g/d)	0.05 \pm 0.00 ^a	0.06 \pm 0.00 ^b	0.05 \pm 0.00 ^a
Nitrogen content (%)	1.74 \pm 0.05 ^a	2.33 \pm 0.08 ^b	1.72 \pm 0.04 ^a
Phosphorus content (%)	0.29 \pm 0.02 ^b	0.21 \pm 0.04 ^a	0.21 \pm 0.03 ^a

Different superscript letters within a row indicate statistically different mean values at $p < 0.05$

than PU and AM. However, phosphorus concentration was high ($p < 0.05$) in PU tissues than in SW and AM (Table 2).

The slope of length against weight (b) did not vary ($p > 0.05$) between the treatments and ranged between 2.78 and 3.0. However, slightly high slope values were obtained in SW, but not significantly different from PU and AM treatments (Fig. 5). Sweet wormwood had the highest maximum growth rate (1.33 g/day) on day 98, whereas pumpkin had the lowest maximum growth rate (1.05 g/day) on day 42. Amaranth treatment had a maximum growth rate of 1.23 g/day on day 98. The growth rate declined gradually in all the treatments after the maximum growth rate was reached (Fig. 6).

Discussion

Water quality is essential in aquaponic systems because all organisms in the system depend on good water quality for optimum growth (Somerville et al. 2014). Therefore, water quality parameters should be maintained within tolerable limits for optimal growth of fish, bacteria and plants in an efficient aquaponic system (Goddek et al. 2016). Water quality parameters in the tested aquaponic system were within acceptable ranges for growth of tilapia and plants.

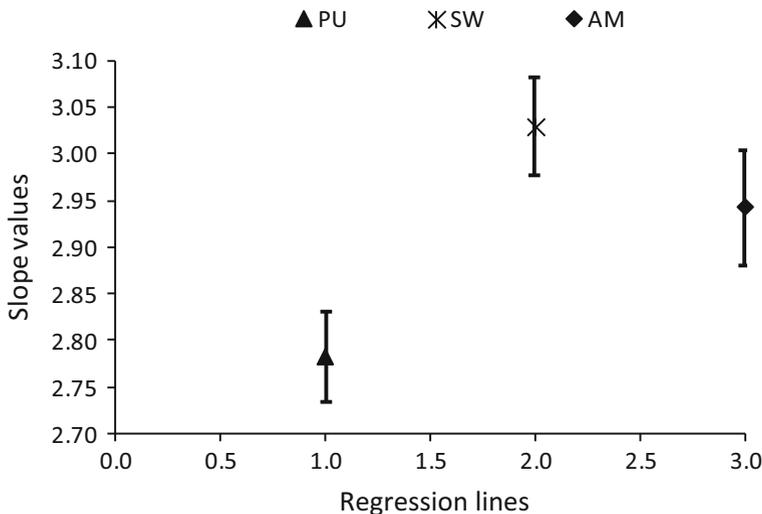


Fig. 5 Slopes of regression lines of lengths against weight of tilapia in PU; pumpkin, SW; sweet wormwood and AM; amaranth treatments

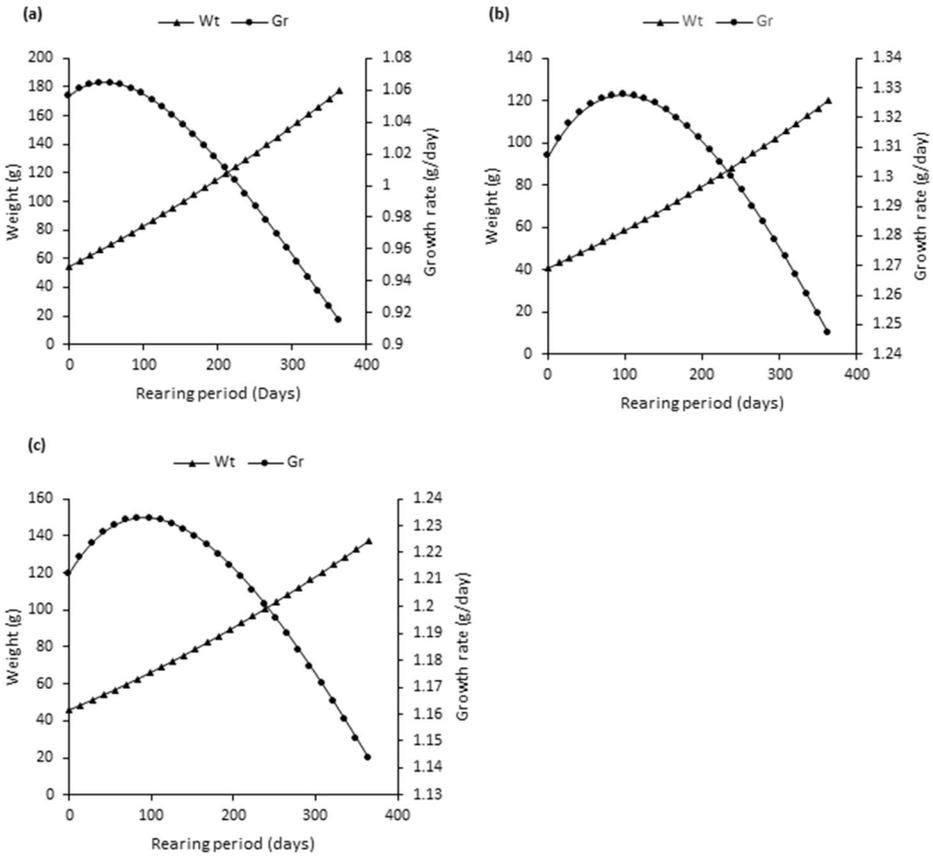


Fig. 6 Maximum growth rate (Gr) and weight (Wt) of *O. niloticus* (estimated from von Bertalanffy growth function) in **a** PU; pumpkin, **b** SW; sweet wormwood and **c** AM; amaranth treatments

However, dissolved oxygen (DO) levels were slightly outside the recommended range considering that dissolved oxygen in fish tanks and hydroponic inlets should be maintained between 5 and 6 mg/L for optimum growth of most warm water fish and hydroponic plants (Wongkiew et al. 2017). The low DO levels in the sump can be attributed to biological processes including fish respiration, oxidation of ammonia in the sand filter in addition to organic load production in the system (Espinosa-moya et al. 2018; Wongkiew et al. 2017). Furthermore, DO levels decrease in the biofilters and hydroponic subsystems due to the activities of microbial organisms including nitrifiers and heterotrophs as well as absorption by plant roots (Gichana et al. 2019; Wongkiew et al. 2017). Plant roots require oxygen to carry out metabolic processes such as aerobic respiration (Schroeder and Lieth 2004). Low dissolved oxygen levels in solution decrease the permeability of plant roots to water which can subsequently reduce nutrient absorption and growth of plants (Estim et al. 2018). Hydroponic plants generally experience low dissolved oxygen periods which may result in root rot and nitrogen loss via denitrification (van Patten 2002). It is, therefore, necessary to maintain enough oxygen around the root mass through the installation of aeration systems or flood and drain mechanism in the hydroponic subsystem (Timmons et al. 2002). In our study, the concentration of dissolved oxygen at the hydroponic inlet was lower (3.81 ± 0.27 mg/L) than

typical ranges (5–6 mg/L) for hydroponic systems concentration (Timmons et al. 2002). The oxidation of ammonia to nitrates (nitrification) in the biofilters possibly contributed to the low dissolved oxygen levels at the hydroponic inlets. Nitrification is an oxygen demanding process because 5 mg of oxygen is needed to oxidize 1 mg of ammonia to nitrate. In addition, 5 mg of oxygen are required to satisfy the demand of aerobic bacteria (Helfrich and Libey 2013). Submerged aerobic biofilters with fixed substrates are prone to solid accumulation because common solid removal techniques such as the sand filtration are ineffective in removing fine solids (Martins et al. 2010; Al-Hafedh et al. 2003). Therefore, the frequent clogging of the disc diffusers resulting from fine solid accumulation affected their effectiveness which in turn reduced the dissolved oxygen levels in the biofilter. Although the aerators were subjected to frequent cleaning, the production of air bubbles from the discs reduced with time suggesting that cleaning was not an effective method to unclog the disc diffusers. The flood and drain mechanism irrigates the plants by filling the hydroponic units with nutrient-rich water followed by a period of draining which introduces air into the root zone (Pattillo 2017). The periodic flooding followed by air exposure introduces oxygen to plant roots and bacteria living in the media-filled grow bed. However, the mechanism floods the plants with nutrient-rich water and allows aeration for a short period compared with constant flow systems with more contact time to remove nutrients and constant aeration (Lennard and Leonard 2004). Lennard and Leonard (2004) demonstrated that constant flow systems improved aeration and allowed hydroponic plants to assimilate more nutrients from the culture water.

The water temperature decreased gradually at the end of the experiment because the last water sampling was conducted in June, which is one of the coldest months in Kenya. However, the temperature range in our study was within the optimum range for growth of tilapia (24–32 °C) and for most hydroponic plants (21.1–24 °C) (El-Sayed and Kawanna 2008; Rakocy et al. 2010). However, fish growth rates and feed utilization efficiency reduce significantly at lower suboptimal (24 °C) and upper suboptimal (32 °C) temperature levels (El-Sayed and Kawanna 2008). pH is one of the most important water quality parameters that control the performance of aquaponic systems. pH affects fish metabolism, the activity of nitrifiers and heterotrophs and influences the availability of nutrients to plants (Zou et al. 2016). pH was slightly alkaline and within 6–9, typical ranges for the culture of fish (Colt 2006). The slightly alkaline pH was also within recommended levels for the activity of nitrifiers and heterotrophs (7.5–8.0) (Kim et al. 2007). However, the mean pH range (7.74 ± 0.06 – 7.94 ± 0.06) in the hydroponic subsystem is considered high for hydroponic cultivation. Ideally, pH should be slightly acid (5.5–5.8) to increase the nutrient absorption capacity of plants (Bugbee 2004). Zou et al. (2016) established that when pH was maintained at 6.4 nitrogen utilization efficiency (NUE) of plants cultivated in a media-based aquaponic system increased to 50.9% but the NUE dropped to 47.3% and 44.7% when pH was increased to 7.4 and 8.0 respectively. But most aquaponic studies recommend an alkaline medium between 6.5 and 9.0 (Estim et al. 2018). Though high pH levels can affect nutrient absorption capacity and plant growth (Rakocy et al. 2010), no signs of nutrient deficiencies were observed on the tested plants.

Relatively high ammonia concentrations in the sump could be attributed to waste metabolites from the fish tanks which are released in form of ammonia through excretion and decomposition of uneaten feed and any organic matter (Yildiz et al. 2017). Minimal water exchange (once in 2 weeks) can be another reason for elevated ammonia levels in the aquaponic system. Furthermore, high ammonia levels at the beginning of the experiment suggest that the nitrifying bacteria had not established properly, although the fish were

stocked 1 month before the start of the experiment to allow the bacteria to naturally colonize the biofilter substrates. This is because the nitrifying bacteria have long start-up and multiplication periods (DeLong and Losordo 2012). Therefore, the nitrifying bacteria that had established in the biofilter substrate could not effectively oxidize ammonia to nitrates (Hu et al. 2015). Besides, the higher than recommended ammonia levels at the hydroponic inlets can be linked to ineffective fine solid removal techniques such as sand filtration that was used in the tested system (Martins et al. 2010). The accumulation of fine solids supports the growth of heterotrophs which influence the rate of ammonia oxidation and in turn reduce the efficiency of the biofilter (Michaud et al. 2006). Moreover, nitrite accumulation on day 45 can be as a result of incomplete denitrification in the sand filter, which acted as a mechanical filter to remove undesirable solid particles. This indicates that the solid particles favoured the growth of denitrifiers which reduced nitrate to nitrite with no further reduction (Stief 2001).

Plants have the capacity to absorb more nutrients than they require for normal metabolic functions through a mechanism called luxury consumption. The mechanism enables plants to absorb nutrients during periods of adequate supply and then store the excess nutrients in plant tissues for later use in times of shortage (Cronk and Fennessy 2001). Furthermore, the strategy allows plants to meet immediate nutrient demands and sustain the growth of plants when nutrient concentrations are low (Adler et al. 2000). Therefore, the ability of the tested plant species to assimilate nutrients from aquaculture wastewater and concentrate them in plant tissues can be as a result of luxury consumption. Luxury consumption of nutrients probably sustained the growth of plants although the concentrations of nutrients at the influent to the hydroponic subsystems were lower than standard hydroponic systems (Rakocy et al. 2004). Luxury uptake can be a means by which plants prepare for unfavourable environmental conditions. Besides, plants that can concentrate excess nutrients in their tissues tend to have high nitrogen utilization efficiency (NUE) and rapid growth rates (Baligar et al. 2001). Our findings indicate that sweet wormwood had high NUE due to the significantly high nitrogen content which translated to high growth rates than amaranth and pumpkin. Studies using mangroves (Agraz-Hernández et al. 2018) wetland plants (Chen et al. 2009) have demonstrated that luxury uptake contributes to the removal of nutrients from wastewater. The substantial removal of phosphorus on day 15 in all the hydroponic subsystems can be attributed to increased demand for root development when plants are young, whereas high phosphorus removal efficiency of pumpkin can be an indication of luxury uptake of phosphorus to offset anticipated need during the fruiting stage (Buzby and Lin 2014).

The effectiveness of hydroponic plants in removing nutrients from aquaculture wastewater is influenced by several factors including (1) surface area of plant roots, (2) the growth stage and nutrient requirement of the plants, (3) microbial activity in the hydroponic subsystem, (4) flow rates and (5) hydraulic loading rate (HLR) (Boxman et al. 2017; Buhmann and Papenbrock 2013; Endut et al. 2010). Plants with a large surface per unit volume of the hydroponic unit effectively remove nutrients from aquaponic systems because the large surface area of the root system provides more attachment sites for microbial organisms including ammonia-oxidizing bacteria (Endut et al. 2009). The trend in nutrient removal of the tested plant species can be attributed to the surface area of plant roots as well as the growth stage of the plants because the nutrient requirement of plants increases with growth stage (Jones et al. 2015). Therefore, the large surface area of fully developed plant roots at the end of the experiment probably provided a large area for the attachment and growth of microbial

organisms which in turn reduced nutrient concentrations in the hydroponic system. Besides, nutrient removal increased with experimental period due to the nutrient requirement of the plants. Buzby and Lin (2014) opined that nutrient removal is greater when plants are growing because the nutrient needs are high.

Microbial activities play a major role in the removal of nutrients particularly nitrogen in media-based aquaponic systems (Boxman et al. 2017; Trang and Brix 2014). The high ammonia and nitrate concentration at day 15 suggests that microbial organisms such as ammonia-oxidizing bacteria and denitrifiers had not established properly resulting in low nutrient removal efficiency (Wongkiew et al. 2017). The trend in ammonia and nitrate levels suggest that microbial activity that oxidizes ammonia and converts nitrates to nitrogen gas favoured the removal of nutrients from the hydroponic subsystem. Similar media-based aquaponic studies demonstrated that microbial organisms play a significant role in removing nutrients from aquaculture wastewater (Gichana et al. 2019; Boxman et al. 2017; Hu et al. 2015; Trang and Brix 2014). Gichana et al. (2019) demonstrated that plant uptake did not contribute significantly to the overall removal of nutrients in a media-based system because a hydroponic subsystem without plants played a role in the removal of nutrients from the effluent water. In Trang and Brix (2014), plants absorbed only 6% of nitrogen and 7% of phosphorus while 45% of nitrogen and 9% of phosphorus was unaccounted for suggesting that the unaccounted nitrogen and phosphorus was probably bound in the media substrate or lost through denitrification. Hu et al. (2015) reported 51.2% and 57.3% of ammonia and nitrogen loss respectively due to denitrification in tomato- and pak choi-based aquaponic systems. The accumulation of suspended solids in media-based systems boost the development of anoxic microsites which in turn provides favourable conditions for the denitrification process which removes a significant amount of nitrogen from aquaponic systems (Boxman et al. 2017; Wongkiew et al. 2017). This suggests that microbial processes in the plant root zone play a key role in removing nutrients in media-based aquaponic systems (Boxman et al. 2017).

Water flow rate is one of the most important operational conditions that can influence the nutrient removal in aquaponic systems because it determines the optimum contact time for nutrients and microorganisms in the culture solution (Nuwansi et al. 2015). High flow rates minimize the contact time between nutrient-rich water and plant roots and wash away microbes and sediments attached to roots which consequently reduce nutrient extraction efficiency of plants. Conversely, low flow rates may result in oxygen deficiency due to the accumulation of organic matter which may increase denitrification and loss of nitrogen from the aquaponic system (Endut et al. 2009). Therefore, an optimum flow rate should be maintained to increase the nutrient extraction efficiency of plants. In commercial recirculating systems, the flow rate is regulated to achieve optimal dissolved oxygen levels in the system as well as rapid removal of wastes and fish metabolites (Boxman et al. 2017; Nuwansi et al. 2015). Flow rates are typically based on the turnover rates of fish tanks which can vary from 15 to 60 min (Ebeling and Timmons 2012). Ideally, high flow rates should be maintained in RAS to exchange the rearing tanks once every 30–45 min to increase oxygen levels and waste flushing from the culture system (Summerfelt et al. 2004). The effect of increasing flow rates resulting in a significant increase in DO concentrations has been demonstrated (Endut et al. 2009). However, low flow rates resulted in low dissolved oxygen conditions which promoted denitrification and significant removal of nitrate in low flow rates of 0.8 L/min and 1.6 L/min (Endut et al. 2009). Boxman et al. 2017 recommended a high flow rate of 1 L/min and a fish tank turnover rate of 38 min to maintain optimum conditions in the culture system. In aquaponic systems, slow flow rates and low hydraulic loading rates are preferred for better

nutrient removal (Boxman et al. 2017). Previous studies demonstrated that nutrient uptake was dependent on flow rate as the nutrient removal decreased with increasing flow rate (Nuwansi et al. 2015). Endut et al. (2009) showed that the removal of phosphates and nitrate increased with increasing flow rates from 0.8 to 1.6 L/min and decreased with increasing flow rates from 1.6 to 4.0 L/min, whereas ammonia and nitrites removal increased with increasing flow rates. In addition, Endut et al. (2009) reported better ammonia (64–78%) and nitrite (67–89%) removal efficiency than the present study although our flow rate (1.42 ± 0.23 L/min) was within the range reported by Endut et al. (2009). The constant flow regime maintained in Endut et al. (2009) than the flood and drain mechanism in the present study because constant flows increase contact time for plants and microorganisms to remove more nutrients from the culture water (Lennard and Leonard 2004). Nitrate removal efficiency in this study was 65.5–76.3%, higher than 42–65% reported by Endut et al. (2009) due to the low oxygen levels in the hydroponic subsystems which provided favourable conditions for removal of substantial amount of nitrogen from the aquaponic system through the denitrification process.

The flow rate per unit surface area of hydroponic grow bed commonly known as hydraulic loading rate influences the contact time of nutrients and the microbial communities in the hydroponic subsystem (Endut et al. 2009). At high HLR, the recirculating water passes rapidly through the hydroponic bed reducing the contact time for microbial processes to become effective. Furthermore, high HLR washes out microbial organisms as well as sediment attached on the biofilter substrate plant roots (Endut et al. 2010), whereas low HLR increases the contact time between the nutrient-rich water and bacteria. Low HLR can increase sediment accumulation and lead to low oxygen levels which can result in denitrification and loss of nitrogen to the atmosphere (Wongkiew et al. 2017). Therefore, relatively high ammonia levels at the hydroponic outlets can be attributed to the high hydraulic loading rate (2.72 m/day) in the present study. The high HLR probably reduced the contact time of nutrients and microbes which in turn reduced the ammonia removal efficiency of the plant species. Endut et al. (2010) found that nutrient removal increased with increasing HLR from 0.64 to 1.28 m/day, whereas a further increase in HLR from 1.28 to 3.20 m/day resulted to significantly low nutrient removal efficiency of water spinach. Previous constructed wetland studies showed that HLRs between 0.018 and 1.95 m/day successfully reduced nutrients from aquaculture wastewater but higher HLRs reduced the removal efficiency of nitrates (Lin et al. 2005; Xu et al. 2014).

The growth performance of *O. niloticus* was comparable between the aquaponic systems suggesting that the plant species treatment did not significantly affect the growth of the fish. However, Al-Hafedh et al. (2008) obtained better specific growth rate (1.0–1.1%/day) and feed conversion ratio (1.5–1.7) in an aquaponic system for *O. niloticus* with comparable initial mean weight (74.8 g) to those of the present study. Besides, the feed conversion ratio was higher than recommended 1.5–2.0 for tilapia cultured in intensive systems (Stickney 2005). Water quality parameters in the aquaponic system particularly high ammonia levels as well as temperature and DO values below the optimum for *O. niloticus* probably influenced the growth of fish. Although tilapia can tolerate a wide range of water quality conditions, poor water quality limits feed intake and affect fish growth (Colt 2006). Moreover, exposure of fish to high ammonia levels above the acceptable limits (< 0.09 mg/L), DO levels below 3.5 mg/L and suboptimal temperature probably reduced fish growth rates and feed utilization efficiency. Other factors apart from the measured parameters probably influenced the growth and survival of fish in the present

study. Martins et al. (2009) reported reduced growth rates of *O. niloticus* cultured in recirculating systems than in flow-through systems. Growth retardation in recirculating systems was due to the accumulation of substances including pheromones and cortisol (Martins et al. 2009) heavy metals (Davidson et al. 2009), steroids (Mota et al. 2015). Furthermore, Mota et al. (2015) reported a 42% and 83% reduction in feed intake and growth respectively in *O. niloticus* in a recirculating system designed for maximal accumulation of substances in water.

A minimal water exchange (biweekly) possibly allowed substances to accumulate in the aquaponic system which in turn affected the growth of fish. Reduced growth rates of Nile tilapia in low water exchange recirculating systems were also reported (Obirikorang et al. 2019). Obirikorang et al. (2019) concluded that, although Nile tilapia is described as a hardy fish because it can tolerate a wide range of culture conditions, long-term exposure to poor culture conditions can result in reduced growth rates and poor health. The slight difference between the standard growth performance indices including weight gain and specific growth rate (SGR) and the VBGF parameters (asymptotic weight, maximum growth rate and slope of regression curve) is attributed to the data used to compute the parameters (Hopkins 1992; Lugert et al. 2014). The weight gain and SGR are calculated based on stocking and harvest data and do not consider intermediate data, whereas VBGF parameters consider all the data (stocking, intermediate and harvest data). Therefore, the intermediate date probably influenced the slope of the regression lines which suggest that fish in SW performed better than fish in PU and AM systems. Most aquaculture studies use the relative (% increase in body weight) and specific growth rates (%/day) because they are easy to compute and compare results (Lugert et al. 2014). Lugert et al. (2014) stated that the von Bertalanffy growth function is a suitable growth model since it provides reliable information for sound management decisions in recirculating aquaculture facilities.

Conclusion

The findings of this study show that pumpkin, sweet wormwood and amaranth were able to absorb nutrients from aquaculture wastes. Nutrient removal was reflected by the gradual decrease of nutrient concentrations in the hydroponic outlets with time. Plant species did not impact ammonia, nitrite and nitrate removal but significantly influenced the nitrogen content in the plant tissues and the growth rate of the plants. Sweet wormwood had significantly high nitrogen content which translated to high growth rates. High phosphorus removal was obtained in pumpkin compared to sweet wormwood and amaranth treatment supporting the idea that fruiting plants require more phosphorus than leafy plants. Most water quality parameters except ammonia and dissolved oxygen were within safe limits for the growth and survival of tilapia. The study demonstrated that high ammonia levels as well as low dissolved oxygen and suboptimal temperature had a significant influence on the growth rate of *O. niloticus*. Based on our current findings, pumpkin, sweet wormwood and amaranth can be used to reduce nutrients in aquaculture wastewater. However, it is recommended to have a lower hydraulic loading rate than (2.72 m/day) to increase retention time for bacteria to effectively remove ammonia and nitrate from the system. This will in turn maintain the water quality conditions and improve the growth performance of fish.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.

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