

Original Research Article

Phytoremediation Potential of Selected Plants & Growth of *Oreochromis niloticus* (Linnaeus, 1758) in Aquaponic Systems

ABSTRACT

Aquaponic plants utilize dissolved nutrients from fish wastewater, promoting fish health and improving water quality. However, the nutrient removal capacity of specific plant species within aquaponics remains underexplored. This study investigated the potential of three plant species: sweet wormwood (*Artemisia annua*), pumpkin (*Cucurbita pepo*) and amaranth (*Amaranthus dubius*) for biofiltration within a media-based aquaponic system. We evaluated the effectiveness of plants in reducing ammonia, nitrite, nitrate and phosphorus. In addition, the growth performance of plants and Nile tilapia (*Oreochromis niloticus*) within the system was evaluated. The experiment adopted a randomized design with three treatments (plant species) and three replicates per treatment. *Artemisia annua* recorded significantly higher removal rates for ammonia ($52.5 \pm 19.9\%$), nitrate ($61.6 \pm 9.02\%$), and nitrite ($41.9 \pm 8.7\%$) compared to other plant species. In contrast, *C. pepo* exhibited the lowest nutrient removal efficiency. Most water quality parameters, except for ammonia and dissolved oxygen, remained within the optimal range for *O. niloticus* growth during the experiment. Significantly higher fish growth rates (0.33 ± 0.006 g/day) were observed in *A. annua* than other treatments. Similarly, *A. annua* produced the highest plant yield (0.49 ± 0.02 kg/m²), while *C. pepo* yielded the least (0.29 ± 0.00 kg/m²). All the studied plants reduced aquaponic system waste, with *A. annua* exhibiting significantly higher biofiltration efficiency, promoting increased fish growth and plant yield. This demonstrates their potential for sustainable aquaculture through wastewater treatment and healthy fish production in media-based systems.

Keywords: *Artemisia annua*; hydroponic; nitrification; nutrients; recirculating aquaculture; sustainable food production; water quality.

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1. INTRODUCTION

Alarming trends in population growth, climate change, pollution, and resource depletion, coupled with rising food insecurity, are driving a resurgence of research in food production. In recent decades, researchers have developed new and innovative technologies for producing food that, incessantly may be the key to meeting the increasing food demand for the ever-expanding global population [1]. Aquaponics, which combines recirculating aquaculture and hydroponics, is gaining popularity as a sustainable food production method. It has the potential to save water, reduce the need for chemical fertilizers, and increase food production in controlled environments. However, ongoing research and development are critical to maximizing its global impact [2,3].

In these systems, nutrient-rich aquaculture wastewater serves as nutrients for plants grown in the hydroponic subsystem. Microbial activities as well as nutrient absorption by plants in the aquaponics system reduce the dissolved nutrient concentration in the culture water which in turn improves the overall environmental conditions for fish [4,5]. Aquaponics, a system that combines fish and plant cultivation, provides a sustainable approach to agriculture. It uses significantly less water than conventional methods and produces a broader range of crops and fish, increasing overall productivity[6,7]. This closed-loop system also reduces wastewater discharge, lowering the risk of environmental pollution. Furthermore, the unique environment of aquaponics reduces the risk of both soil-borne diseases that commonly affect plants grown in traditional hydroponic systems [8,9,10,11].

The synergistic relationship between recirculating aquaculture and hydroponics is well-documented, with numerous studies demonstrating the ability of plants to utilize nutrient-rich aquaculture wastewater for growth while simultaneously improving water quality [6, 12,13,14,4,15,8]. However, optimizing this synergy necessitates the selection of appropriate plant species. Optimizing plant selection in aquaponics necessitates prioritizing species with rapid maturation periods and high adaptability to both the controlled aquaponic environment and the prevailing local climate [13,14]. Continued research efforts are crucial to expand the available plant varieties for aquaponic investors and farmers. This will enable the selection of species that optimize production efficiency, resource utilization, and overall system sustainability.

This study evaluated the suitability of three plant species for integration into aquaponic systems. Sweet wormwood (*Artemisia annua*) a highly prized medicinal herb native to China and adaptable to subtropical environments, such as Kenya. *Artemisia annua* holds significant medical importance as the source of artemisinin, a crucial component of artemisinin-based combination therapy (ACT), the primary treatment for malaria [16]. Pumpkin (*Cucurbita pepo*) and amaranth (*Amaranthus dubius*) are popular indigenous vegetables with significant economic value (Abukutsa-Onyango, 2007). These three plant species are in high demand in both domestic and export markets, creating a significant opportunity for local farmers in developing countries to increase their income [16,17]. Aquaponic systems show promise in meeting this demand by allowing for high yields of pest-free and chemical-free crops[6].

Previous studies demonstrated the successful cultivation of *A. annua* in aquaponic systems due to its extensive root network. The root structure provides a large surface area for the colonization of nitrifying and denitrifying bacteria, crucial for the biofiltration process [2]. However, more information and a better understanding of its biofiltration efficiency and growth performance in this environment is still required. This study aims to address the knowledge gap by evaluating: (1) the growth performance of *A. annua*, *C. pepo* and *A. dubius*, (2) the effect of different plant species on water quality and (3) their corresponding effects on the growth performance of *O. niloticus* in a media-based aquaponic system.

2. MATERIALS AND METHODS

2.1 Study Location and Components

The 60-day experiment was conducted at the Aqualife fish farm in Machakos County, Kenya (-1.525134° latitude, 37.185891° longitude). The growth and yield of *A. annua*, *C. pepo* and *A. dubius* were evaluated in the *O. niloticus* - aquaponic system. *C. pepo* and *A. dubius* seeds were purchased from a local supplier (Simlaw seeds, Kenya) while *A. annua* seeds were obtained from Anamed International e.V (Schafweide, Germany). This choice was made because the Germany variety had desirable characteristics including high quality, high yield,

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and disease resistance, which were lacking in local varieties. Monosex (all male) *O. niloticus* fingerlings were obtained from Aqualife fish farm.

2.2 Experimental Design

Nine identical coupled (continuous recirculation between aquaculture and hydroponic subsystems) gravel based aquaponic systems were constructed under a greenhouse to provide uniform conditions for both fish and plant growth. Each system consisted of three 500 L plastic fish tanks and a 0.1125 m³ rectangular hydroponic unit (Figure 1). A 210 L plastic barrel filled with sand of different sizes was used for solid removal and a biofilter was constructed from the same plastic barrel and filled with thoroughly rinsed and sundried pumice stones to serve as biofilter substrate. A cold start method was used to activate the biofilter. The activation involves stocking of fish without an activated biofilter to encourage the growth of bacteria in the biofilter substrates [18]. To establish a natural population of bacteria in the biofilter substrate, 50 *O. niloticus* fingerlings were stocked in each culture tank one month before the experiment began. Stocking densities in the culture tanks were 4.7 kg/m³, 5.56 kg/m³, and 5.25 kg/m³ in *C. pepo*, *A. annua* and *A. dubius* treatments respectively. Body weights and total body lengths were taken and recorded for all fish before stocking. [The average weights of fish at the start of the experiment were 92.8 ± 4.2g, 103.4 ± 8.1g and 99.8 ± 6.3g in *C. pepo*, *A. annua* and *A. dubius* treatments, respectively.]

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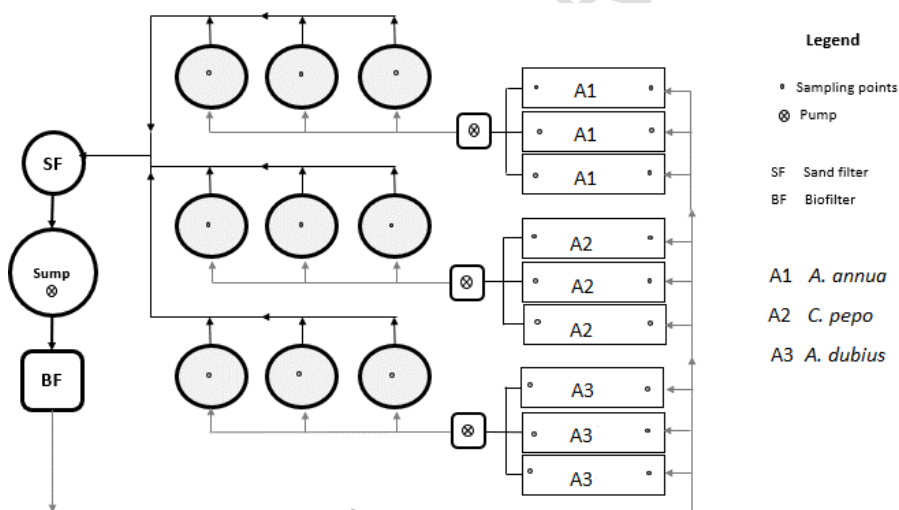


Fig. 1. A schematic diagram of the experimental aquaponics system. (Not to scale). Fish tanks are represented by grey circles, whereas hydroponic subsystems are represented as rectangular boxes. The flow of water is represented by grey and black lines with arrows. Grey arrows represent water inlets, while black arrows represent water outlets.

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. Polyvinyl chloride pipes with ball valves were installed to circulate water in the system. A constant water flow rate of 1.42 ± 0.23 L /min was maintained in all the hydroponic subsystems, and no additional fertilizers or pesticides were used. The flow rate was controlled by ball valves. The flow rate

from the hydroponic units ranged between (1.29 – 1.38 L/min). Water from the fish tanks flowed through the sand filter by gravity to the sump, where a centrifugal pump (DPP 60, 0.5 HP, 2500L/ hr, 0.37kW) delivered effluent water from the pump (6 ± 0.24 L/min) to the biological filter. Then the filtered water was channeled by gravity to the hydroponic subsystems and 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 was exchanged in each fish tank with clean water biweekly during fish sampling. The outlet from each hydroponic subsystem was constructed as a bell siphon with auto-mechanical water movement initiating the flood and drain mechanism modified from Bruno [19] (Figure 2).



Fig. 2. Schematic layout of a hydroponic unit with a bell siphon

Three treatments representing *C. pepo*, *A. annua* and *A. dubius* respectively were replicated three times in the hydroponic subsystems. The fish were broadcast fed twice a day (09:00h and 16:00h) to satiation with 30% crude protein commercial pelleted feed (Raanan, Israel) diet during the study period. *C. pepo*, *A. annua* and *A. dubius* seeds were sown in three seedling trays (filled with loam soil) three weeks before the start of the experiment. Healthy seedlings were then transplanted into the nine hydroponic subsystems.

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2.3 Water Quality Parameters

Selected water quality parameters (temperature, pH, dissolved oxygen, and conductivity) were measured insitu twice daily (0900 and 1600 hrs) in the fish tanks and hydroponic subsystems using Hach probes (HACH HQ40d Portable meter, Loveland, Colorado, USA). Water samples were collected in triplicates every two weeks from the fish tanks, inlet, and outlet of the hydroponic subsystems for a period of two months. The samples were analysed for ammonia, nitrate, nitrite and phosphorus according to standard methods [20] using benchtop Hanna multiparameter photometer (HI83200). Nutrient (ammonia, nitrite, nitrate and phosphorus) removal efficiencies of the experimental plants were calculated as the change in nutrient concentration in the hydroponic subsystem inlets (treated water exits the biofilters) and outlets (treated water exits the hydroponic subsystems) [4].

$$\text{Nutrient removal efficiency (\%)} = \frac{C_i - C_e}{C_i} \times 100 \quad (1)$$

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

2.4 Fish and Aquaponic Plants

All fish in each tank were sampled biweekly and body weight and standard length (SL) recorded. The weight was recorded to the nearest 0.1 g and the mean weight calculated. Fish were harvested after 60 days, and final wet weight taken and recorded. Fish performance was evaluated using standard growth performance indices such as weight gain, specific growth rate (SGR) feed conversion ratio (FCR) and survival rate (SR). The performance indices were calculated using the following equations.

$$\text{Weight gain (g)} = \text{Final weight (g)} - \text{initial weight (g)} \quad (2)$$

$$\text{FCR} = \frac{\text{Total weight of dry feed given (g)}}{\text{Total weight gain (g)}} \quad (3)$$

$$\text{SR (\%)} = \frac{N_0 - N_t}{N_0} \quad (4)$$

$$\text{SGR} = \frac{\ln W_f - \ln W_i}{t} \times 100 \quad (5)$$

where N_0 and N_t are fish numbers at time 0 and at time t and W_i and W_f are initial and final mean wet weight in g; \ln = natural logarithm; t = time in days

Plant height (cm) was measured biweekly, and weight (g) measured at the beginning and end of the experiment. The final wet weight of all 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 based on the equation below:

$$\text{RGR} = (\ln W_2 - \ln W_1) / (t_2 - t_1) \quad (6)$$

where W_2 and W_1 are weights at time t_2 and t_1 , which are initial and final periods and \ln is the natural logarithm. Plant yield (kg/m^2) was calculated using the fresh weight obtained per square meter in each treatment.

2.5 Statistical Analysis

Data was presented as means and standard deviation (SD) of three replicates. Percentage data was subjected to arcsine transformation before statistical analyses. Normality and homogeneity of means were carried out using Shapiro–Wilks and Levene tests respectively. Repeated measures ANOVA was used to test the difference in nutrient removal efficiency, water quality parameters, plant growth parameters and fish growth among treatments. Post hoc multiple comparison of means was performed where there were significant differences using Tukey's HSD test. Differences between means was considered significant at $\alpha=0.05$. Statistical analysis was performed using the Statistical Package for Social Science (SPSS) Statistics for Windows (version. 21.0, IBM Corp., Armonk, NY, USA).

3.RESULTS

Water quality parameters in the fish tanks are presented in Table 1. Temperature, pH, and conductivity values were not significantly different ($P > 0.05$) in the three aquaponic treatments. Temperature values ranged from 21.2 to 24.6 °C and pH fluctuated between 6.88 to 7.77 in all the systems. Dissolved oxygen fluctuated between 2.18 to 5.63 mg/L, but significantly higher levels were recorded in the *C. pepo* and *A. annua* aquaponic systems. Ammonia was significantly higher in the *C. pepo* but low in *A. annua* system, but nitrite levels were not significantly different.

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Table 1. Water quality parameters in the fish tanks. Mean values (\pm standard deviations) within a row with different superscripts are significantly different ($a > b > c$, $P < 0.05$).

Parameters	Treatments		
	<i>C. pepo</i>	<i>A. annua</i>	<i>A. dubius</i>
Dissolved oxygen	5.63 \pm 0.69 ^c	4.49 \pm 0.59 ^b	4.25 \pm 0.81 ^a
Temperature ($^{\circ}$ C)	24.45 \pm 0.81 ^a	24.57 \pm 0.84 ^a	24.52 \pm 1.07 ^a
pH	7.60 \pm 0.23 ^a	7.52 \pm 0.42 ^a	7.66 \pm 0.07 ^a
Conductivity (μ S/cm)	1385.54 \pm 18.24 ^a	1391.24 \pm 15.53 ^a	1389.08 \pm 18.85 ^a
Ammonia (mg/L)	1.23 \pm 1.07 ^b	1.09 \pm 0.58 ^a	1.25 \pm 0.20 ^b
Nitrates (mg/L)	3.15 \pm 0.51 ^b	3.24 \pm 0.39 ^c	3.95 \pm 0.26 ^a
Nitrites (mg/L)	0.29 \pm 0.19 ^a	0.23 \pm 0.05 ^a	0.20 \pm 0.12 ^a
Phosphorus (mg/L)	1.87 \pm 0.74 ^a	2.19 \pm 1.00 ^b	2.00 \pm 0.88 ^b

Nutrient concentrations were significantly high at the hydroponic inlet and significantly low ($P < 0.05$) in the hydroponic outlets (Figure 3). An increasing trend in nitrate, nitrite and phosphorus concentration was observed within the system over time. The ammonia concentration in the *A. annua* treatments decreased gradually over the sampling period, reaching the lowest level at week 8 with an average value of 0.07 ± 0.06 mg/L. Significantly low ammonia and nitrate levels as well as high ($P < 0.05$) phosphorus levels were recorded in the *A. annua* treatment during the study period. Phosphorus was significantly low in the *C. pepo* treatment. There were significant ($P < 0.05$) interactions between aquaponic treatments and sampling period for all nutrient concentrations.

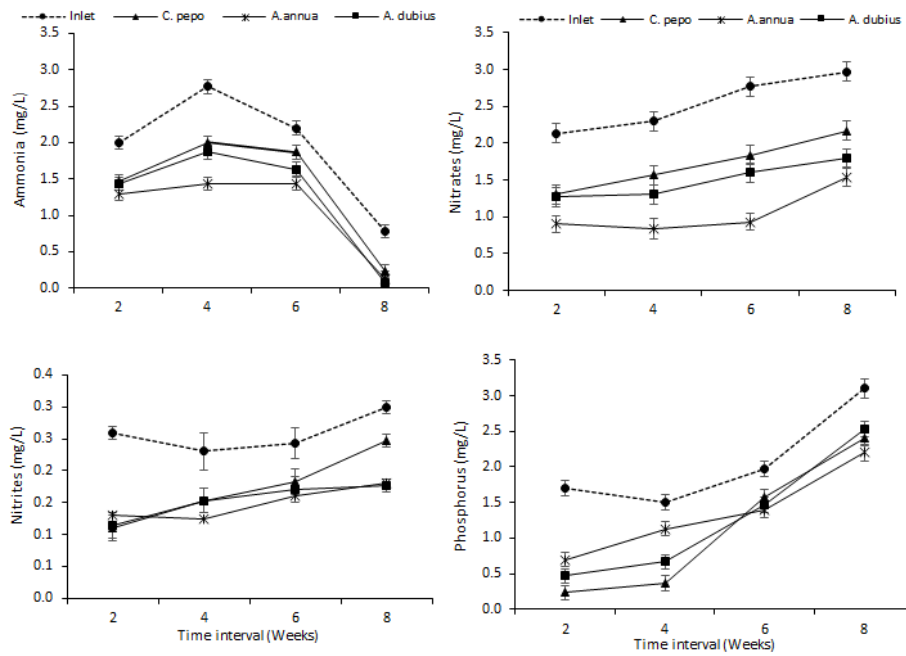


Fig. 3. Nutrient trends (ammonia, nitrates, nitrites, and phosphorus) at the hydroponic inlet and outlets (*C. pepo*, *A. annua* and *A. dubius*). Data points represent the mean of three treatment replicates and error bars indicate standard deviation.

Figure 4 illustrates the nutrient removal efficiency of *A. annua*, *C. pepo*, and *A. dubius* within the aquaponic systems. Among the three plant species tested, *A. annua* outperformed *C. pepo* and *A. dubius* at removing ammonia ($52.5 \pm 19.9\%$), nitrites ($41.9 \pm 8.7\%$), and nitrates ($61.6 \pm 9.02\%$). Conversely, the *C. pepo* system demonstrated a higher efficiency in removing phosphorus ($51.3 \pm 32.4\%$) than *A. annua* ($36.2 \pm 15.4\%$) and *A. dubius* ($43.3 \pm 26.1\%$).

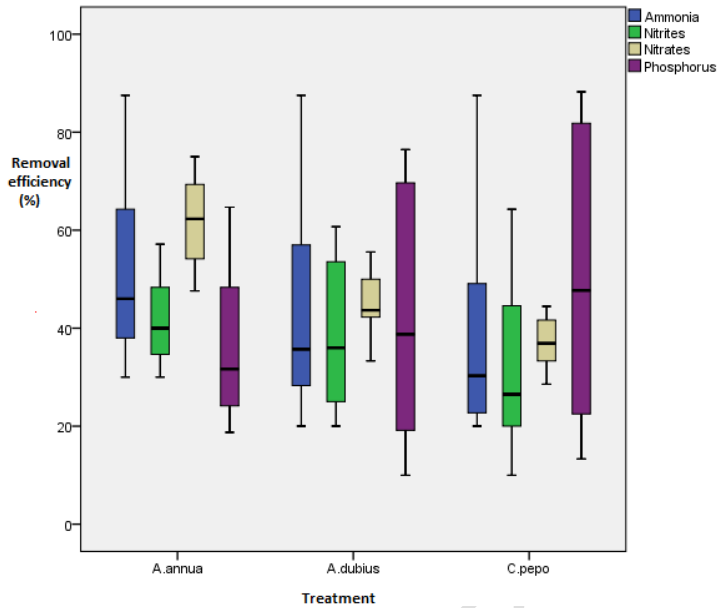


Fig.4. Nutrient removal efficiency of plant species in the aquaponic system.

Oreochromis niloticus growth performance (final weight, weight gain, specific growth rate) in the *A. annua* treatment was significantly high ($P < 0.05$) compared to other treatments (Table 2). Additionally, the *A. annua* system exhibited significantly ($P < 0.05$) lower feed conversion ratio compared to the other systems, indicating improved feed efficiency. Survival rates, on the contrary, did not differ significantly across all treatments, implying that overall mortality was low throughout the experiment.

Table 2. Growth performance of *O. niloticus* in the aquaponic systems. All values are means \pm standard deviations obtained from three replicates.

Parameters	Units	Treatments		
		<i>C. pepo</i>	<i>A. annua</i>	<i>A. dubius</i>
Final weight	(g)	113.50 \pm 5.54 ^a	130.25 \pm 5.76 ^b	112.21 \pm 7.64 ^a
Weight gain	(g)	32.35 \pm 4.79 ^b	26.22 \pm 4.60 ^b	15.55 \pm 1.03 ^a
Feed conversion ratio (FCR)		2.39 \pm 0.33 ^b	2.21 \pm 0.44 ^a	3.10 \pm 0.26 ^b
Specific Growth Rate (SGR)	(%/d)	0.33 \pm 0.07 ^b	0.39 \pm 0.06 ^b	0.20 \pm 0.02 ^a
Survival rate	%	88.15 \pm 4.63 ^a	92.59 \pm 3.39 ^a	82.96 \pm 3.39 ^a

Different superscript letters (a, b) within a row indicate statistically different mean values at $P < 0.05$; a > b > c.

Plant growth parameters were significantly different between the three treatments (Table 3). The final weight, weight gain and yield were significantly higher in the *A. annua* than in *C. pepo* and *A. dubius* systems. Similar results were observed for the relative growth rates.

Table 3. Plant growth parameters in the aquaponic systems. All values are mean \pm standard deviation obtained from three replicates.

Parameters	Units	Treatments		
		<i>C. pepo</i>	<i>A. annua</i>	<i>A. dubius</i>
Final weight (Fresh)	(g)	333.33 \pm 11.72 ^b	367.00 \pm 16.09 ^c	217.67 \pm 2.52 ^a
Weight gain (Fresh)	(g)	312.40 \pm 13.05 ^b	349.20 \pm 17.28 ^c	202.00 \pm 2.00 ^a
Relative growth rate	(g/d)	0.05 \pm 0.00 ^b	0.05 \pm 0.00 ^b	0.04 \pm 0.00 ^a
Yield	(kg/m ²)	0.45 \pm 0.02 ^b	0.49 \pm 0.02 ^c	0.29 \pm 0.00 ^a

4. DISCUSSION

Most water quality parameters were within acceptable limits for *O. niloticus* and plant growth. However, two critical aquaculture parameters, ammonia and dissolved oxygen, differed slightly. Ammonia concentrations exceeded the recommended 1 mg/L level for *O. niloticus* growth [21]. This suggests that nitrifying bacteria, responsible for ammonia conversion in biofilters and plant roots, had developed, but their activity was insufficient to maintain ammonia levels at the required minimum [12,22]. The efficiency of the biofilters were probably influenced by fine solid accumulation and low dissolved oxygen levels in the aquaponic systems. These conditions do not favour the growth of nitrifying bacteria that convert toxic ammonia to nitrates and nitrites during nitrification [23]. Additionally, the observed ammonia levels could be as a result of minimal water exchange (once in two weeks) during the study period. Minimal water exchange rates may increase the accumulation of substances such as ammonia in recirculating aquaculture systems [24].

Dissolved oxygen (DO) levels were lower than recommended limits considering that oxygen should be maintained between 5 to 6 mg/L for optimum growth of fish and hydroponic plants [24]. However, *O. niloticus* can tolerate oxygen levels as low as 1.0 mg/L but levels below 3.5 mg/L can affect growth and food conversion ratio [23]. Low DO in aquaponic systems can be attributed to fish respiration, plant root respiration, activities of nitrifiers and heterotrophs, and high organic loads [24]. When oxygen levels are low, root respiration reduces. This lowers the plant's ability to absorb water and nutrients, ultimately resulting in stunted growth. Furthermore, low oxygen levels can result in root rot and nitrogen loss via denitrification [26]. Therefore, optimum DO levels should be maintained in the aquaponic systems for optimal growth of both plants and fish. If optimal dissolved oxygen (DO) levels were maintained throughout the experiment, nitrifying bacteria could have converted ammonia more efficiently [12,22]. Consequently, lower ammonia levels and

improved water quality might have been observed, potentially leading to enhanced growth rates for both fish and plants[6].

Plants play a major role in the removal of nutrients in the aquaponic systems because their roots provide a surface area for the attachment of bacteria responsible for the removal of both nitrogen and phosphorus from water [27]. The consistent low nutrient concentration from the hydroponic outlets is an indication of nutrient removal through the nitrification and denitrification processes in the plant roots [7, 22,4]. Plants with an extensive root network system effectively remove nutrients from the water because the roots provide a large surface area for the attachment of more nitrifying and denitrifying bacteria [28]. Therefore, the developing roots of the young plants at the start of the experiment probably favoured the attachment of fewer bacteria resulting in lower nitrification rates. The extensive root network of *A. annua* might have favoured the efficient removal of nutrients from the *A. annua*-based aquaponic system compared to other systems. The nutrient trend can also be explained by the growth stage of the plants because nutrient requirement of plants increases with growth stage [29]. Nutrient removal increased with growth stage probably due to the nutrient requirements of the plants. High ammonia levels at the start of the experiment suggests that the nitrifiers had not established properly in the plant roots resulting in low nitrification rates. Our findings are consistent with Wongkiew [23] and Wafula [12] who reported high ammonia levels during the start-up periods in a recirculating aquaculture system because the nitrifying bacteria takes a long time to establish and multiply. Low phosphorus concentration at the start of the experiment can be attributed to increased requirement for root development. In addition, young plants may engage in luxury uptake of phosphorus to counterbalance phosphorus need at a later stage [30].

Oreochromis niloticus exhibited improved growth in the *A. annua*-based aquaponic system compared to other treatments. This enhanced performance can be attributed to *A. annua*'s effectiveness in removing nutrients from water and maintaining favorable conditions for *O. niloticus* growth. However, the observed growth rate in the present study was lower (0.20 - 0.39%/day) than findings from previous aquaponic studies (0.7% and 2.5%/day) for *O. niloticus*, even though the initial fish weights were similar [31]. Furthermore, the feed conversion ratio (FCR) was slightly higher than the recommended range of 1.5 to 2.0 for *O. niloticus* raised in tanks [32]. Relatively high ammonia levels probably influenced the growth of *O. niloticus* in the aquaponic system. Exposure of fish to high ammonia levels above the acceptable limits (< 1 mg/L) have been shown to reduce growth and feed utilization efficiency [33]. Related studies have also reported reduced growth and feed intake in recirculating system with minimal water exchanges [34,24]. Ammonia impairs the fish's ability to extract energy from its food, resulting in a reduction in feed conversion efficiency. Even when fish maintain food intake levels, stressful conditions have been shown to reduce feed conversion efficiency, resulting in decreased growth rates [35,36]. Our findings suggest that although *O. niloticus* can tolerate a wide range of culture conditions, long exposure to below optimum culture conditions can result in reduced growth and feed intake.

The growth performance of the various plants in the current study indicates that they can be successfully cultivated in aquaponic systems. At the end of the experiment,

increased growth, weight gain, and yield were recorded in the *A. annua* aquaponics system. Gichana [34] reported comparable results [weight gain (390.7 ± 26.8 g and yield (0.56 ± 0.03 kg/m²) for *A. annua* in aquaponic conditions. The relatively low weight gain observed in *A. dubius* and *C. pepo* suggests that these plants may have limited ability to extract sufficient nutrients from the aquaponic solution. This could be attributed to their smaller root systems compared to the well-developed root network of *A. Annua*[34]. Despite their different growth rates, the tested plants contributed to nutrient reduction, demonstrating their potential as biofilters in aquaponic systems. In conclusion, the findings from this study provide valuable insights for establishing and managing plant life in freshwater aquaponic systems. Understanding these factors enables the appropriate selection of plant species in aquaponic systems. Plants with desirable growth characteristics and efficient nutrient uptake can be selected while capitalizing on their biofiltration capabilities.

5. CONCLUSION

This study demonstrated that *C. pepo*, *A. annua*, and *A. dubius* can effectively reduce nutrients in aquaponic systems without affecting with the growth of *O. niloticus*. Notably, *A. annua*'s high nutrient uptake likely contributed to improved water quality, which might have contributed to the observed increased growth rates in *O. niloticus*. However, ammonia levels exceeded recommended levels for optimal *O. niloticus* growth. This finding emphasizes the need for additional research to improve conditions for both fish and plants. One potential area of investigation is improving the aeration system to promote nitrification, the process by which harmful ammonia is converted into less toxic nitrates. Furthermore, research is required to assess the nutritional content of the plants and the flesh quality of *O. niloticus* to ensure that they meet the established standards for aquaponic products. These quality parameters are critical to consumer acceptance and the overall success of aquaponic production.

Ethical Statement: The study was carried out in accordance with the international, national and institutional guidelines for the care of experimental animals.

REFERENCES

1. Food and Agricultural Organisation (FAO). The state of food security and nutrition in the world report 2021. Transforming food systems for food security, improved nutrition and affordable healthy diets for all. Food and agricultural organisation, rome, italy; 2021. <https://www.fao.org/3/cb4474en/cb4474en.pdf>
2. Gichana Z, Liti D, Drexler S, Zollitsch W, Meulenbroek P, Wakibia J, et al. Effects of aerated and non-aerated biofilters on effluent water treatment from a small-scale recirculating aquaculture system for Nile tilapia (*Oreochromis niloticus*). *Die Bodenkultur: Journal of Land Management, Food and Environment*. 2019; 70(4):209-219. Available: <https://doi.org/10.2478/boku-2019-0019>
3. Espinosa-Moya A, Álvarez-gonzález A, Albertos-alpuche P. Growth and development of herbaceous plants in aquaponic systems. *Acta Univ. Multidisp. Sci. J*. 2018; 28: 1–8.
4. Boxman SE, Nystrom M, Capodice JC, Ergas SJ, Main KL, Trotz MA. Effect of

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- support medium, hydraulic loading rate and plant density on water quality and growth of halophytes in marine aquaponic systems. *Aquaculture Research*. 2017; 48(5):2463–2477. <https://doi.org/10.1111/are.13083>
5. Effendi H, Wahyuningsih S, Wardiatno Y. The use of Nile tilapia (*Oreochromis niloticus*) cultivation wastewater for the production of romaine lettuce (*Lactuca sativa* L. var. longifolia) in water recirculation system. *Applied Water Science*. 2017; 7(6):3055–3063. <https://doi.org/10.1007/s13201-016-0418-z>
 6. Gichana, Z. (2024). Water Quality and Growth Performance of Nile Tilapia (*Oreochromis niloticus*), Chia (*Salvia hispanica*) and Lemon Grass (*Cymbopogon citratus*) in a Media-based Aquaponics System. *Asian Journal of Biology* 20(5):12-22.
 7. Wafula AE, Gichana Z, Onchieku J, Chepkirui M, Orina SP. Opportunities and challenges of alternative local biofilter media in recirculating aquaculture systems. *Journal of Aquatic & Terrestrial Ecosystems*. 2023;1(1):73-81.
 8. Nuwansi KKT, Verma AK, Prakash C, Tiwari VK, Chandrakant MH, Shete AP, et al. Effect of water flow rate on polyculture of koi carp (*Cyprinus carpio* var. koi) and goldfish (*Carassius auratus*) with water spinach (*Ipomoea aquatica*) in recirculating aquaponic system. *Aquaculture International*. 2016; 24: 385-393 <https://doi.org/10.1007/s10499-015-9932-5>
 9. Somerville C, Cohen M, Pantanella E, Stankus A, Lovatelli A. Small-scale aquaponic food production. Integrated fish and plant farming. FAO Fisheries and Aquaculture. Rome: FAO. 2014; <https://doi.org/10.1002/pssb.201300062>
 10. Bakiu R, Shehu J. Aquaponic systems as excellent agricultural research instruments in Abania. *Albanian J. Agric. Sci*. 2014; Special ed: 385 -389.
 11. Rakocy JE, Masser MP, Losordo TM. Recirculating aquaculture tank production systems: Aquaponics- integrating fish and plant culture. SRAC Publication - Southern Regional Aquaculture Center. 2006; (454):16.
 12. Wafula AE, Gichana Z, Onchieku J, Orina P, Nyakeya K, Musa S. Biochar-based biofilter media improves water quality in recirculating aquaculture systems. *Journal of Crops, Livestock and Pests Management*. 2023;1(1):79-90
 13. Ogah SI, Salleh M, Nurul KSM, Edaroyati MWP. Biological filtration properties of selected herbs in an aquaponic system. *Aquaculture Research*. 2020; 00: 1–9. <https://doi.org/10.1111/are.14526>
 14. Gichana Z, Liti D, Wakibia J, Ogello E, Drexler S. Efficiency of pumpkin (*Cucurbita pepo*), sweet wormwood (*Artemisia annua*) and amaranth (*Amaranthus dubius*) in removing nutrients from a smallscale recirculating aquaponic system. *Aquaculture International*. 2019; 27:1767–1786.
 15. Goddek S. Three-loop Aquaponics System : Chances and Challenges. In *Aquaponics Research Matters*. In Proceedings of the international conference on Aquaponics Research Matters, Ljubljana, Slovenia. 2016; Mar (Vol. 22). <https://doi.org/10.13140/RG.2.1.3930.0246>
 16. Pulice G, Pelaz S, Matías-Hernández L. Molecular Farming in *Artemisia annua*, a Promising Approach to Improve Anti-Malarial Drug Production. *Frontiers in Plant Science*. 201; 7:239.
 17. Abukutsa-onyango M. The diversity of cultivated African leafy vegetables in three communities in western Kenya. *African Journal of Food, Agriculture, Nutrition and Development*. 2007; 7(3):1–12
 18. Delong DP, Losordo TM. How to start a Biofilter. SRAC Publication - Southern Regional Aquaculture Center. 2012; 3:1–4.
 19. Bruno RW, Chen PC, Lai V, Loc H, Delson N. (2011). *Aquaponics Ebb and Flow Mechanisms* ECOLIFE Foundation. MAE 156B: (Ed.), *Fundamental Principles of Mechanical Design II*. 2011;85.
 20. APHA. *Standard Methods for the Examination of Water and Wastewater* (21st ed.).

- Washington, DC: American Public Health Association. 2005.
21. Hargreaves JA, Tucker CS. Managing ammonia in fish pond. SRAC Publication - Southern Regional Aquaculture Center. 2004; (4608):8.
 22. Gichana ZM, Liti D, Silke D, Waikibia J, Waidbacher H. Waste management in recirculating aquaculture system through bacteria dissimilation and plant assimilation. *Aquaculture International*. 2018; 26:1541–1572.
 23. Wongkiew S, Hu Z, Chandran K, Lee JW, Khanal SK. Nitrogen transformations in aquaponic systems: A review. *Aquacultural Engineering*. 2017; 76: 9–19. <https://doi.org/10.1016/j.aquaeng.2017.01.004>
 24. Mota VC, Limbua P, Martins CIM, Eding E, Verreth AJ. The effect of nearly closed RAS on the feed intake and growth of Nile tilapia (*Oreochromis niloticus*), African catfish (*Clarias gariepinus*) and European eel (*Anguilla anguilla*). *Aquacultural Engineering*. 2015; 68:1–5.
 25. Delong DP, Losordo TM, Rakocy JE. Tank Culture of Tilapia. Southern Regional Aquaculture Center. 2009; (282).
 26. van Patten G. Soilless Gardening. Hydroponics For the Rest of Us. In D. Parke (Ed.), *The Best of The Growing Edge*. New Moon Publishing, Inc. 2002.
 27. Endut A, Lananan F, Abdul HS, Jusoh A, Wan Nik WN. Balancing of nutrient uptake by water spinach (*Ipomoea aquatica*) and mustard green (*Brassica juncea*) with nutrient production by African catfish (*Clarias gariepinus*) in scaling aquaponic recirculation system. *Desalination and Water Treatment*. 2016; 57(60):1 - 10. <https://doi.org/10.1080/19443994.2016.1184593>
 28. Endut A, Jusoh A, Ali N, Wan Nik WB, Hassan A. A study on the optimal hydraulic loading rate and plant ratios in recirculation aquaponic system. *Bioresour. Technol*. 2010; 101:1511–1517
 29. Jones C, Olson-rutz, K, Dinkins C. Nutrient Uptake Timing by Crops, to Assist with Fertilizing Decisions; Montana State University: Bozeman, MT, USA, 2015; p. 8.
 30. Buzby KM, Lin LS. Scaling aquaponic systems: Balancing plant uptake with fish output. *Aquacultural Engineering*. 2014; 63:39 - 44. <https://doi.org/10.1016/j.aquaeng.2014.09.002>
 31. Al-Hafedh YS, Alam A, Beltagi MS. Food production and water conservation in a recirculating aquaponic system in Saudi Arabia at different ratios of fish feed to plants. *Journal of the World Aquaculture Society*. 2008; 39(4):510–520. <https://doi.org/10.1111/j.1749-7345.2008.00181.x>
 32. Stickney RR. *Aquaculture: an introduction text*. Cambridge, USA: CABI publication. 2005.
 33. Colt J. Water quality requirements for reuse systems. *Aquacultural Engineering*. 2006; 34(3):143–156. <https://doi.org/10.1016/j.aquaeng.2005.08.011>
 34. Gichana Z, Meulenbroek P, Ogello E, Drexler S, Zollitsch W, Liti, D. et al. Growth and Nutrient Removal Efficiency of Sweet Wormwood (*Artemisia annua*) in a Recirculating Aquaculture System for Nile Tilapia. 2019; *Water* 11(5):923.
 35. d'Orbcastel ER, Lemarié G, Breuil G, Petochi T, Marino G, Triplet S. Effects of Rearing Density on Sea Bass (*Dicentrarchus Labrax*) Biological Performance, Blood Parameters and Disease Resistance in a Flow Through System. *Aquat Living Resour*. 2010; 23:109–17. doi: 10.1051/alr/2009056
 36. Paspatis M, Boujard T, Maragoudaki D, Blanchard G, Kentouri M. Do Stocking Density and Feed Reward Level Affect Growth and Feeding of Self Fed Juvenile European Sea Bass? *Aquaculture*. 2003; 216:103–13. doi: 10.1016/S0044-8486(02)00417-9 58.

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