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Performance Evaluation of Gravel and Clay Pebbles Growth Beds in Aquaponics for Okra (*Abelmoscus esculentus*) and Catfish (*Clarias gariepinus*) Growth and Production

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Abstract The study evaluates the comparison of gravel and clay pebbles as a plant growth bed for cultivating African catfish (*Clarias gariepinus*) and okra (*Abelmoscus esculentus*). A recirculation aquaponic system (RAS) with a bell siphoned was used for a 16-week study period with three various media: gravel (M1), clay pebbles (M2), and a mixture of both with the ratio of 1:1 (M3). The performance comparison of different media growth beds was assessed through the growth and production of catfish and okra, as well as the effectiveness of nutrient removal from the fish tank. The results reveal statistically better okra planting performance in media containing clay pebbles M2 and M3 in terms of plant height, leaf area, and stem circumference. The specific growth rate (SGR) of okra exhibited a notably higher value in M2 and M3, reaching 3.41 ±0.06 and 3.42 ±0.09 respectively, compared to 2.33 ±0.07 in M1. Similar results of catfish growth were obtained where M2 and M3 have better performance for weight gain, survival rate (SR), and feed conversion ratio (FCR). The highest weight gained 290.50 ± 7.37 and the lowest for FCR 1.30 ± 0.03 was achieved in M3 compared to other media growth beds. Okra production was shown to be significantly greater in the growth bed containing clay pebbles M2 (4.37 ± 0.38 kg/m^2) and M3 (4.89 ± 0.40 kg/m^2) compared to gravel growth bed M1 (2.83 ± 0.24 kg/m^2). The resultant effects of media growth bed varieties on nutrient deficiency showed M3 removed total ammonia nitrogen (TAN), nitrite-N, nitrate-N, and total phosphorus (TP) better with percentage removal of 84.2 ± 1.42 %, 89.5 ± 1.85 %, 86.8 ± 1.22 %, and 93.1 ± 0.48 %, respectively. Therefore, the mixture of gravels and clay pebbles demonstrated a satisfactory performance of okra production as well as nutrient removal of RAS and the production of catfish production was also satisfactory.

Keywords African Catfish, Clay Pebble, Gravel, Media Growth Bed, Okra, Recirculation Aquaponic System

1. Introduction

The mutual relationship between aquaculture and hydroponics where both fish and plants coexist in a balanced ecosystem creates a synergy integrated system known as aquaponics. Nutrients in fish farming wastewater developed by uneaten fish feed and fish excrement are used as fertilizer for plants in hydroponics to grow [1-3]. The sustainable aquaponic environment has beneficial microorganisms living inside the system which has a positive influence on plant growth and thus purifies the water and in turn is reused for fish production [4, 5]. A common example is nitrifying bacteria that convert ammonium into nitrate, the simple and accessible form of nitrogen that is absorbed by plants for growth and production. The recycling of nutrients in symbiosis relationships in aquaponic systems reduces the discharging of nutrients wastewater to the water bodies thus less impact to the environment [6, 7]. Hence, the aquaponic system is a sustainable way to produce food sources from both aquaculture and plants.

The aquaponic system is a possible solution to food production scarcity in developing countries which are limited in land and employees [8, 9]. The key factor to ensure the sustainability of the system among them is establishment of the plant growing area [10]. There are three types of common hydroponic designs used in aquaponic system; media bed or also known as ebb-and-flow bed, nutrient film technique (NFT), and deep water culture (DWC) also known as the raft beds [5, 11]. There are various substrates that are commonly used as growing media in aquaponic such as gravel, sand, perlite, hydroton, volcanic rocks and cocopeat [12-14]. The selection of the right media substrate is crucial for the success of the aquaponic, as it serves as the medium for plants to support and to grow and as bioremediation bed where beneficial microorganisms can colonize [15]. Several considerations should be highlighted when choosing a media substrate for aquaponic; pH buffering characteristics, water retention, porosity for biological filtration, weight and structure, costs and availability. Research performed by [12] showed that a cocopeat, hydroton and husk charcoal as an eggplant planting medium have different reduction rates of nutrients while study from [16] showed the capability of gravel as media growth bed for water spinach and mustard green, was reduced up to 70% of nutrients from fish farming wastewater. These findings showed that the nutrient removal of fish farming wastewater is not solely from plant uptake to growth but also from the growing media filtration. Hydroton or clay pebbles and gravel are commonly used as growth media in aquaponic media beds mainly due to their availability, cost and suitability for various types of plants.

The success of an aquaponic system hinges on various factors, with the selection of appropriate plant and fish species playing a vital role in achieving optimal growth and production [16]. The choice of plant and fish species in aquaponics is a multifaceted decision that requires careful consideration of biological compatibility, nutrient requirements, and environmental conditions. Leafy, herbs and fruity plants have been cultivated in aquaponic system and among them are lettuce (*Lactuca sativa*) [17, 18], basil (*Ocimum basilicum*) [19], water spinach (*Ipomoea aquatica*) [20], mustard green (*Brassica juncea*) [21], okra (*Abelmoscus esculentus*) [13, 22-24] and tomato (*Solanum lycopersicum*) [10, 25]. The suitability of plants in aquaponic generally depends on aquaponic system designs that suit certain plants better. Okra, a popular warm-season vegetable, is known for its rapid growth and adaptability to various climates [13]. Known as one of the nutritious vegetable commodities, okra provides important nutrients of body needs in the form of soluble fiber (mucus and peptin) that can contribute to cholesterol level reduction and diminishes the risk of heart disease [26]. The increasing consumption of okra caused by medicinal purposes and diet diversity worldwide thus raised the necessity of maximal cultivation of okra. Okra is cultivated globally in tropical and warm temperate regions such as Malaysia, for the nutritious, fibrous fruit pods [27]. Okra pod is one of the most affordable sources of vitamins, minerals, and antioxidants essential for good health [28]. Its potential in aquaponic systems is noteworthy, and understanding the distinctions of its cultivation in different media beds is crucial for maximizing yields. In tandem with plant selection, the choice of fish species is equally critical and depends on various factors. Several species of fish are commonly cultivated in aquaponic especially from the freshwater species such as tilapia (*Oreochromis niloticus*), trout (*Oncorhynchus mykiss*) [29, 30], and African catfish (*Clarias gariepinus*) [31, 32]. Traditional aquaculture of catfish which was by tank or pond confronts a difficulty related to water quality and waste management. A frequent change of water caused by inefficient waste management and untreated water before disposal led to environmental pollution issues. However, aquaculture production of catfish globally increased by year due to recognized flesh quality and uncomplicated fish cultivation requirements, thus a sustainable aquaculture practice of catfish farming such as an aquaponic system is crucial to meet the demand. Catfish is a choice food fish species in Malaysia due to their high tolerance to fluctuation and poor water quality, disease resistance, and high demands in the market. Its rapid growth at high stocking densities, ability to breathe air, high food conversion, good meat quality, ability to withstand poor water quality, and tasty flesh make catfish an excellent candidate for aquaculture [33-36]. The interaction between catfish and the chosen media substrate directly influences nutrient cycling, water quality, and, consequently, the overall success of the aquaponic system.

As the global demand for sustainable and resource-efficient agricultural practices continues to rise, optimizing the synergy between plant and fish selection in aquaponic systems becomes imperative [29, 37]. The effectiveness of different media substrates, specifically gravel and clay pebbles, in aquaponic systems remains a critical area of investigation. This study aims to evaluate and compare the growth performance of okra plants and catfish in aquaponic systems utilizing gravel and clay pebbles as growth beds. By examining key parameters such as plant and fish growth, nutrient uptake and water quality, this research seeks to provide valuable insights into the optimal substrate for enhancing overall productivity in aquaponic systems. The findings of this research endeavor aim to contribute valuable insights for aquaponics practitioners, researchers, and stakeholders, fostering a deeper understanding of the intricate dynamics between growth beds, plant species, and fish species in the pursuit of enhancing overall system productivity and sustainability.

2. Materials and Methods

2.1. Experimental Design

This study was conducted within 16 weeks in a bell-siphoned recirculating aquaponics system (RAS) greenhouse, located at Universiti Sultan Zainal Abidin (UniSZA), Gong Badak Campus, Terengganu, Malaysia. Four units of RAS were operated simultaneously during the study as shown in isometric layout in Figure 1. The greenhouse was shielded with a 70% net size high density polyethylene (HDPE) sun screening orchid net to protect plants from environmental factors and external influences such as pesticides.

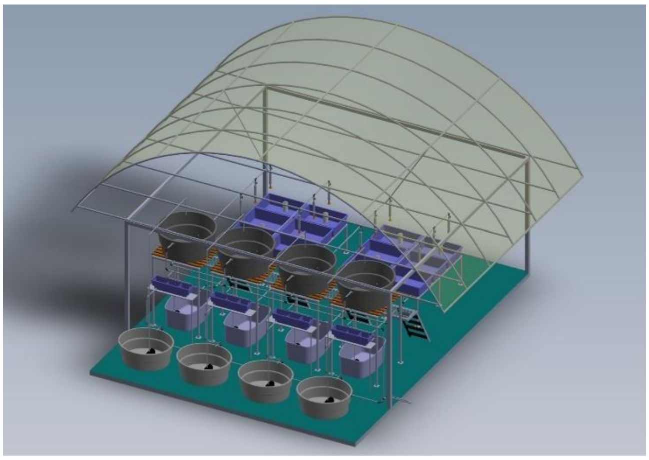

Figure 1. The isometric layout of bell siphoned recirculating aquaponic system (RAS) greenhouse

The single unit of RAS as presented in Figure 2 consists of fish culturing tank, physical filtration unit, growth bed, sump tank, sand filtration unit (mechanical filter), and holding tank. The fish culturing tanks, located at a height of 1.50 m from the ground, comprised of a cylindrical polyethylene (PE) tank with a total capacity of 318 L. An air pump, with an output of 8 W and a capacity of 9.0 L/min, operating at a pressure of 0.014 MPa is employed to supply oxygen to the fish through aeration stones placed in the fish rearing tanks.

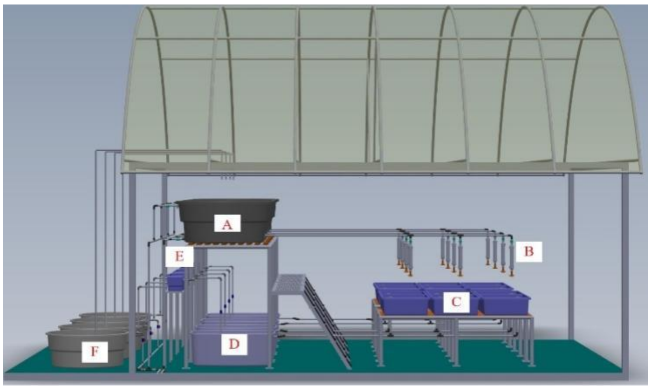

Figure 2. Each unit of RAS consists of fish culturing tank (A), physical filtration (B), growth bed (C), sump tank (D), sand filtration (E) and holding tank (F)

The water from the fish culturing tank flowed gravitationally to the physical filtration unit which is installed in the vertical pipe. The vertical physical filtration unit was filled with high quality cotton wool as a filter media to prevent solid fish feces from penetrating the growth beds. To facilitate the maintenance process, the physical filtration pipe was not tied-off. Once the cotton wool filter is saturated, it will be replaced with a new one to prevent reduction in water flow. Next it passes through vertical physical filtration units and flows into the growing beds which are controlled by ball valve to ensure a uniform flow rate of 360 L/h for all growth beds. This flow rate was selected to ensure that the wastewater was being distributed effectively to the entire system and the bell siphoned was able to operate smoothly.

The growth beds contained growth media and a unit of bell siphon. Two types of plant growth media were used in this study, namely gravel and clay pebbles. The gravel used is of the granite type with medium-fine fraction between 10.0 – 20.0 mm with a porosity of 0.60, while the clay pebbles (Hydrokorrels Bl äton) obtained from the local agriculture market are 8.0 – 16.0 mm in size. Both growth media are cleaned by rising with clean water several times to remove sand or soil and fine particles attached to the growth media before being placed into the growing beds. The plant growth beds used are rectangular plastic containers with measurements of 84.0 x 54.0 x 24.0 cm. The plant growth beds were filled with the media up to a level of 14.0 cm from the bottom of the container. This plant growth media study was conducted by following different media beds; M1: 100% gravel, M2: 100% clay pebbles and M3: mixture of gravel and clay pebbles with the ratio of 1:1. For M3 gravel was placed at the bottom layer of growth bed container while clay pebbles were placed on top of gravel.

The water level in the media growth bed is controlled by a bell siphon, a simple device that is easily built and connected from various sizes of pipe. The siphon allows the growth bed to overflow and drain automatically when water levels reach a certain level. Its mechanism is based on Bernoulli principles, generates a flood, and drains cycles without using electric pump. The overflow and channel cycle are engineered to enhance oxygenation as well as facilitate nutrient absorption by the plants. Following evacuation by the bell siphon, water was channeled into the sump tank (S), which consisted of a cylindrical polyethylene tank. Within this tank, 200 plastic bio-balls were deployed as a medium to foster the development of nitrifying bacteria. Subsequently, water from the sump tank was vertically propelled to the horizontal sand filtration unit to eliminate particulate matter. Eliminating particulates is crucial to avoid blockages in the pipes and other equipment within an aquaponic system. Additionally, the sand filter serves as a biofilter. Subsequently, the water was conveyed into the water holding tank (H) which utilized a rectangular polyethylene tank as a pre-aerated holding reservoir. Positioned on the ground beneath the greenhouse and adjacent to the pipe connecting to the primary tap water source, this tank facilitated efficient water management [18].

2.2. Cultivation of Catfish

Juvenile of African catfish used in this study were supplied by local fish farmers and were transported in polyethylene plastic bags filled with dissolved oxygen gas into three replicate fish culturing tanks. Before commencing cultivation, the juvenile catfish in plastic bags were acclimatized until the water temperature reached equilibrium, ensuring their smooth transition before it was released into the culturing tank. Following the attainment of temperature equilibrium, the water temperature inside the plastic bag closely mirrored that of the fish culturing tank. It is important to reduce the risk of stress on the fish during this process, as stress can contribute to mortality. To ensure that the dissolved oxygen content in the water is sufficient, all fish farming tanks are provided with oxygen through aeration stones connected to air pumps. The fish are fed twice a day with a commercial fish feed that is based on 2-5% of the average weight of fish with an average initial body weight was 4 to 6 g. The fish feed contains crude protein 30.2%, crude fat 4.4%, crude fiber 3.7%, moisture 11.9%, ash 9.9%, calcium 1.4% and phosphorus 1.1%.

To maintain the water level and replace water lost due to evaporation, aerated clean water is periodically added to the fish tank. Fish samples for about 10% from each tank were taken randomly every week to take measurements of weight to determine the amount of fish feed and fish growth rate. The weight of fish feed given is calculated according to the change in fish weight and was recorded. Fish growth was evaluated through the following equations:

$$Weight\ gain\ (\text{kg}) = W_t - W_0 \quad (1)$$

$$Specific\ growth\ rate\ (\%/day) = \frac{\ln W_t - \ln W_0}{t} \times 100 \quad (2)$$

$$Feed\ conversion\ ratio\ (FCR) = \frac{total\ fish\ feed\ consumed{,}g}{weight\ gain{,}g} \quad (3)$$

$$Survival\ rate\ (\%SR) = \frac{fish\ stocked - mortality}{fish\ stocked} \times 100 \quad (4)$$

Where, W_0 is the initial average fish weight, W_t is the final average fish weight and t is the period of the study in day.

2.3. Cultivation of Okra

Okra seeds obtained from the local market were sown in a seed tray for germination. After 2 weeks of germination period, okra seedlings were transferred into growth bed M1, M2 and M3. Okra seedlings were planted inside growing media with 4.0 cm in depth to ensure seedlings are in an upright position with a strong structure. The growth of okra was calculated through measurement of plant height, leaf length and width, and stem circumference using a measurement tape. The stem circumference was measured just below the first node from the growing media surface. The weight of the harvested okra pod was weighed using an electronic balance. The growth rate and average yield were recorded to evaluate the effect of plant growing media on okra growth. Okra's leave area and specific growth rate (SGR) were calculated by following equations:

$$Leaves\ area\ (cm^2) = k \times L \times W \quad (5)$$

Where L is the leaf length in cm, W is a leaf width in cm and k is a coefficient with the value of 0.62

$$Specific\ growth\ rate\ (\%/day) = \frac{\ln H_t - \ln H_0}{t} \times 100 \quad (6)$$

Where H_0 is initial plant height, H_t plant height by time and t is a time in day

2.4. Physical Properties of Media Growth Bed

Sample of gravel and clay pebble was taken to analyze the physical characteristics of both substrates. The analyses were conducted using the ICCROM-UNESCO-WHC ARC Laboratory Handbook. A sample of both growing media of gravel and clay pebble was prepared with some amount and a sample was occupied in a 100 mL beaker. The sample was carefully filled into the beaker to remove any empty spaces, ensuring a complete fill in. The weights of both the empty and filled beakers were measured and recorded. Bulk density was determined by following equation:

$$Bulk\ density\ (g/mL) = \frac{W_2 - W_1}{V} \quad (7)$$

Where W_1 is the weight of the beaker, W_2 is the weight of the beaker and samples, and V is the volume of the beaker.

Before the physical examination was conducted, the growing media samples underwent thorough washing with deionized water to remove any fine particles and dust from their surface. Subsequently, the samples were dried at 60 ℃ in a universal oven (Memmert, UF 110, Germany) for a duration of 24 hours. After the drying process, the samples were located in the desiccator with silica gel for cooling and then the samples were measured using an analytical balance (Shimadzu, ATY224, Philippines). This method was iterated till the variation among two consecutive measurements was below 0.1% of the sample mass within 24-hours period. The resulting constant mass, designated as the dry weight of the samples (m_c), was recorded. Following this, the samples were immersed in deionized water within a glass beaker until they were submerged approximately 2 cm below the water surface for a duration of eight (8) hours. The samples were then removed, weighed, and recorded as saturated mass (m_s). The immersion procedure of the samples was reiterated until the disparity among dual consecutive dimensions was below 1% of the quantity of water captivated. Open-pore volume (V_{op}) was calculated by following equation:

$$V_{op} = m_s - m_c \tag{8}$$

The saturated samples of growing media were located into the graduated cylinder and occupied with deionized water, with the incremental volume shown on the cylinder measured as the apparent volume (V_a) in cubic millimeters, mm^3. The apparent volume represents the total volume of the solid, encompassing the space occupied by the pores. The porosity (%P), denoted by the ratio between the value of open pores (V_{op}) and the apparent volume (V_a) as defined in equation (9), signifies the percentage of interconnected pores space.

$$\%\text{P} = \frac{V_{op}}{V_a} \times 100 \tag{9}$$

The maximum amount of water captivated by a growing medium under saturation conditions at room temperature and standard pressure is well-defined as water absorption capacity (WAC). The WAC is formulated as percentage of dry weight of the sample and calculated as following equation:

$$\%\text{WAC} = \left(\frac{m_s - m_c}{m_c}\right) \times 100 \tag{10}$$

2.5. Water Sampling and Analyses

Water samples were taken from each culture tank, influent and effluent of the growing bed, sump, water holding tank and inflow of culture tank, once a week for chemical analyses. The physicochemical parameters such as dissolved oxygen (DO), pH, and temperature were monitored and conducted using DO portable meter (PRO-YSI, Professional Plus, USA), while pH level was determined by a HACH portable pH meter (HACH, sensION+pH1, USA). Measurements of pH, DO, and temperature were consistently performed between 09:00 and 10:00 am each day. Continuous monitoring of these parameters is imperative to maintain optimal water conditions in the culturing tank for the successful growth of catfish. Additionally, the levels of total ammonia nitrogen (TAN), nitrite nitrogen (NO_2^--N), nitrate nitrogen (NO_3^--N), and total phosphorus (TP) were analyzed using a HACH DR4000 spectrophotometer weekly according to APHA (2005). The efficiency of nutrient removal through comparison between growing media setup was calculated using equation:

$$\%\text{Nutrient Removal Efficiency} = \left(\frac{a - b}{a}\right) \times 100$$

Where a is a concentration of nutrients at initial in mg/L and b is a concentration of nutrients at final in mg/L.

2.6. Data Analyses

The statistical analysis in this study for comparing and evaluating multiple mean values generated from three replicates of the bedding media physical characteristics, catfish and okra plants growth parameters, catfish and okra production and physicochemical parameters were performed using one-way analysis of variance (ANOVA). The data was checked for normal distribution and variance homogeneity, and post hoc tests were performed using Tukey's HSD (Honestly Significant Difference) test to compare the averages to exhibit significant differences among the treatments at significant level of 0.05. The analysis was conducted using statistical software such as Minitab 16 (Minitab, Minitab 16, USA) and Microsoft Excel, with a significance level set at $p \leq 0.05$. The graphing software used for graphical analysis was OriginLab (OriginPro 9.1, USA). Results from both data analysis and the graphing software were employed for presenting the results.

3. Results and Discussion

3.1. Growth and Production Performance of RAS

Table 1 tabulates the physical properties namely bulk density, open pore volume, porosity and water absorption capacity evaluated in this study. The significantly highest mean of bulk density ($P < 0.05$) was 1246.33±6.13 kg/m^3 found in gravels followed by layer of gravel and clay pebble of 851.22±3.14 and clay of pebbles of 456.10 ±5.10 kg/m^3, respectively. Bulk density measurement is defined by how efficiency of particles is packed together in confined medium. It is greatly influenced by the particle shape, size, and distribution of the substrates. The gravels used were in non-uniform square shape and have bigger in diameter, ranging between 16 and 25 mm compared to clay pebble which was ranging between 8 to 15 mm. Clay pebbles are mainly in spherical shape and are more compact particles arrangement but have lighter weight compared to gravel. Obviously, significant disparity in particle size distribution of clay pebbles media resulted in much lower bulk density values compared to gravel. In aquaponic media-based growth bed, the weight and size of particles are a main consideration in the selection of media types.

Table 1. Physical Properties of substrates

Parameters	Gravel	Clay pebble	Gravel and clay pebble layer
Bulk density (kg/m^3)	1246.33±6.13^a	456.10±5.10^c	851.22±3.14^b
Open pore volume (mm^3)	0.03±0.01^c	0.24±0.02^a	0.13±0.01^b
Porosity (%)	2.19±0.03^c	18.43±0.93^a	10.31±0.36^b
Water absorption capacity (%)	1.94±0.06^c	36.90±0.61^a	22.43±0.13^b

Values given are mean from triplicate data (n=3).

The data are expressed as mean ± standard deviation.

Mean with the different superscript is significantly different at the $p \leq 0.05$ level.

The interaction between the growing media and water, and water transport phenomena through pore structure, is characterized by porosity. Clay pebbles in this study have a mean porosity of 18.43±0.93% which was significantly higher than layer of gravel and clay and gravel. The lower bulk density of clay pebbles compared to gravels contributed to a significantly high value of percentage of effective porosity. As required characteristics of growing media in aquaponics, porosity is essential for nitrogen and phosphorus removal, as well as a medium for the growth of microbial.

Water absorption capacity (WAC) is different from one media bed to another, and it also has important agronomic characteristics. It was observed in this study that clay pebbles have significantly higher WAC which is 36.90±0.61% at $p<0.05$ compared to gravels 1.94±0.06%. Hypothetically, growing media with a high WAC could be saturated with water for longer time and reduce a leaching loss of nutrient issues. It is distinct from WAC because of two factors, which are the degree of porosity of the material and the total organic matter of the media particles. According to Table 1, clay pebbles are more porous with value of more than 10% of effective porosity. Clay pebbles productions are mix of raw clay and organic materials thus resulting in capacity of high amount of water retaining. The process of making clay pebbles at high temperature produced honeycombed ceramic pellets with lightweight, porous and high crushing resistance characteristics [38]. The porosity of clay pebbles is suitable for plants to access enough oxygen and wider surface area for microorganism growth [39].

The performance of RAS was evaluated by analyzing the growth of both okra and African catfish as tabulated in Table 2. Okra for both growth beds in M2 and M3 was shown in healthy condition, considerably high growth and no nutrient imbalanced symptoms were observed throughout the study period. Meanwhile, okra in growth bed M1 experienced slow growth with nutrients deficiency signs. It was proven by final plant height where okra plant in M2 was the highest at 150.33 ± 5.49 cm followed by M3 at 146.67 ± 8.82 cm. Results showed plant height in M1 was at the lowest value at 43.33 ± 4.41 cm. The highest okra height was achieved by [13] at 98.75 ± 2.38 cm in the growth bed mixed with gravel and coconut husks. Thus, a growth bed containing clay pebbles promotes much better plant height and can support a plant height of over 150 cm. The highest leaf area was achieved by okra plant in growth bed M2 at average 1007.29 ± 8.89 cm^2 followed by M3 was at 940.95 ± 14.65 cm^2 and the lowest was M1 at 101.27 ± 23.83 cm^2. The result of this study in terms of leaf area was better than the previous study conducted by Azad et al. [13] in their study of okra cultivation with tilapia in aquaponic system. The result for stem circumference showed the same growth traits for plant height and leaf area where the highest value was in growth bed M2 at 7.17 ± 0.52 cm, followed by M3 at 6.63 ± 0.19 cm and M1 at3.93 ± 0.23 cm respectively. These results revealed that growth beds M2 and M3 which contained clay pebbles showed excellent features as media beds for growing okra plant. All the results for okra growth performance showed that there was a significant difference in okra plant height, leaf area and stem circumference between growth bed M2 and M3 compared to M1. One of the failure factors of aquaponic system was mainly because of ineffective solid removal [40]. Clay pebbles have superb water-draining properties compared to gravels and this was advantageous for this media to easily clean the substrate media and no need for maintenance schedule. Poor water drainage for gravel contributes to the growth of algae and moss and thus increasing the quantity of solid waste to get rid of the systems. Therefore, the present study is in line with the conclusion of Zainal Alam et al. [39], which stated that nutrients availability and porous structure of substrates played an important role in plant growth performance.

Table 2. Okra (*A. esculentus*) and African catfish (*C. gariepinus*) growth performance in recirculation aquaponic system (RAS)

Parameters	M1	M2	M3
	Okra		
Final plant height (cm)	43.33 ±4.41^{b}	150.33 ±5.49^{a}	146.67 ±8.82^{a}
Leaf area (cm^2)	101.27 ±23.83^{b}	1007.29 ±8.89^{a}	940.95 ±14.65^{a}
Stem circumference (cm)	3.93^{b} ±0.23	7.17 ±0.52^{a}	6.63 ±0.19^{a}
Specific growth rate, SGR (%/d)	2.33 ±0.07^{b}	3.41 ±0.06^{a}	3.42 ±0.09^{a}
	Catfish		
Weight gain (kg)	152.17 ±10.02^{c}	250.33 ±6.21^{b}	290.50 ±7.37^{a}
Specific growth rate, SGR (%/d)	3.08 ±0.23^{b}	3.62 ±0.21^{a}	3.63 ±0.13^{a}
Survival rate, SR (%)	94.5 ±0.29^{b}	98.0 ±0.29^{a}	98.0 ±0.58^{a}
Fish conversion ratio, FCR	2.17 ±0.14^{a}	1.33 ±0.03^{b}	1.30 ±0.03^{b}

Values given are mean from triplicate data (n=3).

The data are expressed as mean ±standard deviation.

Mean with the different superscript is significantly different at the $p \leq 0.05$ level.

The growth performance of African catfish regarding weight gained (kg) was tabulated in Table 2. Daily feeding rate increased with culture time and accounted for 2–5% of fish body weight. There are significant differences when comparing fish production performances with various substrates used for weight gain. M3 shows the highest weight gain which is 290.50 ±7.37 kg followed by M2 and M1 at 250.33 ±6.21 kg and 152.17 ±10.02 kg, respectively and statistically significant difference between each growth bed. The present study revealed a relatively higher performance in fish weight gain compared to previous study [22, 40] due to the differences of substrate used and longer culture period employed by other studies. The average SGR value of catfish for all fish rearing tanks in this study shows a higher value compared to the study by others research [33, 41, 43]. These are important traits to keep stability and good water quality thus affecting the growth of the fish in free-stress condition and uptake of nutrients and water for plant growth in healthy.

The average fish survival rate (SR) for all tanks was between 94.5 ±0.29 % to 98.0 ±0.58 %, where there was a significant difference in SR between different bedding media. The higher value of SR obtained in this study may be due to the excellent water quality in fish culturing tanks. This suggests that none of the bedding substrates tested in this study had a deleterious effect on fish survival rate. The returned water to fish tanks was treated well during recirculation process and filtration through hydroponic basin and other biological and physical filtration efficiently removed the nutrients. Ammonia's toxicity and cannibalism phenomena are among the factors of catfish death during the growing process in rearing tank. Meanwhile, the feed conversion ratio (FCR) value shows a significant difference between M2 and M3 with M1 with the lowest FCR value at M3 being 1.30 ± 0.03 and the highest at M1 being 2.17 ±0.14. The average FCR value for M2 and M3 is not much different from the ideal value of FCR 1.00 for catfish cultivation in a closed aquaponic system and much better compared to research by [11] with high FCR value of 2.55.

The yield of okra and catfish produced through this study from recirculation aquaponic system is shown in Figure 3. Within the study period, growth bed M3 produced the highest mean yield of okra at 4.89 ±0.40 kg/m^2, followed by M2 at 4.37 ± 0.38 kg/m^2 whereas M1 produced the lowest okra production of 2.83 ±0.24 kg/m^2.

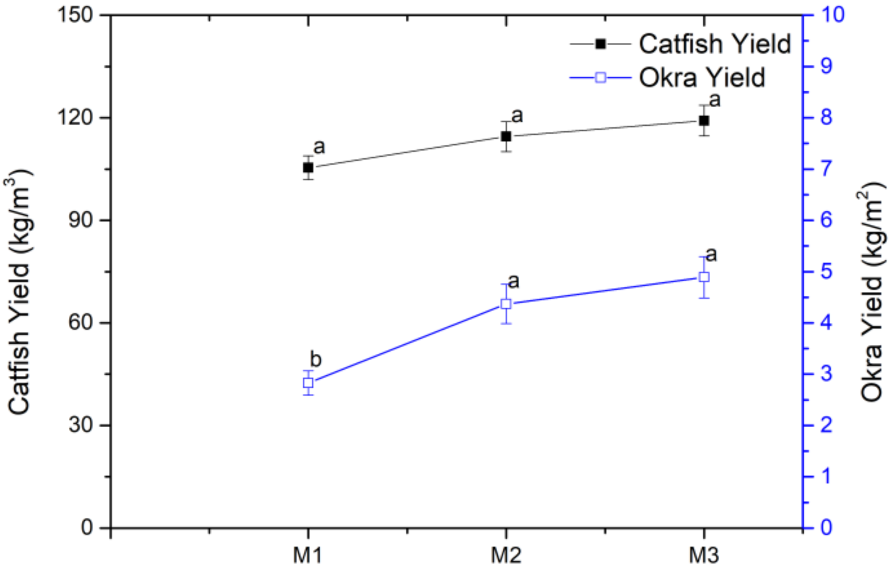

Figure 3. Okra and catfish yield in different bedding media

Okra is a fruit plant that is harvested frequently, several times per week during production. [23] reported of okra that was planted in UVI aquaponic system over 12 weeks yielded 3.04 kg/m^2 which is lower than production of M2 and M3 in this study. The signs of nutrient deficiency of okra plant in gravel alone growth bed of M1 resulting in the low yield of okra during cultivation period. The inability of the gravel to absorb sufficient water containing nutrients needed for the growth of the plant was linked with the low water absorption capacity and effective porosity characteristic. Although okra is a fast growth plant that is cultivated well under warm conditions, enough nutrients and water holding capacity which contributed by types of media also affected the production. In comparison to the study conducted in previous study [42], the various types of fertilizer used resulting in lower yield of okra than in this finding. This study demonstrates a notable economic advantage in terms of okra yield when employing the aquaponic system. By using only wastewater from catfish aquaculture as fertilizer for plants growth, the finding of okra yield suggests a potential for enhanced cost-effectiveness and resource optimization. The economic feasibility of this study approach was required within aquaponic farming systems as sustainable urban modern aquaculture [37].

No significant difference was observed in catfish production with different growth media and the highest yield was achieved by M3 at 119.18 ±4.51 kg/m^3 followed by M2 and M1 at 114.50 ±4.39 kg/m^3 and 105.41 ±3.48 kg/m^3, respectively. The diet was fed to fish for all rearing tanks with the expectation of fish growing at the same rate and uniform yield was achieved. This study was proven to produce significantly high yield of catfish compared to study of [40], which used pumice and charcoal as bedding media and the production of fish at average 3.24 kg/m^3 to 5.22 kg/m^3.

3.2. Water Quality Parameters of Recirculation Aquaponic System (RAS)

The data of influent and effluent for the nitrogen cycle of the recirculating aquaponic system (RAS) is presented in Figure 4. The weekly variation of total ammonia nitrogen (TAN), nitrite nitrogen (NO_2-N), nitrate nitrogen (NO_3-N) and total phosphorus (TP) concentrations was influenced by bedding media as shown in figure. In this study, TAN concentration in the influent exhibited a value of 0.82 ±0.04 to 0.93 ±0.01 mg/L. The concentration of TAN was dropping significantly in the effluents of all bedding media with a range of 0.29 ± 0.03 to 0.48 ± 0.01mg/L for M1, 0.11 ±0.04 to 0.25 ±0.01mg/L for M2 and 0.13 ±0.01 to 0.21 ±0.06 mg/L for M3. These results showed the growth bed containing clay pebbles, M2 and M3 was better with lower TAN concentration in the effluents compared to effluents from the gravel growth bed, M1.

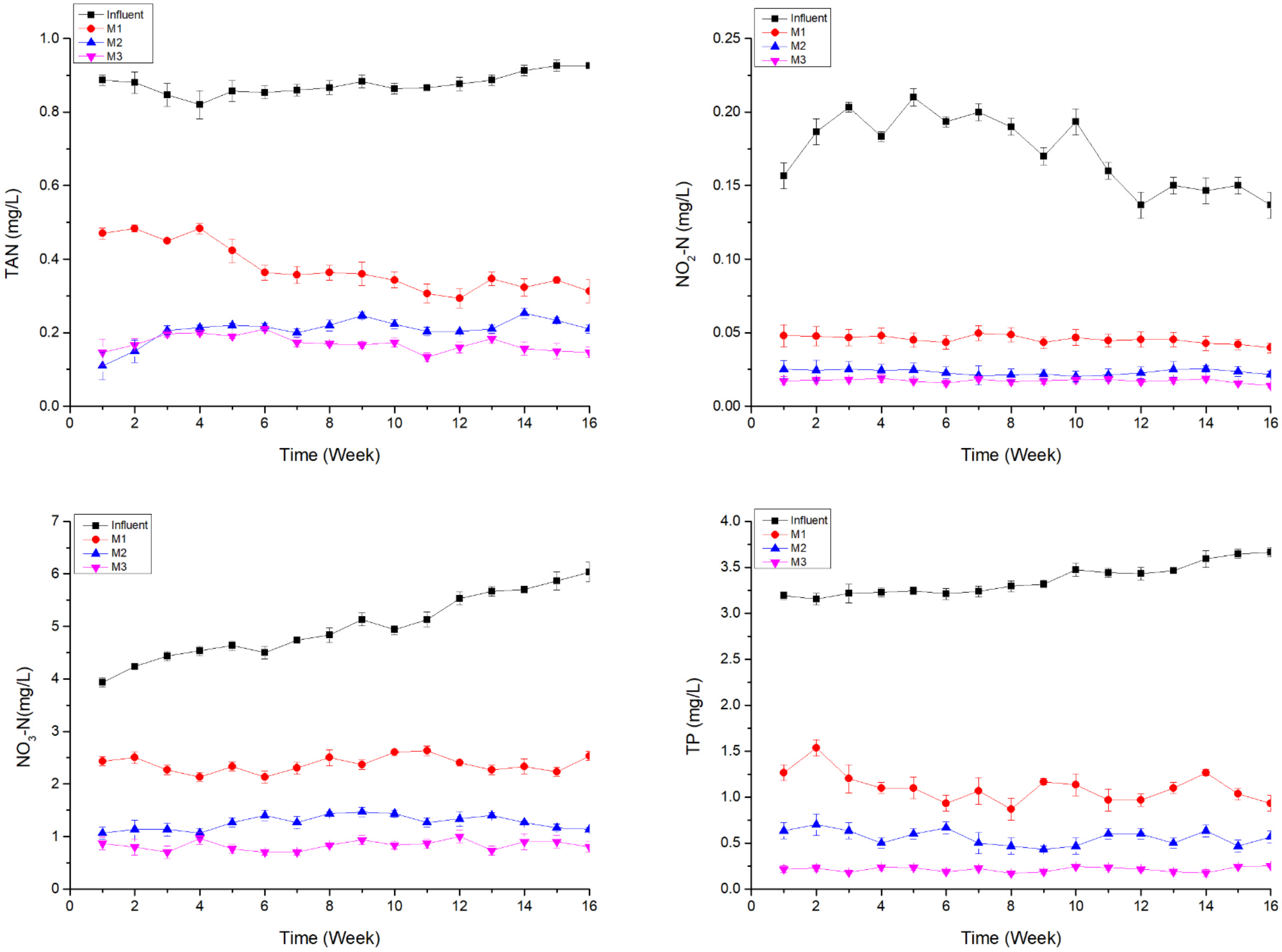

Figure 4. Influent and effluent reduction in the nutrient concentration of TAN, nitrite, nitrate and total phosphorus during 16 weeks of experiment

TAN concentration is a sum of both forms of ammonia exist in water which are unionized ammonia (NH_3) and ionized ammonia (NH_4^+) [18]. The major nitrogen removal mechanism has been classified into two factors; microbial assimilation and plant nitrogen uptake. The effective amount of NH_4^+ removed from wastewater was identified to be converted by nitrifying bacteria into NO_2^- and NO_3^-. The substrate in the growth bed of RAS was utilized as crucial source for the growth of nitrifying bacteria. Thus, high porosity clay pebbles provide a greater area for the development of nitrifying bacteria and enhance the rate of microbial nitrification. The absorption of NH_4+ in aquaponic system through the absorption of plant roots through the support of oxygen and bacteria. However, the uptake of nitrogen and phosphorus by plant is only a fraction of the amount generally removed from the water contaminated with nutrients [11]. This indicates that microbial assimilation in the root zone and associated with hydroponic substrate in which plant was cultivated play a significant role in nutrients reduction.

The lower concentration of TAN in M3 observed in this study could be concluded that well-developed microbial community in mixed hydroponic substrate of gravels and clay pebbles. The combination of different hydroponic substrates creates different aeration levels being an added value for promoted active nitrification process.

The influent concentration of nitrite nitrogen (NO_2^-N) at average of 0.16 ± 0.01 to 0.21 ± 0.01 mg/L within a period of 8 weeks and showed declining trend after week 10 at average of 0.14 ± 0.01 to 0.16 ± 0.01 mg/L. The RAS is a closed loop system that means no water changes during operation and the reduction of the influence concentration of NO_2-N could result from the nitrification process. The concentration of NO_2^-N in the effluent after being filtered through okra growth bed was in the range of 0.039 ± 0.003 to 0.049 ± 0.005 mg/L for M1, 0.025 ± 0.002 to 0.020 ± 0.003 mg/L for M2 and the lowest with the range of 0.014 ± 0.002 to 0.019 ± 0.003 mg/L was in M3. Nitrification is a transformation process of ammonia where microbes oxidize NH_3/NH_4^+ to NO_3^-. Under aerobic conditions, nitrification occurs in two-step-reaction by autotropic bacteria which are *Nitrosomonas* and *Nitrobacter* [13]. *Nitrosomonas* or also known as ammonia-oxidizing bacteria oxidize NH_4^+ to nitrite (NO_2^-) and transform to nitrate by nitrite-oxidizing bacteria, *Nitrobacter*. The low concentration of nitrite in the effluent demonstrated that the transformation reaction of ammonia to nitrite occurs in optimal environments in the growth bed. The microbes for this transformation were grown well in the mixture of gravels and clay pebbles media growth bed as well as allowing oxygen supply sufficiently.

The concentration of nitrate nitrogen (NO_3-N) in the influent was at a range of 3.93 ± 0.09 to 6.03 ± 0.19 mg/L. The result showed a slightly ascending trend of influent NO_3^- due to transformation reaction of ammonia to nitrate. However, there was reduction of NO_3^- concentration in the effluent for all growth beds and the highest reduction was in M3 with a range of 0.70 ± 0.06 to 1.00 ± 0.12 mg/L. NH_4^+ and NO_3^- were a major source of nutrients for plants. NO_3^- is a favorable form of inorganic nitrogen that was released during nitrification process by a reaction from *Nitrosomonas*. Nitrate was taken up by the root plants as a natural fertilizer for growth. The reduction of nitrate may also be contributed by assimilation of microorganisms in the water column or biofilms associated with the root mats of plants [19, 43]. The combination of high porosity and bulk density of clay pebbles and gravels is favorable for microorganisms to actively grow and create more biofilm in the root mats zone. In addition, the physical characteristics of clay pebbles which can retain more water thus remove more nitrate through flowing water. These conditions factors contributed to the lower nitrate concentration in the effluent of mixed media clay pebbles and gravels growth bed.

The total phosphorus (TP) concentration in the influent varied between 3.15 ± 0.06 mg/L to 3.67 ± 0.05 mg/L. The decrement pattern of TP concentrations in the effluent was quite similar to nitrate concentrations trends which indicated M3 removal was better than M1 and M2. After being filtered by media growth bed, effluent concentration of TP for M1, M2 and M3 reduced to the range of 0.87 ± 0.12 to 1.53 ± 0.09 mg/L, 0.43 ± 0.03 to 0.70 ± 0.12 mg/L and 0.17 ± 0.02 to 0.25 ± 0.01 mg/L respectively. The TP is a measure of all forms of phosphorus found in water that includes orthophosphates (PO_4^3-), acid-hydrolyzable phosphates, organic soluble phosphates, and particulate phosphorus. The removal of phosphorus from wastewater occurs by sorption, complexation, precipitation, and assimilation into microbial and plant biomass [42]. Major particulate phosphorus is removed by filtration or sorption in the root system. Through the bacteria activity, organic phosphorus is converted into mineral phosphorus that can be assimilated by the plants [23]. The sustainable RAS allows microorganisms oxidation that converts organic phosphorus to inorganic phosphorus (orthophosphate) and then could be taken up by plants roots [20]. The well development of root mats of okra plant on the media surface increased the uptake of inorganic phosphorus thus resulting in the significant decrease of TP in the effluents.

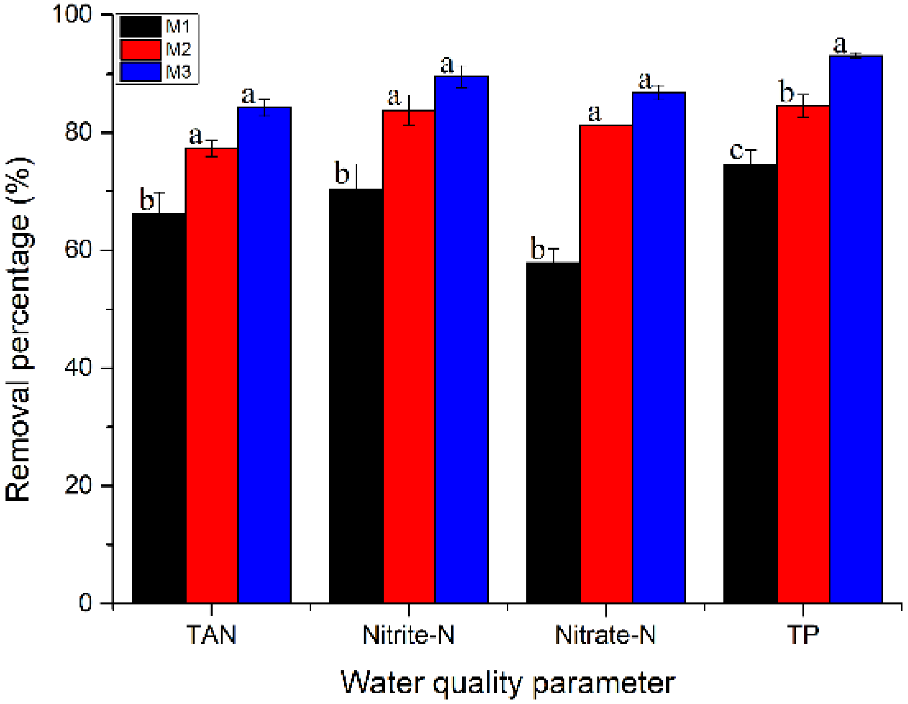

Figure 5. Removal percentage of TAN, nitrite-N, nitrate-N and total phosphorus by gravel, clay pebble and mixture of both in RAS

Figure 5 shows the removal percentage of nutrients concentration by different media growth beds M1, M2 and M3. The result showed statistically significant nutrient removal between clay pebbles and mixture of gravels and clay pebbles growth bed for TAN, nitrite-N and nitrate-N. The highest TAN removal percentage was achieved in M3 84.2 ± 1.42% compared to 77.3 ± 1.41% in M2 and 66.1 ± 3.76% in M1. The removal efficiency was 89.5 ± 1.85% in M3 and 83.8 ± 2.56% in M2 for nitrite-N and were no significant difference but significant lowest removal efficiency for M1 at 70.4 ± 4.24%. The effective removal percentage for nitrate-N was achieved in M2 and M3 at 81.2 ± 0.09% and 86.8 ± 1.22%, respectively meanwhile the lowest percentage was 57.9 ± 2.43% in M1. The removal efficiency for TP shows significant differences for all growth bed M1, M2 and M3 with the highest percentage 93.1 ± 0.48% in M3 followed by 84.5 ± 1.96% in M2 and 74.5 ± 2.55% in M1. The mixture of gravels and clay pebbles growth bed, M3 consistently showed better nutrient removal efficiently for okra planting and catfish cultivation in RAS.

The physical properties of gravel with low water retentive capacity and rocky surface contribute to reducing the media's proficiency to remove the nutrients physically from water flow because of biofilm development and slippery conditions [17]. Clay pebbles through its pore structure allow water movement and create the interaction between media and water. The micro and macropores contained by clay pebble naturally formed interconnected channels that flowing water with rich nutrients can penetrate through it thus forming physical nutrients filtration. Advantageous value could be concluded by mixture of gravel and clay pebbles growth bed which was increasing the water holding capacity and different porosity of media have different effects on nutrients uptake by root plants [38]. This study shows that aquaponics is economically feasible under some reasonable assumptions such certified organic production. This activity is also environmentally advantageous since it reduces considerably the use of water and fertilizers. According to [22], RAS are used as artificially compatible engineered wetland ecosystems with incorporated combinations of plants, gravels, bacteria, substrates, and hydraulic flow system are utilized. The correct selection and maintenance of them to sustain the system by optimizing the physical, chemical, and microbiological processes naturally present in the root zone.

4. Conclusions

Gravel and clay pebble have the ability as a growth media to support root structure for plant growth and to reduce the nutrients concentration from aquaculture wastewater. This study based on 16 weeks experiment suggested that a mixture of gravel and clay pebbles has better okra-catfish growth performance and nutrient reduction than gravel-alone growth media. A significantly higher specific growth rate (SGR) of okra was achieved in in growing bed containing clay pebbles-alone, M2 and a mixture of clay pebbles and gravel, M3 with values of 3.41 ± 0.06 and 3.42 ± 0.09. The highest weight gain of catfish at 290.50 ± 7.37 and the lowest for FCR at 1.30 ± 0.03 was achieved in M3. The combination of clay pebbles and gravel with a suitable effective porosity of 10.31±0.36 and water adsorption capacity (WAC) of 22.43±0.13 have been proven to have high removal capacity of nutrients from aquaculture wastewater. The removal of TAN, nitrite-N and nitrate-N for growth bed M3 was 84.2 ± 1.42%, 89.5 ± 1.85% and 86.8 ± 1.22%, respectively meanwhile for TP removal was 93.1 ± 0.48%. The mixture of both gravel and clay pebbles creates a combination of preferred characteristics thus increasing the reduction efficiency of nutrients concentration from catfish rearing tanks. In conclusion, it is recommended that further analysis can be conducted on various local waste products as potential media bed substrates in aquaponic systems, aiming to provide alternative, cost-effective solutions for aquaponic farmers to enhance system efficiency and sustainability.

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