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Research Article

Growth Performance of Roselle (*Hibiscus sabdariffa* **L.) in Aquaponics and Determination of The Optimal Stocking Density of Nile Tilapia Maximizing Its Productivity**

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Abstract:

This study was performed to evaluate productivity of roselle (*Hibiscus sabdariffa*) in aquaponics and determine the optimal fish stocking density which maximize its growth performance. It was conducted with Nile Tilapia *Oreochromis niloticus* (30.4±4.5 g and 6.5±1.2 cm) using fifteen plant/m² and five levels of fish stocking density (3 kg.m⁻³, 6 kg.m⁻³, 9 kg.m⁻³, 12 kg.m⁻³ and 15 kg.m⁻³). After 45 days, the plants were examined with respect 18 growth and productivity variables. The results obtained show that plants with higher fish stocking density showed better growth performance as plant biomass (2736 \pm 34.9 g/unit), plant height (95.0 \pm 7.1 cm/plant), number of leaves per plant (89.4 \pm 6.5), fresh leaves biomass (932.9 \pm 23.8 g/plant), leaf area (35.8 \pm 4.3 cm²/plant) and plant growth rate ($115.11 \pm 9.4\%$) observed with plants of 15 kg.m⁻³ for plant biomass, plant height, number of leaves, leaf area, and plants of 12 kg.m⁻³ for leaves biomass, plant growth rate and root weight. The linear regression analysis showed a highest correlation between fish stocking density and plant height ($R²$ = 0.9693), leaves number ($R^2 = 0.9209$), leaf weight ($R^2 = 0.878$), plant weight ($R^2 = 0.956$), leaf length ($R^2 = 0.969$) 0.8945) and leaf width ($\mathbb{R}^2 = 0.9234$). Relationships between leaf area and leaf number per plant is also very high (R² = 0.9508). Polynomial regression analysis showed that the optimal fish stocking density maximizing roselle productivity is 14.1 kg.m⁻³ for leaf number and 13.0 kg.m⁻³ for leaf area.

1. Introduction

Currently, one of the greatest challenges for agriculture in the world is to produce food for an ever-growing population by adapting to climate change. In developing countries, and the Democratic Republic of Congo (DRC) in particular, traditional agricultural production systems have already shown their limits in terms of productivity and the environment (Beucher and Bazin, 2012; Lebailly et al., 2014). It would not be able to provide sustainable food production for a population already living in food insecurity. To ensure agricultural production susceptible to meet food needs of the population, alternative production methods that are resilient, productive and environmentally friendly will be needed.

Aquaponics, a modern and innovative agricultural production system, is one such sustainable production method that could be adopted. Aquaponics is an integrated system that is resilient, productive, environmentally friendly and less demanding farm land (Bernstein, 2011; Goddek et al., 2015). It is a sustainable agricultural method merging fish and plant production in an integrated system that recirculates water so that fish effluents transformed by nitrifying bacteria into assimilable nutrients fertilize the plant crop (Richard et al., 2011; Goddek et al., 2015). By extracting dissolved nutrients, plants filter and purify the water, making it clean for reuse in fish farming (Adler et al., 1996; Wongkiew et al., 2017; Thelwell, 2019). Thus, to fight growing food insecurity and the difficulties of obtaining land resources for agricultural in Congolese urban areas, aquaponics appears

therefore as a real alternative solution. For its adoption and expansion, it is therefore necessary that the growth and productivity potential of main crops and the determination of theirs specified techniques production be studied in aquaponics.

According to Pasch et al., (2021), optimal plant growth in aquaponics requires the continuous supply of nutrients in sufficient quantities, otherwise the plants will suffer nutritional deficits. The supply of sufficient nutrients in aquaponics depends mainly on the balance between fish biomass, biofilter and number of plants (Eck et al., 2019; Hossaina et al., 2022). Hence, finding the balance between fish, bacteria and plants is absolutely essential for the harmonious functioning of aquaponics system (Hossaina et al., 2022). This balance is achieved by determining the fish biomass, which corresponds to the number of plants, and maintaining it consistently (Foucard et al., 2016). The calculation of feeding ratio determines the quantity of feed to be fed by fish, which corresponds to the density of plants to be grown in a given space. This quantity of feed will determine the number of fish that need to be reared to consume it. According to Somerville et al., (2014), this method of calculating fish feeding using only applies to mature systems during the growth phase of the fish, and it also requires further consideration before adopting it. The determination of the optimum fish density maximizing plant growth is applied to the systems that have not yet reached maturity (Andriani et al., 2017; Wiyoto et al., 2023).

Some studies have already been carried out on various fish combined with different plant species to determine fish and plant densities for aquaponics production, such as African catfish and basilic (Towa et al., 2022), Nile Tilapia and tomatoes, peppers and cucumbers (Adeleke, 2020), Nile Tilapia and Indian Spinach (Hossaina et al., 2022), Nile Tilapia and lettuce (Sabwa et al., 2022).

Roselle (*Hibiscus sabdariffa* L), a plant of the Malvaceae family, is mainly cultivated for food and medicinal purposes (Ali et al., 2005; Lépengué et al., 2007). In human nutrition, the leaves of the roselle are consumed as cooking vegetables, and the calyxes used for the extraction of "roselle juice" (Lépengué et al., 2011). However, this crop has not yet been studied to assess its growth potential and productivity in aquaponics. Nor is the density of fish required for optimal growth known. Thus, the questions relating to the growth potential of this plant in aquaponics, and the optimal fish density that can maximize its productivity remain unanswered. To fill this scientific gap, the aim of the current trial is to evaluate the growth of *Hibiscus sabdariffa* in aquaponics and determine the optimal fish stocking density to maximize its productivity.

2. Materials and Methods

2.1. Experimental approach

The experiment was carried out in Bandundu (Latitude: 3°19′00″ south, longitude: 17°22′00″ east, altitude above sea level: 286 m) in the Democratic Republic of Congo from February 21 to April 06, 2024. Five treatments, following a completely randomized design, consisted in a combination of varying the stocking density of Nile Tilapia *Oreochromis niloticus* (30.4±4.5 g of average weight and 6.5 ± 1.2 of average length) with 15 plants per meter square of roselle as planting density in ten identical aquaponic systems. The fish densities tested on plant growth performance were: 3 kg.m^{-3} , 6 kg.m^{-3} , 9 kg.m^{-3} , 12 kg.m-3 and 15 kg.m-3 . Each treatment was replicated two times. Fish were stocked in 250-litre tanks, and fed twice a day at 5% of their biomass with a commercial feed containing 35% crude protein.

Roselle seeds were sourced from a local producer and germinated in a nursery of 2 m x 1.2 m (length x width) on mulched soil to minimize evaporation before emergence and a few days after germination. On the eighteenth day after germination, the roselle plants, having reached a height of 10 cm with 7 leaves, were transplanted into the aquaponics culture beds (1.25 cm x 0.8 cm x 30 cm L, W, H respectively) at a rate of 15 plants per square metre at regular spacing of 20 cm x 20 cm. On the tenth day after transplanting in aquaponics, the roselle plants were topped to give ramifications, thus increasing production (Atta et al., 2010). The leaves were dried by exposure to the sun.

2.2. **Data collection**

Water quality was monitored daily for temperature,

pH and dissolved oxygen using a multimeter HANNA. Nitrogen compounds and phosphate were sampled twice weekly using test solutions. For plant, eighteen plant growth and production parameters were measured, the main ones being: number of leaves per plant, fresh and dry weight, leaf weight, leaf length (L), leaf width (W), plant height (H), leaf area (LA), stem diameter (SD). The rectangular technique based on the calculation of individual leaf area integrating their length and width measurements into a regression function was used to determine leaf area (LA) according to equation 1 of Cho et al., (2007). Plant growth rate (PGR) was estimated according to equation 2 (Carberry, 2015).

$$
LA = L \times W \times 0.83
$$
 Equation 1

PGR=Final height-Initial height/Culture duration x 100 **Equation 2**

2.3. Statistical analysis

The data collected are presented as means \pm standard deviation, and subjected to three statistical analyses using R software version 4.3.0 for Windows. Means of water quality and plant growth parameters were processed by one-way analysis of variance (ANOVA), and least significant different test (LSD) was used to identify differences between treatments. Linear regression analysis was used to assess correlations between some plant growth parameters (leaf area, average leaf weight, number of leaves and plant height) and the fish stocking density. Coefficients of determination (R^2) were calculated between the parameters evaluated.

The optimal fish density corresponding to optimal *Hibiscus sabdariffa* growth was determined graphically by applying the second-order polynomial regression model following equation 3 (Khandan et al., 2019).

$$
Yi = ax^2 + bx + c
$$

The coefficients a, b and c of the equation 3 were obtained by multiplying each value in the row of matrix $M⁻¹$ with each value in the column of matrix Y of the equation 4. $M⁻¹$ being the inverse of the matrix M described below and Y being the sum of the products of the fish density variables and the roselle growth variables.

$$
\begin{pmatrix}\n\Sigma x^4 & \Sigma x^3 & \Sigma x^2 \\
\Sigma x^3 & \Sigma x^2 & \Sigma x^1 \\
\Sigma x^2 & \Sigma x^1 & \mathbf{n}\n\end{pmatrix}\n\begin{pmatrix}\na \\
b \\
c\n\end{pmatrix}\n=\n\begin{pmatrix}\n\Sigma x^2 y \\
\Sigma x^1 y \\
\Sigma y\n\end{pmatrix}
$$
\nEquation 4\n
$$
\begin{pmatrix}\n\Sigma x^2 & \Sigma x^1 \\
\Sigma x^1 & \Sigma x\n\end{pmatrix}
$$

3. Results

3.1. Water quality

The water quality parameters of the hydroponic components during experimental period are recorded in Table 1, and concern temperature, pH, dissolved oxygen (DO), electrical conductivity (EC), alkalinity, ammonia, nitrite, nitrate and phosphate. The daily fluctuations of temperature, pH and DO are shown in Figures 1 and 2.

Equation 3

Parameter	Experimental treatments					
	3 kg.m^3	6 kg.m^3	$9 \text{ kg} \cdot \text{m}^3$	$12 \text{ kg} \cdot \text{m}^{-3}$	$15 \text{ kg} \cdot \text{m}^{-3}$	p-value
Temperature, °C	30.4 ± 0.97	30.4 ± 1.07	30.3 ± 1.03	30.5 ± 1.07	30.5 ± 1.03	0.937
pH	$7.75 \pm 0.14^{\text{a}}$	7.58 ± 0.11^a	7.51 ± 0.21^b	7.38 ± 0.3 ^{bc}	7.22 ± 0.22 ^c	< 0.01
DO, (mg/l)	$8.24 \pm 0.43^{\circ}$	$7.16 \pm 1.05^{\rm b}$	6.35 ± 1.31 ^{bc}	6.73 ± 1.33 ^{bc}	$6.35 \pm 1.31^{\circ}$	0.02
EC , (μ S/cm)	691.3 ± 18.5 ^c	523.9 ± 23.1 ^d	658.0 ± 14.6 °	718.2 ± 17.2^b	$786.7 \pm 19.3^{\circ}$	0.000
Alkalinity, (mg/l)	80 ± 7.0 ^d	112 ± 6.3^b	114 ± 6.2^b	88 ± 5.2 ^c	$122 \pm 6.8^{\circ}$	< 0.01
Ammonia (mg/l)	0.89 ± 0.03	0.93 ± 0.07	$0.87+0.02$	0.85 ± 0.09	$0.90+0.02$	0.567
Nitrite (mg/l)	0.03 ± 0.01	0.03 ± 0.03	0.04 ± 0.02	$0.01 + 0.01$	$0.02+0.03$	0.082
Nitrate (mg/l)	1.4 ± 0.01 ^d	9.4 ± 0.03^b	8.8 ± 0.02 ^b	8.1 ± 0.01 ^{bc}	$12.8 \pm 0.04^{\text{a}}$	< 0.01
Phosphate (mg/l)	0.05 ± 0.2 ^c	0.02 ± 0.01 ^c	$0.28 \pm 0.08^{\text{a}}$	$0.29 \pm 0.06^{\text{a}}$	0.11 ± 0.02^b	0.03

Table 1. Experimental water conditions of hydroponic component

Values in the row having different superscripts are significantly different at p˂0.05

Some water quality parameters showed significant differences between treatments (p <0.05). The temperature values are relatively the same between treatments, fluctuating from 30.9 to 32.0°C during the trial period. The pH ranging from 7.22 to 7.75 remained neutral for all treatments and decreases in the time.

Figure 2. Variation of pH (left) and dissolved oxygen (right) during the trial period

Dissolved oxygen decreases with the fish stocking density during the experimental duration. OD is higher $(8.24 \pm 0.43 \text{ mg/l})$ with low density and lower $(6.35 \pm 1.5 \text{ g})$ 1.31 mg/l) with high density. The range of EC was between 523.9 ± 23.1 and 786.7μ S/cm. The alkalinity range was varied between 80 and 122 mg/l. In all treatments, the ammonia and nitrite concentration remained below 1 mg/l and 0.05 mg/l respectively for nitrate and nitrite. Nitrate was very low for the density of 3 kg.m⁻³ $(1.4\pm$ 0.01 mg/l) and higher for 12 kg.m^{-3} (12.8 mg/l). There were no significant differences for ammonia and nitrite between the different treatments $(p>0.05)$ but for nitrate the treatments relating to 15 kg.m^{-3} and 9 kg.m^{-3} , recorded higher nitrate contents face other treatments (p<0.05). The phosphate concentration varied from 0.05 to 0.29 depending on the fish stocking density. There is a significant difference (p˂0.05) between treatments for phosphate concentration (Table 1).

3.2. Roselle growth parameters

The roselle growth parameters studied in this work are presented in Table 2 and some illustration of plants of roselle according with different fish stocking density is showed in Figure 3. Fish stocking density affected several plant parameters of roselle that were significantly different between treatments (Table 2).

Generally, roselle growth parameters per treatment increased with increasing fish stocking density. High values of fresh plant biomass $(2736 \pm 34.9 \text{ g/unit})$, final plant height (95.0 \pm 7.1 cm/plant), stem diameter (3.2 \pm 0.2 cm/plant), number of leaves per plant (89.4 ± 6.5) and leaf area $(35.8 \pm 4.3 \text{ cm}^2)$ were significantly different between treatments, and observed respectively in plants in systems with 15 kg.m⁻³. Apart from these variables, low-density treatments also recorded minimum leaf length and width $(2.9\pm0.7 \text{ cm and } 2.7\pm0.3 \text{ cm})$, number of primary (5 ± 2.6) , secondary (2 ± 0.3) and tertiary (0) branches per plant (Table 2).

Figure 3. Plants of roselle in different fish stocking density

Root weight ranged from the lowest value of 9.7± 2.1 g in the treatment stocked at 3 kg.m^{-3} to the highest value of 12.8 ± 4.3 g in the treatment stocked at 9 kg.m⁻³. The PGR is highest with 12 kg.m^{-3} . The plant growth rate ranging from 67.78% \pm 4.9 % for 3 kg.m⁻³ and 115.11 \pm 9.4 % for 12 kg.m-3 . Dry leaf biomass, average weight of a dry leaf, average leaf length, and average leaf width and leaf area were high in 12 kg.m⁻³ (174, 92 \pm 16.3 g), (0.23 \pm 0.9 cm). There is any change in the characteristics observed with fresh leaves between treatments after the drying of the leaves. The observed characteristics with fresh or dry leaves between treatments are similar, varying between treatments. Analysis of variance showed a significant difference between treatments for all variables (Table 2).

Table 2. *Hibiscus sabdariffa* growth performance

¹LSD: Least significant difference. The values having different superscripts are significantly different at probability of *P<0.05, **P<0.01, ***P<0.001. NS= Not significant

Figure 4. Effects of fish stocking density on plant height (black) and stem diameter (blue)

Figure 6. Effects of fish stocking density on plant average weight (red) and leaf area (black)

Figure 5. Effects of fish stocking density leaves number (black) and leaf average weight (orange)

Figure 7. Effects of fish stocking density on leaf length (green) and width (black)

Figure 8. Relationships between leaf area and leaf number per plant

Linear regression analysis presented in Figures 4-7 was used to show correlations between the effects of fish stocking density on plant height (y=5.5167x+9.41, R^2 =0.9693), stem diameter (y=0.0733x + 1.9, R^2 =0.7908), leaf number (y=0.0303 + 0.503, R^2 =0.9209), leaf average weight $(y=70.2x + 253.6, R^2=0.878)$, plant weight $(y=6.1567 x + 92.73, R^2=0.956)$, leaf area $(y=2.3967x +$ 3.97, $R^2=0.9118$), leaf length (y=0.2033x + 2.57, $R^2=$ 0.8945) and leaf width (y=0.3x + 1.24, R²=0.9234).

The correlation between these variables was very positive. Linear regression analysis also showed a highly positive correlation between leaf area and plant number. The value of the coefficient of determination associated with its function $(y=2.258x + 11.412)$ is 0.9508 for the three treatments relating to high densities (Figure 8).

4. Discussion

The results of this study show that the temperature, pH and DO were remained within the acceptable range for hydroponics. The pH is one of the crucial factors in aquaponics favouring the absorption of nutrients by plants. It also influences dissolved oxygen, and this causes, at low levels, a loss of nitrogen (Luuk et al., 2022; Wiyoto et al., 2023). When pH is ≤ 6 , it affects the solubility of nutrients hence affecting the plant growth and yields negatively (Rakocy, 2010). The optimal pH value for many crops in hydroponics is varying from 6 to 7 (Singh and Bruce, 2016). The pH values of this study ranging 7.22 to 7.75 are neutral and similar range of pH reported by Ekawati et al., (2021) and Adeleke et al., (2023) in spinach and tomatoes culture. Alkalinity is a term used to express the concentration of bicarbonate, and it is also very important for crops. High alkalinity above 75 mg/l increases the pH of the water (Singh and Bruce, 2016), and indirectly, influences the nutrients assimilation. According to Somerville et al., (2014), the alkalinity level in aquaponics should remain between 60 to 140 mg/l, ranging from 80 to 112, the alkalinity of the aquaponic systems recorded in this work was within tolerable limits and its levels were high while the pH was low.

Considering the presence of nitrogen compounds, nutrients very important for the growth of roselle, and resulting by nitrification process (Goddek et al., 2015), the results show that treatments at low densities showed very low concentrations of ammonia, nitrate and nitrite. These low concentrations lead to less efficient plant growth. The results also show that increasing fish density increases nitrate concentration. According to Sabwa et al., (2022), fish density is one of the parameters influencing this phenomenon. The high value of nitrate recorded in the high fish stocking density system is linked to the high fish biomass which received a great amount of feed. These results were also found by Sabwa et al., (2022); Adeleke et al., (2023) who made the same observation.

According to Rakocy et al., (2006), in aquaponics, fish feed is the main input that provides the nutrients necessary for the growth of fish, plants and bacteria. There are two ways in which fish feed is transformed into plant nutrients. Nitrification of metabolic excreta for nitrogen compounds and mineralization of solid waste (rejected food and feces) into minerals (Rakocy, 2007; Somerville et al., 2014).

The results obtained in this study revealed that roselle growth and productivity in terms of plant height, stem diameter, leaf length and width, and leaf area increase with fish stocking density. These results highlight the effects of fish density on these variables, and can be justified by the fact that water from high-density aquaponic systems is richer in nutrients.

Roselle is a plant that gives important ramifications (Atta et al., 2010). Topping is a technique that stimulates the plant to produce twigs (Khattak et al., 2016). Depending on the stocking density of the fish, it was observed that the lowest density (3 kg.m^{-3}) did not result in secondary twigs. The plants in this treatment remained stunted and less nourished due to the lack of nutrient water compared to a larger number of plants. The observed decreasing trend in plant weight, leaf number and leaf area at higher fish densities would be due to the decrease in oxygen and pH also reducing nutrient uptake by plants for the density of 15 kg.m⁻³

Subjecting the leaf number per plant and leaf area data to second-order polynomial regression analysis to determine the optimal fish stocking density maximizing plant growth, it can be seen as shown in Figures 9 and 10 that the optimal plant stocking density maximizing these two variables is 14.1 kg.m^{-3} and 13.0 kg.m^{-3} respectively for leaf number per plant and leaf area.

Figure 9. Second -order polynomial analysis fitting of leaf number per plant to fish stocking density

Figure 10. Second -order polynomial analysis fitting of leaf number per plant to fish stocking density

5. Conclusion

As the world faces climate challenges, the transition from traditional production systems to modern, productive and resilient systems is imperative. In this perspective, the determination of technical approaches for the production of the main crops is necessary to guide farmers in farm exploitation.

Aquaponics is one of the techniques likely to contribute to the supply of food resources at a time of climate challenges, but which is not adopted in developing countries, and in the Democratic Republic of Congo. Roselle is a plant widely consumed as a fresh or dried leafy vegetable. This study was conducted with the aim of demonstrating the potential of roselle productivity in aquaponics. The results obtained show that fish stocking density affects significantly its production. Plant biomass, height plant, number of leaves, leaf area and orders growth and production parameters increase with increasing density of fish. According to the findings of this study, a density of 14.1 kg.m⁻³ or 13.0 kg.m⁻³ is ideal to produce this plant in association with Nile Tilapia in aquaponics.

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