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Article

# Aquaponic culture with application of biostimulants in floating root recirculation systems

# Cultivo acuapónico con aplicación de bioestimulantes en sistemas de recirculación de raíz flotante

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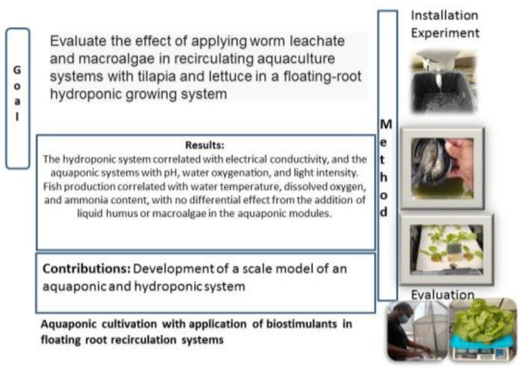
**Abstract**

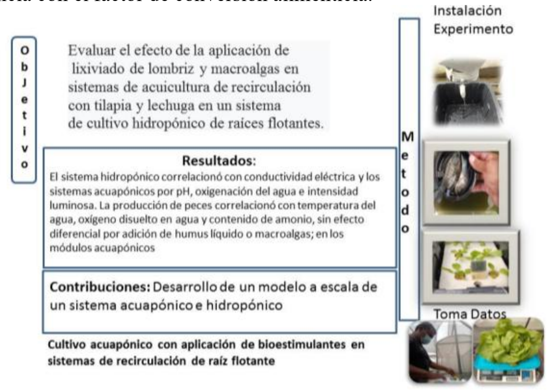
The objective was to evaluate the effect of the application of earthworm leachate and macroalgae [*Macrocystis pyrifera*] [L.] C. Agardh] in recirculating aquaculture systems with tilapia [*Oreochromis niloticus*] and lettuce [*Lactuca sativa* var. capitata] with floating root. An aquaponics system produces food by incorporating components [fish, plants, and microorganisms] into a recirculating system. Lettuce production was compared against a hydroponic system, where a higher weight of lettuce was obtained, followed by aquaponics with macroalgae. The hydroponic system correlated with electrical conductivity and aquaponics systems by pH, water oxygenation and light intensity. Fish production correlated with water temperature, dissolved oxygen in water and ammonium content, with no differential effect due to the addition of liquid humus or macroalgae; in the aquaponics modules; The greatest growth was in December-January where there was a higher absolute growth rate and weight gain without differentiation.




**Acuaponía, Hidroponía, Bioestimulantes, Lixiviado de Lombriz, Macroalga**

**Resumen**

El objetivo fue evaluar el efecto de la aplicación de lixiviado de lombriz y macroalgas [*Macrocystis pyrifera* [L.] C. Agardh] en sistemas de recirculación acuícola con tilapia [*Oreochromis niloticus*] y lechuga [*Lactuca sativa* var. Capitata] en sistema de cultivo hidropónico en raíz flotante. El sistema acuapónico produce alimentos mediante la incorporación de componentes [peces, plantas y microorganismos] en un sistema recirculante. La producción de lechuga se comparó contra un sistema hidropónico, donde se obtuvo mayor peso de lechuga, seguido del acuapónico con macroalgas. El sistema hidropónico correlacionó con conductividad eléctrica y los sistemas acuapónicos por pH, oxigenación del agua e intensidad luminosa. La producción de peces correlacionó con temperatura del agua, oxígeno disuelto en agua y contenido de amonio, sin efecto diferencial por adición de humus líquido o macroalgas; en los módulos acuapónicos; el mayor crecimiento fue en diciembre-enero donde hubo mayor tasa de cr7ecimiento absoluto y ganancia de peso sin diferencia con el factor de conversión alimenticia.




**Aquaponics, Hydroponics, Biostimulants, Worm Leachate, Macroalgae**

**Area**: Advocacy and attention to national problems

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## Introduction

Currently, the agri-food sector faces the challenge of producing sustainable food without harming the environment and reducing the excessive use of agrochemicals by producers seeking higher yields and better crop quality [Gutiérrez et al., 2008]. Aquaponics is a technique that allows organic food to be produced and obtained, which is why it is considered an alternative that reduces the environmental pollution caused by conventional agricultural production, as well as avoiding nitrogen waste discharges into water bodies [Ramírez et al., 2008] from fish farming through a recirculation system [Rivas et al., 2021] that allows the reuse of nutrient-enriched water [Ramírez-Sánchez et al., 2016].

An aquaponics system allows food to be produced by incorporating two or more components into the same production system. The principle is based on water recirculation, harnessing the energy of the system, as well as the combination of fish, plants and microorganisms [heterotrophic bacteria, fungi and other higher organisms] that use organic carbon to break down solid waste [faeces and food scraps] [Colorado et al., 2021].

There are also nitrifying bacteria [Nitrosomonas spp. and Nitrobacter spp.] which, in the presence of oxygen, transform ammonium into nitrites and subsequently into nitrates, allowing these nitrogenous metabolites to be absorbed by plant roots and enabling the return of “clean” water to where the fish are located, functioning as a biological filter that maintains the proper functioning of the production system [Andrade et al., 2015]. The main advantage of producing food in aquaponic systems is the added value that can be given to food, because by not incorporating agrochemicals, the use of pesticides and fertilisers is eliminated, obtaining organic food and avoiding soil and water quality deterioration [Valdez-Martínez et al., 2023].

On the other hand, natural products such as biostimulants and biofertilisers have also begun to be sought and used to improve the process of food production and procurement [Espinosa-Antón et al., 2020] in an environmentally friendly and respectful manner, as well as to improve crop yield and/or quality [Florez-Jalixto et al., 2021].

Biostimulants are classified into: humic substances, products containing amino acids, and products containing hormones. The latter include seaweed extracts containing growth phytohormones [auxins, cytokinins and other derivatives], which are considered useful for plants [Kauffman et al., 2007; Kurepin, et al., 2014; du Jardin, 2015; Alcántara et al., 2019]. Based on the above, the objective was as follows:

## Objective

To evaluate the effect of applying worm leachate and macroalgae [*Macrocystis pyrifera* [L.] C. Agardh] in recirculating aquaculture systems with tilapia [*Oreochromis niloticus*] and lettuce [*Lactuca sativa* var. Capitata] in a floating root hydroponic cultivation system.

## Methodology

The study was conducted at the Bofish Aquaponics Farm [20°33' 22.7" N, 103°25' 48.3 W] in Santa Anita, Tlaquepaque, Jalisco, Mexico, at an altitude of 1,575 metres above sea level, in an area of 17.1 m² inside a reinforced zenithal greenhouse [15 m wide x 45 m long x 6 m high]. Two similar experiments were conducted: the first from October to November 2021 and the second from December 2021 to January 2022.

Organic lettuce seeds [*Lactuca sativa* var. capitata], butter type variety ‘Nancy’ [Isla®] and tilapia [*Oreochromis niloticus*] obtained from the ‘BOFISH’ aquaponics farm were used.

*Obtaining seedlings and estimating the fish biomass ratio per plant, per module.* One hundred and fifty seeds were sown in a 200-cell tray with BP [Berger®] peat substrate. After 33 days, seedlings with 5-6 true leaves [Maroto, 1983] were obtained for transplanting. The roots were washed and placed in polystyrene plates in the aquaponic-hydroponic modules. The ratio of fish [tilapia] biomass to planted plants was determined based on Racocy [2007] proposal for raft systems, of 60 to 100 g of fish food per square metre of cultivation area.

Considering the floating root area of each aquaponic module [0.2318 $m^2$] and the initial amount of food [60 g], this resulted in 13.9 g of food, equivalent to 4% of biomass, which at 100% biomass resulted in 347.5 g of biomass per module, divided between four fish of 86.9 g per module.

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*Installation and management of the aquaponic and hydroponic modules.* The aquaponic modules consisted of nine plastic boxes [102 L], where the tilapia were placed and a biological filter was incorporated into each of the modules, consisting of a 19 L plastic bucket, soft drink lids and anti-aphid mesh [reused materials] where the microorganisms and bacteria responsible for transforming the floating solid waste from the fish water into secondary metabolites useful for aquaponic cultivation were fixed [Figure 1].

The hydroponic modules for the lettuce under the floating root system were constructed in three boxes similar to those containing the fish, placed on top of them and covered with polystyrene plates 5 cm thick, 68 cm long x 42 cm wide, with six holes 20 cm apart for transplanting the plant material. For the recirculation of water with the nutrient solution, the aquaponic and hydroponic modules were interconnected, generating mechanical oxygenation by the fall of water [Figure 1].

*Treatments***.** The treatments and their acronyms are shown in Table 1.

The conventional control was the hydroponic system with nutrient solution [SHSN], which was prepared and applied at the beginning of each experiment [25 days], using per 75 L of water: Monopotassium phosphate [$KH_2PO_4$ ] 15 g, Magnesium sulphate [$MgSO_4$] 38 g, Potassium nitrate [$KNO_3$] 26 g, micronutrients 3.5 g, calcium nitrate [Ca [$NO_3$]$^2$] 53 g, based on Steiner's Universal Nutrient Solution [Juárez et al., 2006].

The aquaponic system without biostimulants [SASB] was used as the aquaponic control. Worm humus [in SAHL] and the macroalgae product [in SAM] Seaweed Dry ® [Macrocystis pyrifera] powder [Table 2] were the treatments to be evaluated within each experiment and the three respective replicates.

They had a capacity of 35 L, so the biostimulants were applied based on that volume of water.

Water quality variables in the aquaponic-hydroponic module and greenhouse environmental variables.

In each of the two experiments and replicates, variables related to water quality were recorded every three days: hydrogen potential [HANNA potentiometer mod. HI98127]; water temperature [°C, thermometer], dissolved oxygen [Smart sensor oximeter mod. AR406, mg $L^{-1}$] electrical conductivity [HANNA conductivity meter mod. HI98304, mS/cm], ammonium [YSI® Mod 9500 spectrophotometer, mg $L^{-1}$] and nitrites and nitrates [Fluval® tests, mg $L^{-1}$].

The following environmental parameters were recorded inside the greenhouse: minimum and maximum temperature, relative humidity [hygrometer with probe Mod. TA318, %] and light intensity [Smart sensor lux meter Mod. AS803, Lux].

*Determination of lettuce yield.* To evaluate the yield [g] of the lettuce crop in the aquaponic and floating root hydroponic modules, three lettuces were taken and weighed for each repetition and treatment at the time of cutting [fresh weight].

*Determination of aquaponic crop yield.* To evaluate the yield of tilapia expressed by the weight and length of each tilapia, two biometric measurements were taken for each repetition of the experiment: the first at the start and the second after 15 days to avoid stress on the fish due to handling. With each biometry, the amount of food to be given daily to the Tilapia during the 25 days of each experiment was recorded. The yield formulas [Table 3] for tilapia proposed by [Ramírez-Sánchez et al., 2016] were used.

*Statistical analysis.*

The possible combinations between the four treatments were evaluated in a completely randomized block design [CRBD] with three replicates each and two dates or experiments. Normality [Shapiro-Wilk] and homoscedasticity of variance [Bartlett's test] tests were performed.

An analysis of variance [ANOVA] and the adjusted Bonferroni method test [STD] were performed to evaluate the biostimulants in the recirculating aquaculture system [RAS] with the yield of lettuce cultivation and tilapia cultivation.

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A permutational analysis of variance [PERMANOVA] was performed to determine the response of the study variables on the fresh weight of lettuce and fish biometrics, in addition to canonical principal coordinate analysis [ACC] based on the Bray-Curtis distance matrix and 1,000 permutations [Anderson and Willis, 2003], to explore the relationships between the parameters studied and the yield of lettuce and tilapia crops through the standardization of total variance and the selection of the variables that concentrated the highest percentage of variability among the four treatments [Ramírez & Nienhuis, 2012]. The analyses were performed with the agricultural packages [De Mendiburu, 2023], ggplots2 [Wickham, 2016], MASS 7.3-58.2 [Ripley, 2022] and Vegan 2.2-1 [Oksanen et al., 2022], in R Studio 4.2.3 [R Core Team, 2023].

**Results**

In both experiments, the PERMANOVA analysis of lettuce weight identified significant differences [p = 0.017] in the electrical conductivity of the water, which influenced the weight of the lettuce. The rest of the variables estimated in the water [pH, water temperature and dissolved oxygen] did not show significant differences, nor did the environmental variables Temperature and Relative Humidity [T and RH].

This indicates that the weight of the lettuce in both experiments was similar and that EC was related to the treatments used: nutrient solution, aquaponic water and the application of macroalgae or worm humus. For Sambo et al., [2019], the characteristics to be controlled in the hydroponic nutrient solution include electrical conductivity [EC], pH and temperature, which affect the chemical balance of the nutrient solution and influence nutrient absorption.

The highest weight values [714.5 g] of lettuce showed a greater correlation with water temperature and electrical conductivity [Ta and EC] for treatments with nutrient solution [SHSN] and aquaponic treatment with macroalgae [SAM] [Figure 2]. The variability in lettuce weight in the aquaponic treatment without biostimulant [SASB] and the aquaponic treatment with worm humus [SAHL] was correlated with pH [6.3–7], oxygenation [2.9–3.9%], and light intensity [5082 to 11506 lux].

The highest electrical conductivity was found in the SHSN treatment, with values of 2.3 to 2.6 dS $m^{-1}$.

In this regard, García-Terrazas et al., [2022] agree that the highest fresh weights of lettuce were found in nutrient solution with an EC of 2.5 dS $m^{-1}$, although they attribute this good tolerance to salinity to the variety of lettuce used [Paris Island Cos romaine lettuce].

With regard to the treatments, significant differences were obtained in the ANOVA and Tukey's test at a 5% probability. The treatment with nutrient solution [T1:SHSN] yielded the highest lettuce weight [244 g], followed by the treatment with macroalgae [T4:SAM] [216 g], aquaponics [T2:SASB] [193 g], and finally the treatment with liquid humus [T3:SAHL] [176 g]. In the last three treatments, their average EC values were 0.9, 1.0 and 1.3 dS $m^{-1}$, respectively.

Bautista et al., [2021] reported that lettuce in a hydroponic system [HS] had a higher yield [1,847 kg/$m^2$] compared to the aquaponics system [AS with tilapia, 1,080 kg/$m^2$], which they attributed to the fact that the HS had the necessary nutrients from the start of cultivation due to the use of fertilizer.

In the present study, among the aquaponic systems, the one that received macroalgae [SAM] obtained the highest fresh weight [645.5 g], statistically similar to SHSN [714.5 g] with an average difference of 27.9 g, while the greatest difference was found with SASB [577.7 g], with 67.4 g less.

In relation to water temperature, [Ramírez-Sánchez et al., 2016] point out that between 21.1 and 23.3 °C there is optimal plant growth, at higher temperatures there is low growth and susceptibility to pathogens begins. In this regard, Urdiales-Ponce and Espínosa-Ortega [2018] pointed out that the optimal temperature for lettuce development is between 15 and 25 °C, with lettuce able to withstand maximum temperatures of 26 °C and minimum temperatures of 7 °C, confirming that temperature is a variable that affects plant development. In the hydroponic modules where the lettuce was grown, the water temperature was the same as that recorded in the aquaculture area due to the recirculation system.

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The overall yield [fresh weight] of the lettuce crop showed a normal distribution [p = 0.26] and homogeneity of variances [p = 0.58]. The ANOVA for the fresh weight of lettuce showed significant differences [p = 0.038] between the conventional SHSN control and the aquaponic treatments [SASB, SAHL, and SAM] [Figure 3].

During the first experiment, a higher fresh weight of lettuce was obtained than in the second, despite having the same average temperature, probably due to a greater amount of light compared to the second [9,999 and 7,225 lux, respectively]. Neff and Fankhauser [2000] point out that light, in addition to being an indispensable source of energy for plant photosynthesis, is also an important factor for plant growth, development and morphology, responding to light intensity [Fukuda et al., 2008].

Fuentes-Morales et al., [2022] obtained the highest fresh weight in lettuce under an intensity of 5700 lux; at 2100 lux, the highest plant height was observed, and it was the treatment with the highest energy consumption. On the other hand, with regard to the results on tilapia growth, in the PERMANOVA analysis [p = 0.001], only water temperature [p = 0.001] showed highly significant differences, while dissolved oxygen [p = 0.079] showed a tendency to show differences.

The principal coordinate graph [Figure 4] confirms that in both experiments, water temperature influences the length and weight of tilapia, in addition to dissolved oxygen and ammonium.

The weight and length of fish increased with higher dissolved oxygen content and/or lower water temperature [Table 2], such that in the second experiment [December-January], fish weighing 116 g and measuring 16.4 cm were obtained at an average temperature of 18.6 °C and 4% dissolved oxygen; higher values than in the first experiment [October-November], where fish weighing 89 g and measuring 11 cm were obtained at 23.8 °C and 3.7% oxygenation.

The ammonium concentration reached an average of 0.2 and 0.4 mg $L^{-1}$ in each experiment, respectively.

According to Lama et al., [2025], an optimal temperature range between 18 and 30°C is recommended for aquaponic systems; according to Santoyo-Telles et. al., [2019], the appropriate water temperature for tilapia farming in aquaponic systems is 28 to 32°C. In this study, temperatures of 18° to 22° were recorded. Despite this, the tilapia did not show stress or symptoms of disease due to the low water temperatures, although there was an effect on performance [weight and length], because tilapia are ectothermic organisms [characterized by their dependence on external sources for heat], which generated metabolic differences related to the food consumed, reflected in performance [Ramírez-Sánchez et al., 2016].

According to the ANOVA for the weight and length of the tilapia, there were no statistically significant differences between the treatments [p = 0.9316] despite the normal distribution of the data and homogeneity of variances.

The feed conversion ratio obtained in the three aquaponic treatments [Figure 5] falls within the range reported by Sánchez-Sequeira [2006] as optimal [1.5-1.9], with no statistical difference between treatments of 1.87 to 1.9, except for SASB in the second experiment with 1.8. Comparatively, the FCA [feed conversion factor] values obtained were higher than those reported by Pérez et al., [2015] in a similar growth period of 25 days from 0.82 to 0.91 under a semi-intensive system and similar to those obtained by Zafra et al., [2019] with an average of 1.76 in a closed system.

For the absolute growth rate [AGR], there were also no differences between the aquaponic systems [without biostimulant, humus or algae] but there were differences between experiments, with higher values in the second experiment [December-January] at 0.4 g day $^{-1}$ than in the first experiment, which remained between 0.22 and 0.26, and in the second experiment between 0.6 and 0.63, lower than those obtained by Burgos et al., [2023] of 0.72 to 0.92 in red tilapia [*Oreochromis mossambicus*]. Similarly, there were no differences in weight gain between the aquaponic treatments except between experiments, with higher values in the second experiment of 15 to 15.9 compared to 5.25 to 6.6 in the first experiment; in the latter, similar to those obtained by Burgos et al., [2023] of 6.2 to 6.8 in red tilapia [*O. mossambicus*].

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## Conclusions

The highest lettuce weight was obtained in the hydroponic system with nutrient solution, where EC had the greatest influence, while the second-best weight was obtained when macroalgae were applied. The aquaponic treatments were affected by the pH and oxygenation of the water, as well as the light intensity.

Fish production in aquaponic systems was influenced by water temperature, followed by dissolved oxygen in water and ammonium content, with no differential effect from the addition of liquid humus or macroalgae in the aquaponic modules.

The best growth was observed during the months of December-January [second experiment], where higher TCA and GP were observed with no difference in the Feed Conversion Ratio [FCR].

## Declarations:

### Conflict of interest:

The authors declare that they have no conflict of interest.

### Authors' Contribution

*Chavez-Rangel*: Contributed to the idea of the Project, which is part of his master's thesis.

*Arellano-Rodriguez*: As thesis director Contributed to the documentary review, and monitoring of the work and additions modifications

*Neri-Luna*: Contributed to the introduction and the analysis of the results.

*Rodriguez*: Contributed to the statistical analysis and review of results

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## Abbreviations

| | |
|---|---|
| ACC | Canonical principal coordinate analysis |
| AGR | Absolute growth rate |
| ANOVA | analysis of variance |
| CRBD | Completely randomized block desing |
| EC | Electrical conductivity |
| HANNA | Potentiometer |
| pH | Hydrogen Potential |
| PERMANOVA | Permutational analysis of variance |
| R1, R2, R3 | Repetitions |
| RH | Relative humidity |
| RAS | Recirculating aquaculture systems |
| SAHL | Aquaponic system plus liquid humus |
| SAM | Aquaponic system plus macroalgae |
| SASB | Aquaponic system without biostimulants |
| SHSN | Hydroponic system with nutrient solution |
| T | Temperature |

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### Basics

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