Report regarding project: **En digitaliserad strategi för hållbar livsmedelsproduktion med PA projekt nummer 1402/Trg/2022**

Main Applicant
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Introduction

Aquaponics, as an integrated production system of fish and vegetables, is used as a model system in the current project, with the potential to be developed as a future circular food production system in urban and pre-urban areas. However, aquaponics is facing challenges regarding plant nutrition and product quality. Tools to improve and manage these drawbacks are thus of great importance. The current project aims to develop an early warning system to detect biotic and abiotic stress in aquaponics using Artificial Intelligence (AI). The sustainability of AI as a monitoring tool is evaluated concerning (1) type of crop, (2) type of fish feed and (3) vertical farming conditions. The spread of pathogens, plant growth and development, and plant quality are included in the evaluation.

Objectives

The multidisciplinary approach of the application was to meet the horticultural obstacles in achieving sustainable fertilization combined with efficient use of resources with focus on 1) AI as a monitoring system and 2) aquaponics as an aquacultural source for plant fertilization to provide a sustainable and resilient food production solution that:

- Reduces, reuses, and reutilizes industrial and agricultural waste streams in food production system to increase nutrient and water use efficiencies.
- Provides opportunities for digital and sustainable cultivation able to detect abiotic and biotic disturbance in the cultivation system
- Enhances food security and quality through combination of the two big food production systems

Research questions to be answered:

1- Can AI be used as a tool to detect biotic and abiotic stress in aquaponic cultivation?
2- Can the AI monitoring system detect nutrient unbalance in aquaponic system with respect to the type of crop and their nutrient demands during the cultivation period?
3- Can good plant growth and quality be achived through the application of AI monitoring system using aquacultural fertilizers?

Output from the project

1- Scientific baselines for application and functionality of aquaponics systems in relation to light conditions, different types of horticultural crops and fish feed.
2- Identification of abiotic factors related to nutrient balance and AI monitoring.
3- Supported by AI application, identification of factors with positive impacts on plant quality in aquaponic systems compared to hydroponic cultivation conditions
4- Scientific baselines related to the health aspects of aquaponic system and in relation to the the microbial conditions in the system

5- Publications
    a. Scientific publications in Pree-reviewd journals
        i. Review article about AI application in aquaponic systems is under review in Journal of Aquacultural reports.
        ii. One manuscript from experiment with impact of LED light on plant and fish growth in aquaponic system submitted to Fronties in Plant Science
        iii. Two manuscripts under preparation to be submitted during the summer of 2025.
            1. One manuscript with experiment with fish fertilizers using two different crops, Tatsoi and Tomato
            2. One manuscripts with different fish feed.

    b. Popular Sciences publications
        i. Fact sheet to growers 2025:1. DOI: https://doi.org/10.54612/a.4hae6at1tc
        ii. One article to the grower journal Viola (www.viola.se) to be published during the spring 2025.

6- Students thesis
    a. Two students thesis at master level has been performed

7- New collaborations
    a. The projects opened up new collaborations with
        i. SLU-Alnarp
            1. ***Yenitze Fimbres,*** Postdoc. Department of Biosystems and Technology, LTV faculty regarding aquaponic system and analyses
            2. ***Vishnukiran Thuraga,*** Postdoc. Department of Plant breeding, LTV faculty regarding AI analyses

        ii. SLU-Uppsala
            1. Aleksandar Vidakovic, Department of Applied Animal Science and Welfare; Aquakultur, VH faculty regarding fish feed
        iii. Gothenburg University regarding fish health in aquaponic systems
            1. Jonathan Roques, Department of biology and environmental science regarding fish growth and health. Connected to student master thesis

Co- funding

The project shared co-funding with other project where Samar Khalil was also the main applicant as follows:

1- Postdoc grants form Carl Tryggers stiftelse, where the postdoc Yentize Fimbres was the owner of the stipendium
2- Grants from Crafoordska stiftelse to share running costs
3- Grants from Partnerskap Alnarp for performance of student master thesis to strength the research area and cover the analyses costs regarding fish quality in relation to the use of alternative fish feed

Project performance

The project has been performed as Postdoc studies. The following experiments and tasks were conducted in the project:

1- Review article regarding aquaponic and artificial intelligence (AI):

The Postdoc work started with research and a literature review. This review critically evaluates the primary factors influencing the scalability of aquaponics, emphasizing the essential role of technology in augmenting production efficiency and strengthening essential facets of production and management. The overarching aim is to comprehensively assess the challenges confronting aquaponics and elucidate the potential of technology to mitigate these obstacles. While technology has long been integrated into specific aspects of aquaponics systems, its nascent integration into other domains underscores the need for further development and refinement.

2- Experiment with aquaponic systems

a. Aquaponic system designed in vertical farming mode using LED light under control conditions using Tilapia as model fish, Tatsoi as model crop and commercial fish feed. The aim of this experiment is to investigate the impact of LED and vertical design as factors applied in urban environment on cultivation conditions in aquaponic system.

b. Aquaponic system conducted in the greenhouse using light conditions usually applied in the greenhouse, Tilapia as model fish, Tatsoi as model crop and commercial fish feed. The aim of this experiment is to investigate the impact greenhouse conditions with light usually used in greenhouse cultivation on cultivation conditions in aquaponic system.

c. Experiments in the greenhouse using nutrients from fish water as organic plant fertilizers. The aim of this experiment is to investigate the impact of extracted fertilizers on plant growth. Tomato and Tatsoi were used as model crops

d. Aquaponic system conducted in the greenhouse using Tilapia as model fish, Tatsoi as model crop and mussel meal as alternative fish feed. The aim of the experiment is to investigate the impact of alternative feed on the cultivation conditions in aquaponic system

3- The system design used in the experiments

The aquaponic system consisted of a fish tank, biofilter, sump tank, and hydroponic units connected to the fish tank (Figure 1) was used in the experiments. Three replicates of this set-up were used.

Figure 1: Experiments performed with aquaponic and vertical system setup.

4- Analyses performed in the experiments

The experiments were based on the following analyses:

1- Water quality assessments were conducted by collecting samples to analyze the ammonium (NH_4), nitrite (NO_2), nitrate (NO_3), and phosphate (PO_4) using a Hach kit. The analyses included daily monitoring of physicochemical parameters such as oxygen, temperature, and pH in each unit using an electrical multiparameter. The commercial lab LMI (Helsingborg – Sweden) performed nutrient analyses concerning macro- and micronutrients.

2- Growth analyses of fish and plants expressed as yield, biomass, and total weight of plants and fish. The fish and plants' size and weight were measured weekly.

3- Artificial intelligence was also applied to monitor plant health regarding leaf area, chlorophyll content and photosynthetic activity. A large leaf surface area means more photosynthetic cells and a higher capacity to capture light, which increases photosynthetic activity and thus the plant's nutrient and energy supply.

4- Microbial analyses were performed regarding the occurrence of root pathogens belonging to the fungal group *Oomycetes* in hydrponic system and the plant part in aquaponic system. The content of nitrification bacteria in biofilter in aquaponic system and of human pathogens (*Entrobacteriacea*) in fish tank was also evaluated.

5- Obtained results

The achieved results from the project indicate that

- Application of AI is an effective tool to investigate plant growth in aquaponic systems
- Combination of aquaponic systems with LED increases leaf area, chlorophyll content and photosynthetic activities in the leaves, which leads to greater biomass
- Combination of aquaponic systems with LED light has a positive effect on plant growth and

increases the possibility of applying aquaponic systems under vertical and urban conditions

- Variation in greenhouse conditions needs to be optimized with respect to light to optimize plant growth in aquaponic systems in greenhouses
- Combination of aquaponic systems with mussel meal as alternative fish feed increases leaf area, chlorophyll content and photosynthetic activities in the leaves, which leads to greater biomass
- Fertilization with nutrients from fish water varies depending on plant species and proportions and fertilization with 100% fish nutrients is not suitable for nutrient uptake and plant growth
- Better plant growth and yield in aquaponic compared to hydroponic

6- Factors of optimized and crucial characters

The performed experiments identified different factors of crucial characters need to be considered to improve growth and quality in aquaponic systems and thereby plant fertilization.

a. pH

This factor is crucial for both fish, plants and for the nitrification process. In all the experiments, the pH values were measured at daily level and adjusted according to the cultivation conditions to the level between 6-7. These results advocate for the need of monitoring system to maintain the pH conditions in the system,

b. Temperature

In general, the temperature is a crucial factor for the fish. In the performed experiments Tilapia, warm-water fish, were used. These fish require a water temperature between 25-27^{0}C for their growth. In controlled conditions in the biotron, a water temperature of 25^{0}C could be achieved through climate installation of the growth chamber. This could not be achieved in the greenhouse and heaters were used in both fish tanks and biofilter tanks to increase and maintain an optimal temperature for the fish. These results advocate for the need of monitoring system to maintain the temperature conditions and the potential to enhance resource efficiency if the aquaponic is applied in controlled cultivation conditions.

c. Light

Light conditions has shown to be of crucial characters in our experiments. The AI analyses showed that Tatsoi plants grown in aquaponics systems in controlled conditions with LED light had significantly higher chlorophyll content, larger leaf area and higher photosynthetic activity (Figure 2). These parameters indicate that the continuous nutrient supply in aquaponics systems interacted with LED light provides plants with access to nutrients in optimal amounts, which may lead to higher chlorophyll content and thus better photosynthetic capacity and growth compared to those grown in a control system (hydroponics).

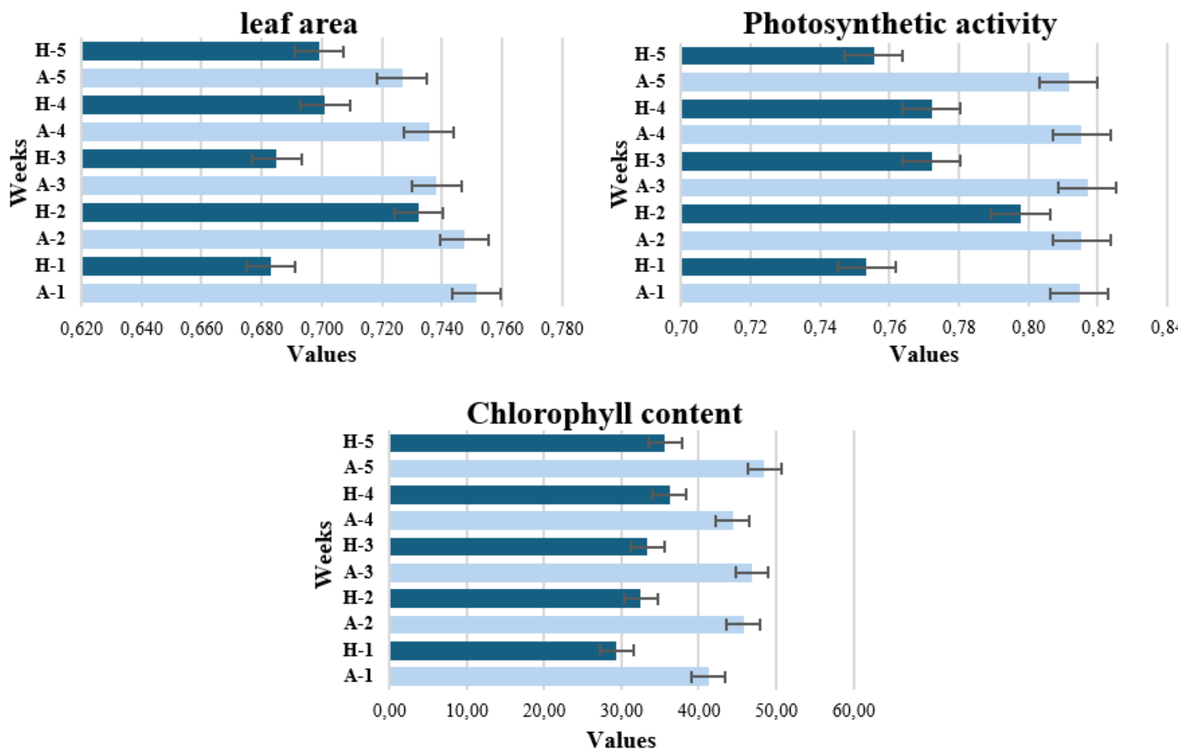

Figure 2. Leaf area, photosynthetic activity and chlorophyll content in Tatsoi plant cultivated for five weeks in aquaponic (A) and hydroponic (H) system in controlled cultivation conditions in the biotron

Unlike the biotron experiment, the results from the greenhouse experiment showed no differences in chlorophyll content in aquaponics grown plants compared to hydroponics grown plants alone (Figure 3). However, chlorophyll content was higher when measured at the end of the cultivation period in the aquaponics system compared to the beginning. Leaf area was larger at the beginning of the cultivation period in both aquaponics and hydroponics grown plants, but decreased in all plants during the rest of the cultivation period (Figure 3). Photosynthetic activities were higher in hydroponics grown plants at the beginning of the cultivation period compared to plants in the aquaponics system. However, the activities of aquaponics grown plants increased towards the end of the cultivation period (Figure 3). These results indicate that the application of aquaponics systems with LED lighting increases the possibility of more stable plant growth and biomass during the growing period. This could be an advantage that enhances the use of aquaponics systems in vertical farming and in urban environments. In greenhouses without LED lighting, the system stabilizes towards the end of the growing period, indicating the sensitivity of aquaponics-grown plants to the effect of light conditions and its variability.

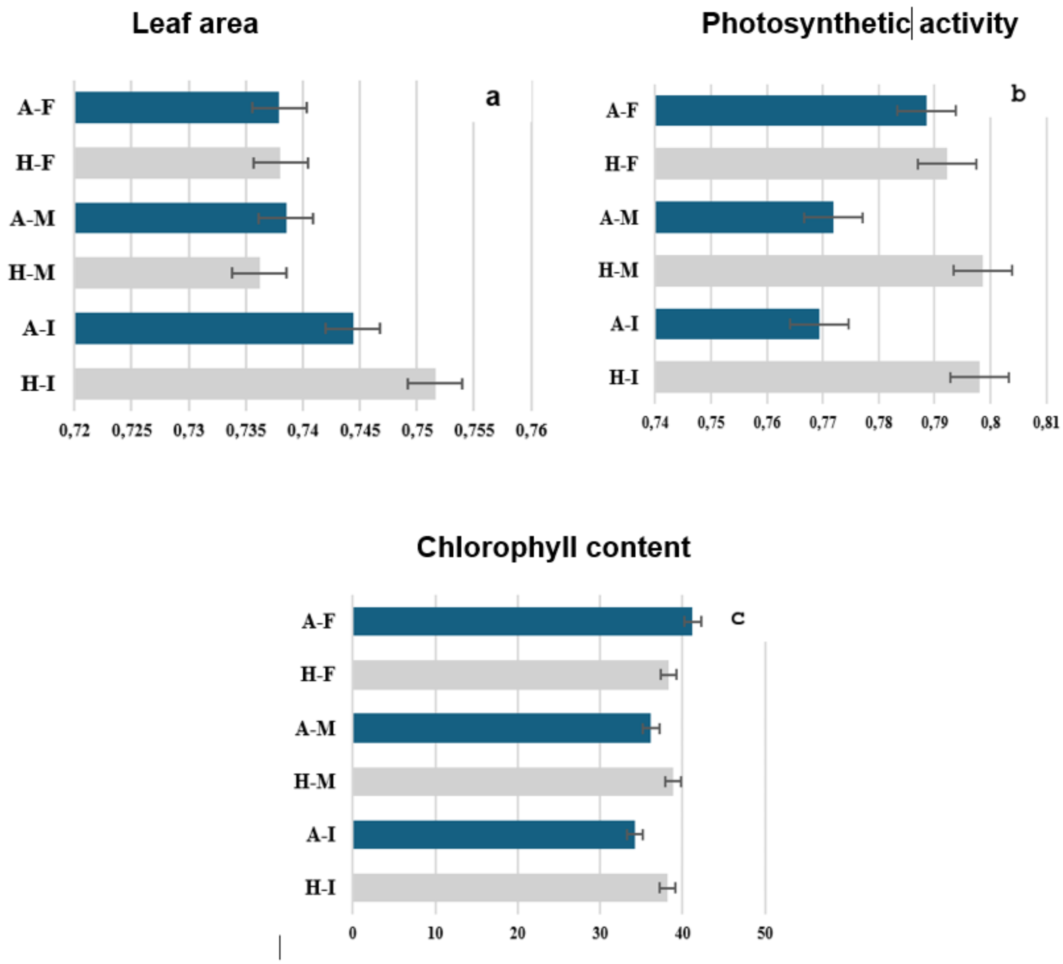

Figure 3. Relationship between (a) leaf area, (b) photosynthesis activity and (c) chlorophyll content during a five-week cultivation period in aquaponic (A) and in hydroponic (H) systems in greenhouses with normal light conditions. Measurements were made at the beginning of the cultivation period (I), in the middle (M) and at the end (F).

d. Nutrient content

The crucial part in the nutrient content and dynamic is the nitrogen content and nitrification process. In all our experiments, the time needed for the nitrification process to start is between 3-4 weeks. Monitoring and measurements of the levels of nitrate, nitrite and ammonium indicated fluctuations in these contents throughout the cultivation period at both greenhouse and biotron conditions (Figure 4). These results advocate for the need of monitoring system to maintain the nitrogen conditions in the system.

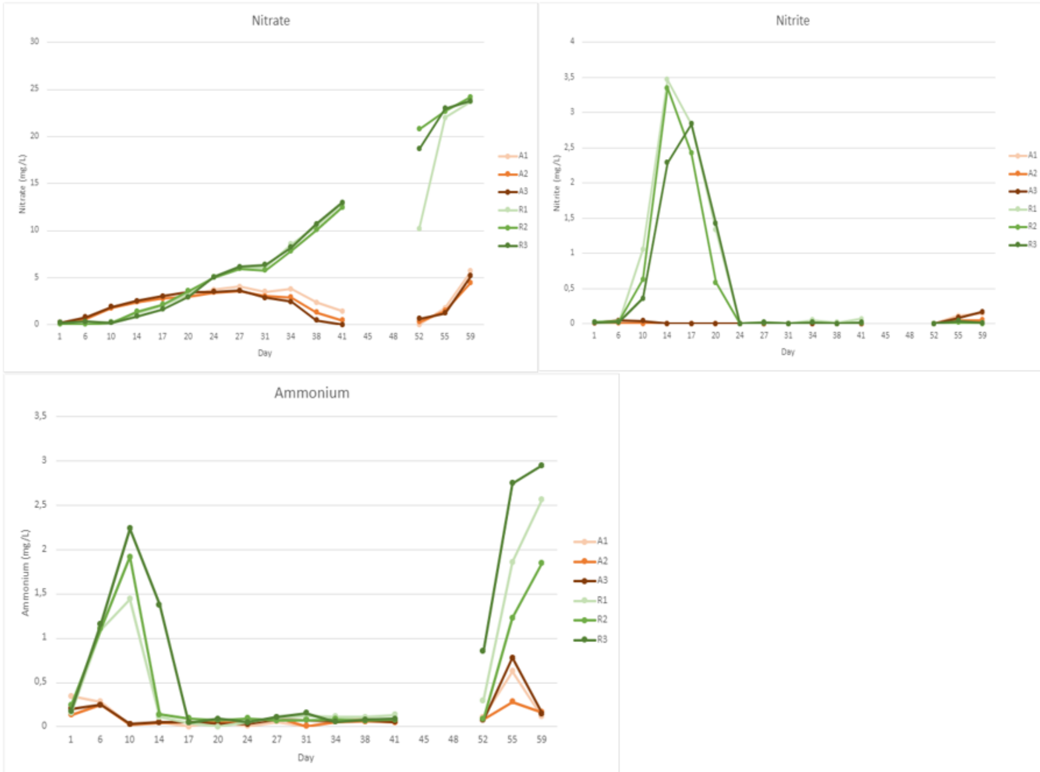

Figure 4. The content in mg /l of nitrate, nitrite and ammonium in aquaponic (A) system compare to recirculated aquaculture system RAS (R) in the fish water in greenhouse experiment of Tatsoi using commercial fish feed.

e. <u>Fertilization with 100% fish water</u>

The nutrients from fish water can also be used directly as organic fertilizer for plants. In a greenhouse experiment, we investigated the fertilization effect of fish water on plant growth of tatsoi and tomato plants. The plants were grown in pots with peat as a substrate and irrigated with nutrient solution from fish water in the proportions of 100%, 75%, 50% and 25%. The mixture was carried out with standard nutrient solution for hydroponic cultivation which was also used as a control. Analyses with AI showed that fertilization with 100% fish water reduced the chlorophyll content in both tatsoi (Figure 5) and tomato plants (Figure 6). Chlorophyll content was highest in tatsoi plants fertilized with 75% nutrients from fish water. In tomato plants, chlorophyll content was highest in plants fertilized with 75%, 50% and 25% fish nutrition at the end of the growing period (Figure 6). No differences were identified in leaf area or photosynthetic activity between tatsoi or tomato plants.

This may indicate high nutrient levels in 100% fish water which the plants cannot absorb and thus affect chlorophyll content and photosynthesis. The use of 75% fish water for tatsoi seedlings was best to achieve an optimal chlorophyll content, however higher dilutions of fish water cannot be applied. However, in the case of tomato seedlings the use of 75%, 50% and 25% fish water can be used to achieve an optimal chlorophyll content.

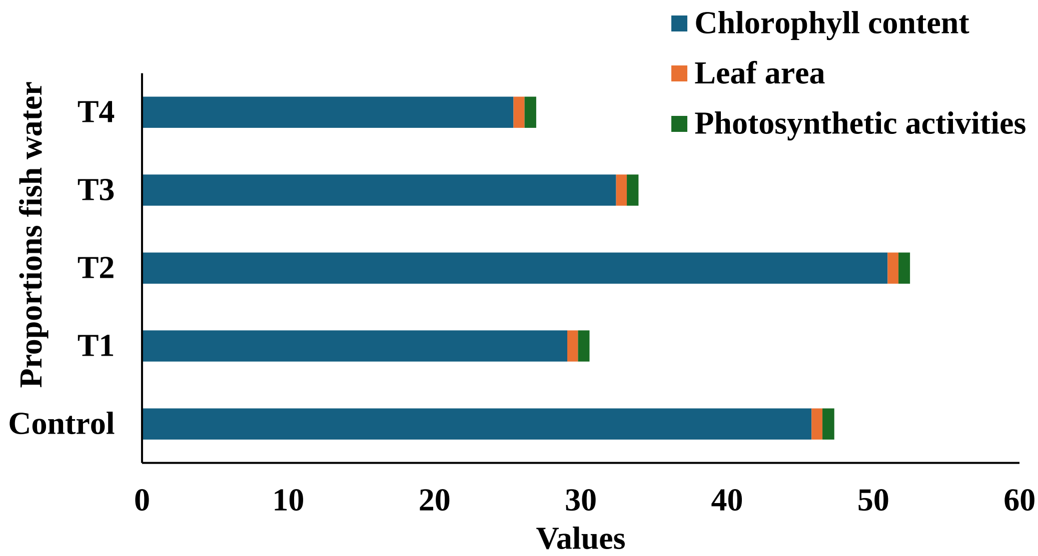

Figure 5. Measurements of chlorophyll content, leaf area, photosynthetic activities of tatsoi plants grown in peat and fertilized with nutrients from fish water in proportions T1= 100% fish water, T2= 75% fish water, T3= 50% fish water, T4= 25% fish water. The fish water was proportioned with standard nutrient solution for hydroponic cultivation that was used as a control.

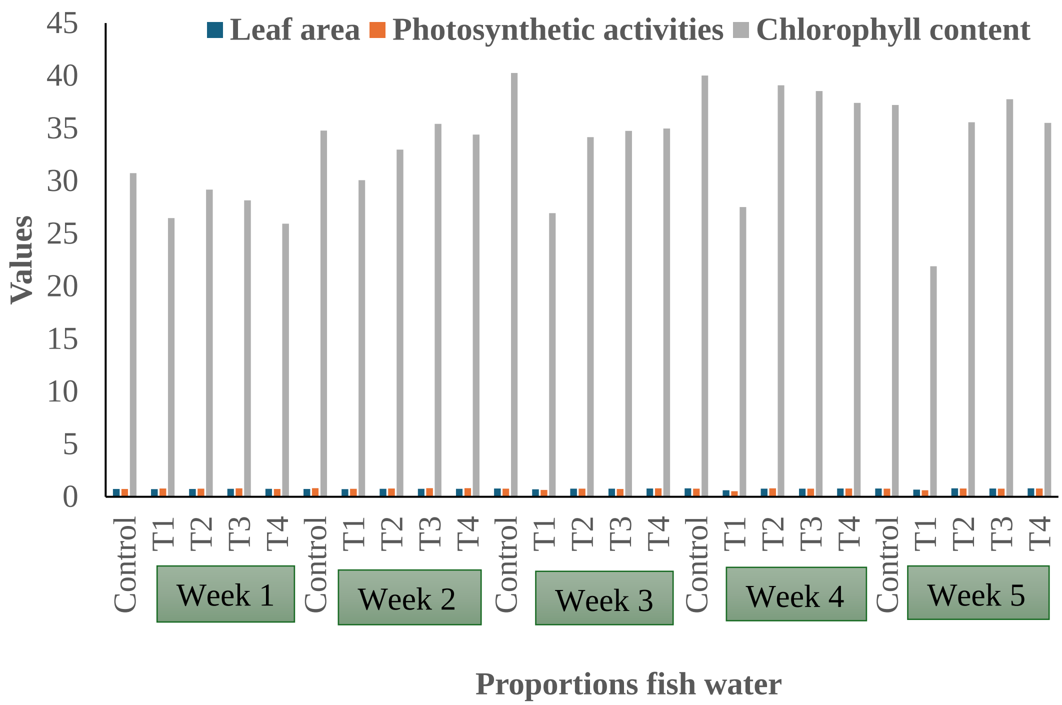

Figure 5. Measurements of chlorophyll content, leaf area, photosynthetic activities of tomato plants grown in peat and fertilized with nutrients from fish water in proportions T1= 100% fish water, T2= 75% fish water, T3= 50% fish water, T4= 25% fish water. The fish water was proportioned with standard nutrient solution for hydroponic cultivation that was used as a control.

f. Microbial content

The occurrence of human pathogens included in the bacterial family *Entrobacteriacea* could be indicated during the cultivation period in the fish tanks as a source of these pathogens (Table 1) in the greenhouse experiments with aquaponic using normal light or fish water as fertilizers. Indication of these pathogens were alos indicated when altervative feed with mussel meal was used. However, no detection of these pathogens was indicated in LED light experiments. This might indicate that the growth conditions under LED light have a reduction effect towards theses pathogens. The content of the nitrification bacteria enhanced during the cultivation period indicated the efficiency of the biofiltration process. On the other hand, the occurrence of plant pathogens related to the fungal group Oomycetes could be indicated in the control system with hydroponic and systems using fertilization with fish water but not in aquaponic system.

Table 1. *The occurrence of root pathogens (Oomycetes), nitrification bacteria and human pathogens (Entrobacteriacae) in aquaponics (A) and hydroponics systems (H). The samples were collected at three different occasions (O) beginning (O1), middle (O2) and end (O3) of the experiment duration. The symbols + or - indicates positive and negative occurrence respectively.*

	Oomycetes		Nitrification bacteria		Entrobacteriacea	
	A	H	A	H	A	H
Biotron With LED light	-	-	´+	-	-	-
Greenhouse	+	+	´+	-	+	-
Mussel feed	-	+	´+	-	+	-
Fish fertilizers	+	+	´+	+	+	-

7- Plant and fish growth

In all our experiments, the total productivity of plants was significantly higher in aquaponics compared to that in hydroponics. However, this was performed with Tatsoi plants as a leafy vegetable crop. Aquaponic needs to be optimized for the production of other vegetables and crops that strengthen the food security aspects.

8- Fish feed

Mussel meal as alternative fish feed has been applied in the current project with good results on plan growth and nutrient dynamic. However, the microbial aspects are of concern for further investigation