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Effect of Inactivation kinetics of Bacteria on Water Quality in the Aquaponic System

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THE AIM of the present study was to determine the food safety status and the effectiveness of ultraviolet treatment as a food safety intervention in reducing the microbial loads of the water system in a model aquaponic unit by using ultraviolet (UV) sterilizer. The experimental system was designed, manufactured and implemented at the laboratory of the Department of agricultural Engineering, Faculty of Agriculture, Kafrelsheikh University, Kafrelsheikh Governorate, Egypt during the 2021 season. Water samples were collected during the production period of Nile Tilapia from ponds and microbial analysis included the total bacteria count, total coliform, the prevalence of total bacteria counts, and coliforms in the systems in triplicates. A significant increase was observed in microbial counts over the trial period, in the absence of ultraviolet (UV) treatment. So, UV sterilization significantly reduced the total bacteria counts and coliform counts when compared with the absence of ultraviolet (UV) samples. The regression analysis showed relationships between Q (ID) and its impact on log total bacteria and log total coliform and Log I when the height of the lamp equals 10, 20, and 30 cm. The estimation coefficient (R^2) was the highest at the height of the lamp 20 cm between Q (ID) and Log I (D) for total coliform and it was 0.9696, whereas, the p-values were lower than the level of significance (0.05) for all parameters. The statistical analysis indicated a statistically significant variation for all parameters.

Keywords: Aquaculture, Aquaponics, Coliform, Food Safety, Product safety.

Introduction

Human population growth around the world has driven to investigation of modern agricultural systems to meet the expanding request for food *(Molden 2007)*. In 2022 and for the first time in history, aquaculture passed capture fisheries as the main producer of aquatic animals. Universal aquaculture production arrived at an unprecedented 130.9 million tonnes, of which 94.4 million tonnes are aquatic animals, 51 percent of the total aquatic animal production *(FAO. 2024)*. For illustration, systems that coordinate plants with fish production are perceived as environmentally friendly and sustainable *(Rakocy et al. 2006)*. Aquaponics includes combining fish and soilless plant production, either in a two-loop (decoupled) or a single-process loop (coupled) design *(Goddek et al. 2019; Love et al. 2015 and Maucieri et al., 2018)*. The features of aquaponics over recirculating aquaculture systems (RASs) and hydroponic systems incorporate the capability of breeding fish, while simultaneously growing consumable plants, which remove nutrients from the water *(Tyson et al. 2011)*. Hence, vegetable planting no longer requires fertilization, and fish cultures do not need water changes as habitually. This change permits fish, cultivated crops, and microorganisms to form mutually advantageous symbiosis and concordant coexistence of environmental balance relationships. It is a working mode of sustainable healthy food production *(Azad et al. 2016)*. In aquaponics, water quality was better in terms of lower contents of ammonia, nitrite, and nitrate compared to RAS. And then, plants in aquaponics improved fish growth due to better water quality. Plants grow equally well in aquaponics as in hydroponics and alter the microbial communities of rainbow trout in aquaponics *(Atique, 2023)*. In the face of soil pollution, dry season, and climate alteration, aquaponic systems have attracted expanding attention due to their resource savings, high efficiency, and low utilization, and they have become the drift and direction of modern agricultural improvement *(Mchunu et al. 2018)*. Hence, the system allows for the continuous production of quality fish and vegetables while limiting water replacement. In expansion, there is a

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reduced requirement for formulated fertilizers *(Wongkiew et al. 2017).* Aquaponics is an integrated system for crop and fish production, operating according to the concept of a circular economy for food production. It combines fish farming and aquaculture, and the two systems are linked by water recycling and diluted nutrients. The rationale for integrated aquaculture systems is to take advantage of the shared resources between plant production and aquaculture, such as nutrients and water, to develop and achieve economically viable and environmentally sustainable primary production practices. The metabolism of fish and uneaten feed enriches the water with essential nutrients for plant growth. The nitrogen cycle is dominant in aquaculture, which involves the conversion of ammonia produced by fish into nitrate, a useful nitrogen source for plants, by bacteria through the process of nitrification. In this way, plants, fish, and bacteria coexist in a balanced system. Converting waste into resources makes aquaculture a promising and environmentally friendly technology that, under certain conditions, may allow for economic benefits compared to conventional production systems *(Tsoumalakou et al., 2022; Palm, K. and Kotzen, 2023).* An aquaculture system is an ecosystem consisting of fish, plants, and bacteria that includes both autotrophic and heterotrophic microbes. These bacteria are essential to maintaining an aquatic ecosystem *(Blancheton et al., 2013; Eck et al., 2019 Schmautz et al., 2017).* Successful aquaculture depends on the complex microbial ecosystem it contains. Much appreciated in this microbial ecosystem, the mineralization of nutrients required for plant production and biological water cleaning are given. However, whereas a few species of these microorganisms in the system are useful, others may be harmful to human health. Aquaponics – a coordinated combination of the recirculation aquaculture system (RAS) and soilless organic cultivating – is gaining the consideration of scientists, entrepreneurs, producers, and consumers. It is an imperative and possibly sustainable method for producing environmentally friendly organic food near consumers *(Vermeulen and Kamstra 2013).* Bacteria play a greatly important role in the optimal advancement of species in aquaponics *(Alderman 2015).* Pathogenic bacteria can be added to the food supply chain at the pre-harvest, harvest, dispersion, and capacity phases of production *(FAO/WHO 2008; Moriarty et al. 2018; Mori and Smith 2019).* Microbes perform the important role of fundamental biological filtration of water to provide the required nutrients for plant growth Therefore, microbes in aquaponics may affect the system performance, water quality, and the growth and quality of the plants and fish *(Kasozi et al., 2021).* Although good agricultural practices and rigorous post-harvest cleansing, foodborne illness outbreaks of E. coli O157:H7 from fresh produce have happened in many agricultural production systems *(Mori and Smith 2019)*. In common, different bacteria and coliforms exist throughout aquaponics systems *(Rakocy et al. 2006)*. Be that as it may, the microbial safety concerns have been alleviated in part by the results of studies that show a lower risk of microbial contamination of the products from aquaponics systems, as compared to products grown in soil-based systems *(Fox et al. 2012; Mori and Smith 2019).* Keeping up an optimal water temperature of 22 − 24 °C, pH in the range of 5.6 − 7.3 and DO of 3 − 10 mg/L for tilapia and crops is a compromise between the needs of plants and fish. The other levels of resilience are alkalinity of 50 − 250 mg/L, CO2 of 0 − 30 mg/L, hardness of 50 − 350 mg/L, salinity of 0 −10 ppt, nitrite concentrations of 0 − 0.8 mg/L *(Nelson, 2008).* Ultraviolet- C (UV-C) disinfection is a physical strategy that plays an important role in water treatment *(Chevrefils et al. 2006).* In aquaculture facilities, this technology is used for the prevention of bacterial, viral, and fungal illnesses *(Kasai et al. 2002; and Gullian et al. 2012).* This technique's efficiency relies on the processed water's UV-C transmittance. The UV-C transmittance is adversely affected by strong assimilation by dissolved organic matter (DOM) and diffusing by suspended solids *(Gullian et al. 2012; U.S. Environmental Protection Agency 2006).* Metabolic products in aquaponic microbial communities play a crucial role in different molecular processes. These processes incorporate the transformation of nitrogenous compounds, the consumption of organic matter, the mineralization of complex organic molecules *(Timmons and Ebeling 2010)*, the utilization of dissolved oxygen, the renewal of water alkalinity, the consumption and the production of carbon dioxide *(Ebeling et al. 2006).* These processes are critical, as they all directly affect plant improvement and the welfare of the fish grown in such systems. Microbes transform fish metabolites into compounds that plants utilize for their growth *(Schmautz et al. 2017)*, and in this way, they are fundamental for the proper working of the system *(Somerville et al. 2014).* Vegetables grown in aquaponics systems are for the most part consumed. The World Health Organization (WHO) and the Food and Agriculture Organization (FAO) have prioritized minimizing microbial contamination dangers of leafy vegetables *(FAO/WHO 2008)*. So, the overall objective of this research is to study the effectiveness of ultraviolet treatment as a food safety intervention in reducing the microbial loads of the water system in a model aquaponic system by using an ultraviolet (UV) sterilizer. The main concept of the unit is to be easy to use, making it accessible to all users the recirculation aquaculture system, and can get it installed smoothly without

requiring additional knowledge of this system. Furthermore, to obtain food safety and Reduce colon diseases, which can help with maximizing growth and health for humans.

Materials and Methods

Ultraviolet (UV) sterilizer unit

Two units were made from local materials (Galvanized sheet). Each unit was designed in the form of a cuboid cross-section. Its dimensions are 63 * 56 * 15 cm. It has several holes with a diameter of 5 cm for lamps at three heights of 10, 20, and 30 cm, and holes of outlet water at four heights of 2.5, 5, 7.5, and 10 cm with a diameter of 1 cm, all these at four times as 2-, 4-, 6- and 10-min. Fig. 1 shows a photograph of the structure of the unit, and Fig. 2 shows a schematic diagram isometric of the unit.

The UV Water is a robust unit used for the disinfection of water. Disinfection of the water takes place when the water flows past the built-in UV Lamp. There are various models for different flow rates, all easy to install and made to the highest quality with a stainless-steel housing and external control box with monitoring capabilities.

The UV lamp (Philips Lightning IBRS 10461 – 5600VB NL ' TL' 20 W/ 52) was used for the sterilizer of water. It blows on three heights 10, 20, and 30 cm from the water surface within the unit. Fig. 3 shows a photograph of the UV lamp.

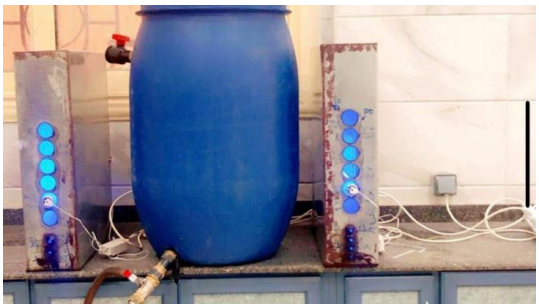

Fig. 1. A photograph the structure of the unit.

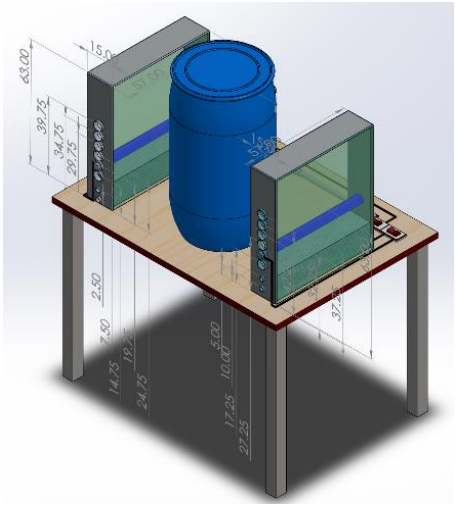

Fig. 2. A schematic of the diagram isometric for the structure of the unit.

Fig. 3: shows a photograph of the UV lamp (Philips Lightning).

Water samples

Water samples were collected during the production period of Nile Tilapia from ponds of the Faculty of Aquatic and Fisheries Science, Kafrelsheikh University, and microbial analysis was conducted for the total bacteria count and total coliform and the prevalence of total bacteria counts, coliforms in the systems in triplicates. Sampling analysis has been conducted in KafrEl-Sheikh Company for water and wastewater - Central Laboratory for Drinking water.

Experiment variables and procedures

The experimental system was designed, manufactured, and implemented at the laboratory of the Department of Agricultural Engineering, Faculty of Agriculture, Kafrelsheikh University, Kafrelsheikh Governorate, Egypt. The experimental work of the present study was carried out during the period from July 2021 to October 2021. Study experimental variables are presented in Table 1.

Table 1. Study experimental variables

Height of lamp (HL, cm)	Height of water (HW, cm)	Exposure time (t, min)
10	2.5	2, 4, 6 and 10 min
	5	2, 4, 6 and 10 min
	7.5	2, 4, 6 and 10 min
	10	2, 4, 6 and 10 min
20	2.5	2, 4, 6 and 10 min
	5	2, 4, 6 and 10 min
	7.5	2, 4, 6 and 10 min
	10	2, 4, 6 and 10 min
30	2.5	2, 4, 6 and 10 min
	5	2, 4, 6 and 10 min
	7.5	2, 4, 6 and 10 min
	10	2, 4, 6 and 10 min

Instrumentation and Measurements

Water Quality Parameters including temperature, dissolved oxygen, pH, ammonia, and salinity (Ec) were mainly measured for water used. The total bacteria count and total coliform bacteria were measured in the water before and after treating it with an ultraviolet (UV) sterilizer.

Water quality

The water quality parameters were recorded throughout the experiment by laboratory devices and they were as shown in Table 2.

Table 2. Water quality parameters (mean±SD) for water used.

Parameter	value
Temperature (C)	19±2
pH	7.1±0.5
Dissolved Oxygen (mg/L)	5.2±0.3
Salinity salinity (Ec), ppm	600±5
Total Ammonia (mg/L)	0.43±0.05

Microbiological Analysis

The sample was taken after each transaction and saved in an icebox to save the samples, then transported into the laboratory to conduct the microbial analysis, total number of bacteria count/ml (T. B. CFU/ml), and total coliform count / 100 ml (T. C. CFU/100 ml).

Inactivation Kinetics

Linear regression was used to obtain the time-based (kt, s) inactivation constants by linearly fitting the log inactivation, log (I), to the UV exposure time as shown in Equation (1). Time-based inactivation was the most appropriate calculation for the full-scale UV-LED sterilizer as the dose was difficult to determine directly ***(Jarvis, et, al. 2019).***

$$\textbf{Log (I) = log } (N_0 / N_t) = k_t \times t \quad \text{..... equ (1)}$$

Whereas log (I) is the log inactivation of total bacteria (PFU/mL) and total coliform (PFU/100 mL), the N_0 is the initial concentration, N_t is the concentration after a specific UV exposure time, k_t (s^{-1}) is the time-based inactivation constant and t, is time (s).

In this study the flow of water was used as an expression of time, therefore N_Q is the concentration after a specific UV exposure time, k_Q (s/L) is the flow of water-based inactivation constant and Q is the flow of water (L/s).

$$\textbf{Log (I) = log } (N_0 / N_Q) = k_Q \times Q \text{ ..... equ (2)}$$

Statistical Analysis

The bench-scale experiments were conducted as factorial experiments. All experiments were duplicated independently with three method replicates for each sample. Further statistical analyses were completed using SPSS® Statistics 25 (IBM, Portsmouth, UK) and it was used for linear regression and to calculate standard deviations. The significance of the effect of ultraviolet (UV) sterilizer on the inactivation kinetic efficiency was determined using Welch's one-way analysis of variance (ANOVA) ($p < 0.05$).

Results and Discussion

To benchmark the performance of the UV-LED sterilizer, the flow water rate-based inactivation kinetic efficiency of the bench-scale sterilizer was considered. The UV lamp was used as a water sterilizer, it has fixed wavelength light emitted from it. This performance was then benchmarked at three different heights 10, 20, and 30 cm from the water surface in a UV sterilizer on total bacteria count (CFU/ml) and total coliform (CFU/100 ml). Through comparison of the flow water rate -based performance of the bench-scale UV-LED sterilizer for the different heights UV on in unit sterilizer.

Effect of height of lamp on total bacteria count (CFU/ml) and total coliform (CFU/100 ml):

When the lamp height was 10 cm as shown in Figure 4a, it is observed that both the count total of bacteria and the count of coliform increase with increasing the water flow rate. This means that the rate of passage of water with the constant height of the lamp affects the count of bacteria after they pass through the sterilizer, so the relationship between them is direct. The lowest count of total bacteria and count of total coliform was 24 CFU/ml and 5 CFU/100 ml, respectively, at a consumption of 0.2 L/min, and the largest count of total bacteria and coliform bacteria were 415 CFU/ml and 50 CFU/100 ml, respectively, at a consumption of 4.2 L/min.

When the lamp height was 20 cm as shown in Figure 4b, it is observed the same trend that observed at 10 cm, the total count of bacteria and the count of coliform bacteria increased with the increase in the water flow rate. This means that the rate of passage of water with the constant height of the lamp affects the count of bacteria after they pass through the sterilizer, so the relationship between them is direct. The lowest count of total bacteria and count of total coliform were 20 CFU/ml and 7 CFU/100 ml respectively, at a consumption of 0.2 L/min, and the largest count of total bacteria and coliform bacteria were 215 CFU/ml and 54 CFU/100 ml respectively, at a consumption of 4.2 L/min.

When the lamp height is 30 cm as shown in Figure 4c, it is observed that the total count of bacteria and the count of coliform bacteria increase with the increase in the water flow rate. This means that the rate of passage of water with the constant height of the lamp affects the count of bacteria after they pass through the sterilizer, so the relationship between them is direct. The lowest count of total bacteria and count of total coliform were 120 CFU/ml and 28 CFU/100 ml respectively, at a consumption of 0.2 L/min, and the largest count of total bacteria and coliform bacteria were 305 CFU/ml and 62 CFU/100 ml respectively, at a consumption of 4.2 L/min.

Effect of UV-LED Sterilizer for all Parameters under Study

Figure 5 shows the total bacterial count (CFU / ml) and the total coliform count (CFU/100 ml) for all study parameters such as time (min), H.L (cm), and H.W (cm). The highest value of number of total bacteria count was 415 CFU / ml at the parameter of study 2 min, 10 cm, and 10 cm for t, H.W, and H.L, respectively compared to the value of the total bacterial count which was 450 CFU/ml before treatment using the sterilization unit. The lowest value of the rate of change percentage for total

bacterial was 20 CFU / ml at the parameter of study 10 min, 2.5 cm, and 20 cm for t, H.W, and H.L, respectively. The highest value of the total coliform count was 62 (CFU/100 ml) at the parameter of study 2 min, 10 cm, and 30 cm for t, HW, and H.L, respectively compared to the value of the total bacterial count which was 63 CFU / 100 ml before treatment using the sterilization unit. The lowest value of the total number of the coliform count was 5 (CFU/100 ml) at the parameter of study 10 min, 2.5 cm, and 20 cm for t, H.W, and H.L, respectively.

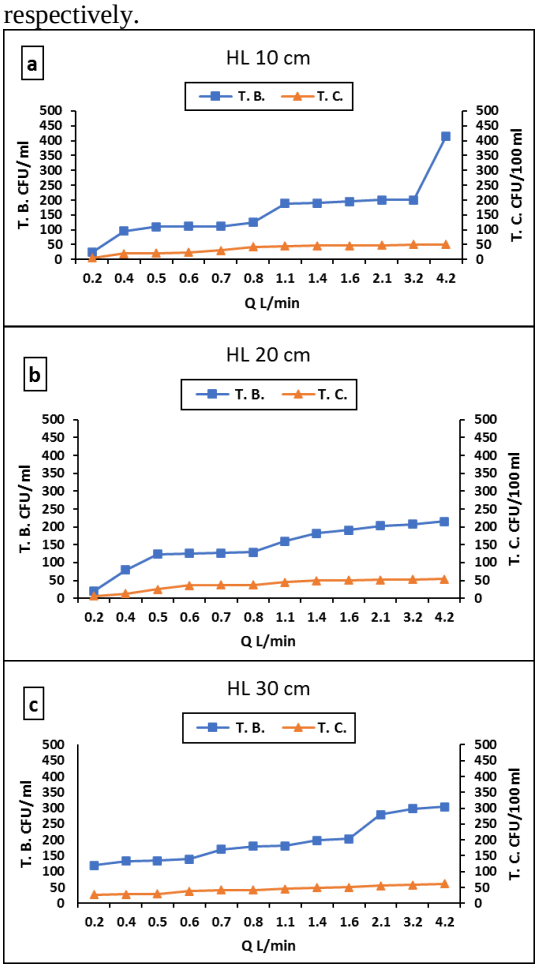

Fig. 4. Total count of bacteria (CFU/ml) and total count of coliform (CFU/100 ml) for water flow rates at different heights of the lamp 10, 20, and 30 cm.

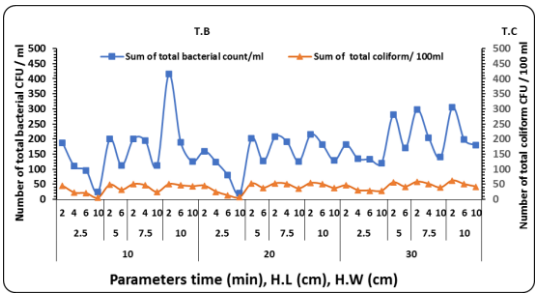

Fig. 5. The total bacteria count (CFU / ml) and the count of coliform (CFU/100 ml) for all study parameters.

Effect of Inactivation Kinetics of total bacteria and total coliform at different water flow rates

The regression analysis showed a logarithmic function for all relationships between Q (ID) and its impact on log total bacteria and log total coliform when the height of the lamp equals 10, 20, and 30 cm. The regression analysis showed a power function for all relationships between Q (ID) and Log I when the height of the lamp equals 10, 20, and 30 cm as shown in Table 3.

The regression analysis at the height of lamp10 cm of log total bacteria between Q (ID) and log T. B. (D) and Log I (D) was shown in Table 3, Figure 6, and Figure 7. The estimation coefficient (R^2) for Q (ID) with log T. B. (D) and Log I (D) were equal to (0.835 and 0.9273), respectively, whereas the p-values were equal to (0.00 and 0.00) which were lower than the level of significance (0.05) for Q (ID) with log T. B. (D) and Log I (D).

The regression analysis at the height of lamp10 cm of log total bacteria among Q (ID) and log T. C. (D) and Log I (D) is shown in Table 3, Figure 8, and Figure 9. The estimation coefficient (R2) for Q (ID) with log T. B. (D) and Log I (D) were equal to 0.73 and 0.9619, respectively, while the p-values were equal to 0.00 and 0.00 which were lower than the level of significance (0.05) for Q (ID) with log T. C. (D) and Log I (D), respectively.

Table 3. Regression analysis for Q (ID) and its impact on total bacteria, total coliform, and K_Q when the height of the lamp equals 10, 20, and 30 cm.

Path Analysis	M ± SD	β_0	β_1	R^2
$HL = 10\ cm$				
Q → Log T. B (D)	2.14 ± 0.30	2.14	0.306	0.835
Q → Log (I) (D)	0.513 ± 0.296	0.512	(-0.305)	0.9273
Q → Log T. C (D)	1.49 ± 0.29	1.492	0.279	0.73
Q → Log (I) (D)	0.31 ± 0.289	0.308	0.279	0.9619
$HL = 20\ cm$				
Q → Log T. B (D)	2.11 ± 0.28	2.11	0.273	0.7244
Q → Log (I) (D)	0.54 ± 0.28	0.542	(-0.271)	0.8726
Q → Log T. C (D)	1.53 ± 0.28	1.527	0.269	0.7549
Q → Log (I) (D)	0.28 ± 0.28	0.273	(-0.269)	0.9696
$HL = 30\ cm$				
Q → Log T. B (D)	2.27 ± 0.14	2.271	0.152	0.9343
Q → Log (I) (D)	0.38 ± 0.14	0.383	(-0.151)	0.832
Q → Log T. C (D)	1.63 ± 0.12	1.633	0.128	0.9203
Q → Log (I) (D)	0.17 ± 0.12	0.167	(-0.128)	0.7379

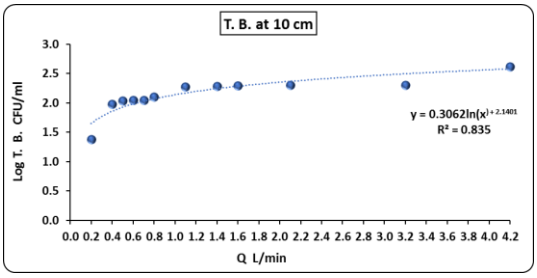

Fig. 6. Regression analysis for Q (ID) and its impact on log total bacteria (Log T. B. CFU/ ml) when the height of the lamp is equal to 10 cm.

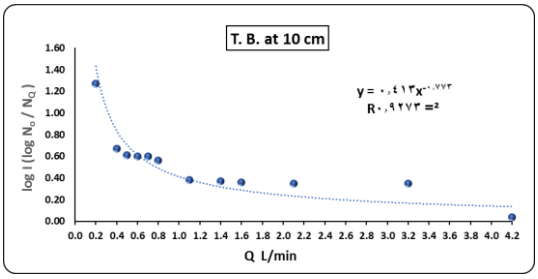

Fig. 7. Regression analysis for Q (ID) and its impact on log I (log N_O/N_Q) when the height of the lamp is equal to 10 cm.

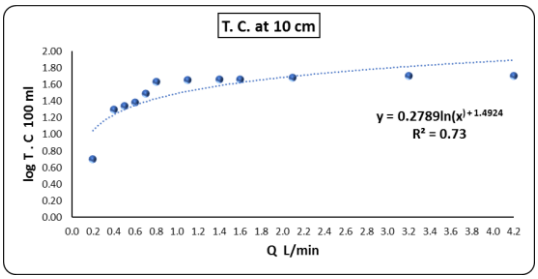

Fig. 8. Regression analysis for Q (ID) and its impact on log total coliform (Log T. C. CFU/ 100 ml) when the height of the lamp is equal to 10 cm.

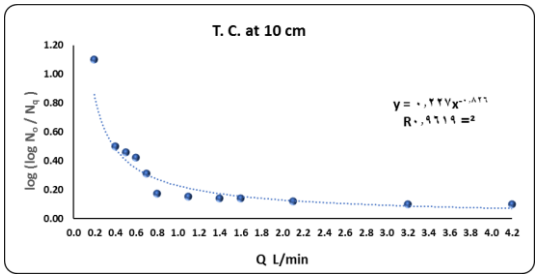

Fig. 9. Regression analysis for Q (ID) and its impact on log I (log N_O/N_Q) when the height of the lamp is equal to 10 cm.

The regression analysis at the height of lamp 20 cm of log total bacteria among Q (ID) and log T. B. (D) and Log I (D) was shown in Table 3, Figure 10, and Figure 11. The estimation coefficient (R^2) for Q (ID) with log T. B. (D) and Log I (D) were equal to 0.7244 and 0.8726, respectively, however, the p-values were equal to 0.00 and 0.00 which were lower than the level of significance (0.05) for Q (ID) with log T. B. (D) and Log I (D), respectively.

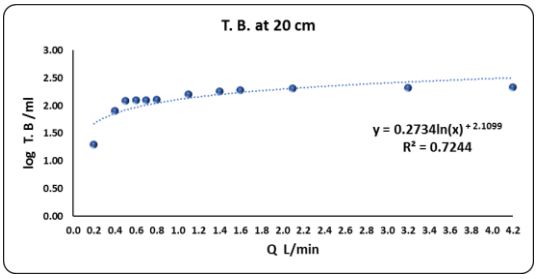

Fig. 10. Regression analysis for Q (ID) and its impact on log total bacteria (Log T. B. CFU/ ml) when the height of the lamp is equal to 20 cm.

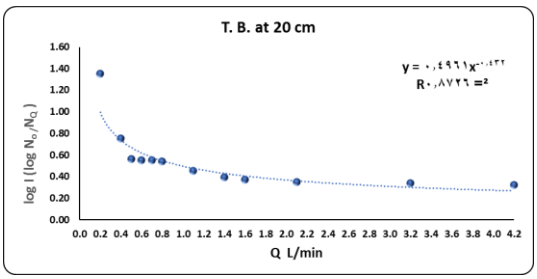

Fig. 11. Regression analysis for Q (ID) and its impact on log I (log N_O/N_Q) when the height of lamp equal to 20 cm.

The regression analysis at height of lamp 20 cm of log total bacteria among Q (ID) and log T. C. (D) and Log I (D) was presented in Table 3, Figure 12 and Figure 13. The estimation coefficient (R^2) for Q (ID) with log T. C. (D) and Log I (D) were equal to (0.7549 and 0.9696), respectively, but the p-values were equal to (0.00 and 0.00) which were lower than the level of significance (0.05) for Q (ID) with log T. C. (D) and Log I (D), respectively.

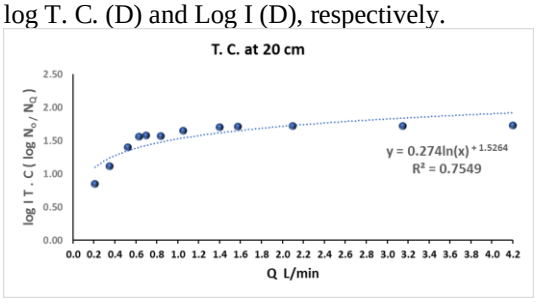

Fig. 12. Regression analysis for Q (ID) and its impact on log total coliform (Log T. C. CFU/ 100 ml) when the height of the lamp is equal to 20 cm.

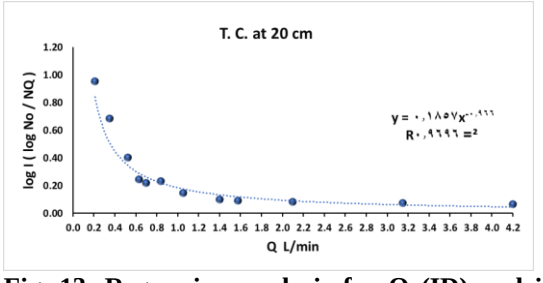

Fig. 13. Regression analysis for Q (ID) and its impact on log I (log N_O/N_Q) when the height of the lamp is equal to 20 cm.

The regression analysis at the height of lamp 30 cm of log total bacteria among Q (ID) and log T. B. (D) and Log I (D) was presented in Table 3, Figure 14, and Figure 15. The estimation coefficient (R^2) for Q (ID) with log T. B. (D) and Log I (D) were 0.9343 and 0.832, respectively, while the p-values were equal to 0.00 and 0.00 which were lower than the level of significance (0.05) for the log total bacteria.

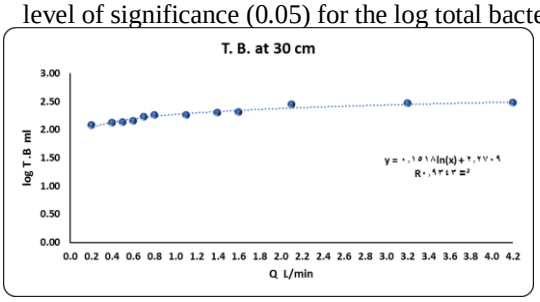

Fig. 14. Regression analysis for Q (ID) and its impact on log total bacteria (Log T. B. CFU/ ml) when the height of the lamp equal to 30 cm.

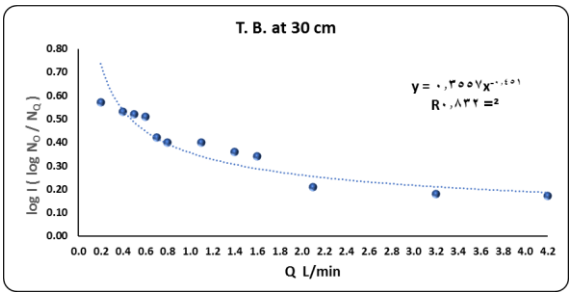

Fig. 15. Regression analysis for Q (ID) and its impact on log I (log N_O/N_Q) when the height of the lamp is equal to 30 cm.

Data presented in Table 3, Figure 16, and Figure 17 cleared the regression analysis at the height of lamp 30 cm of log total bacteria among Q (ID) and log T. C. (D) and Log I (D) as shown in. The estimation coefficient (R^2) for Q (ID) with log T. B. (D) and Log I (D) were 0.9203 and 0.7379, respectively, however, the p-values were 0.00 which were lower than the level of significance (0.05) for the log total coliform.

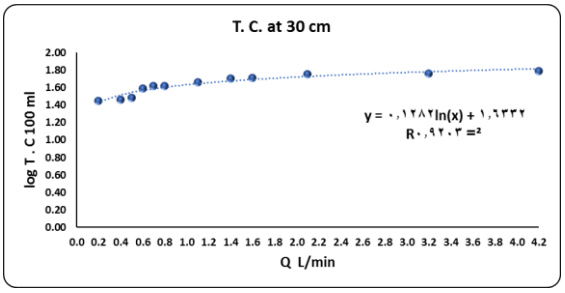

Fig. 16. Regression analysis for Q (ID) and its impact on log total coliform (Log T. C. CFU/ 100 ml) when the height of the lamp is equal to 30 cm.

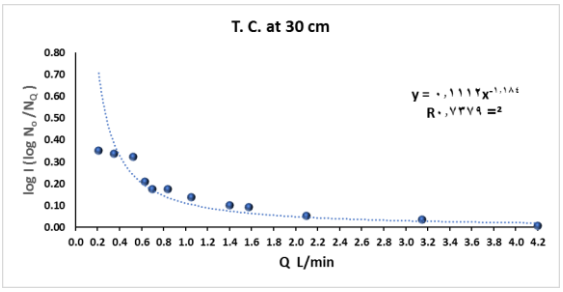

Fig. 17. Regression analysis for Q (ID) and its impact on log I (log N_O/N_Q) when the height of lamp equal to 30 cm.

Conclusion

This study showed that the model used, which is a sterilizer that used ultraviolet rays to reduce the microbial load in aquaponic water, at heights of 10, 20, and 30 cm. The lower height (10 cm) was better than all treatments before use. Through a homemade experimental unit, it is hoped that this unit will contribute to the development of standard experimental procedures and validation protocols for UV water disinfection. Finally, the simple concept and modular materials available make it accessible to everyone and any farmer can use it to purify water, reduce harmful microbial load, achieve a good healthy pattern of plants grown and produced from aquaponics and thus obtain healthy and safe food. However, it is recommended that more prototypes be studied and developed for larger scale under commercial use and be suitable for different sizes of LED UV sterilizers suitable for large aquaponic units.

Consent for publication:

All authors declare their consent for publication.

Author contribution:

The manuscript was edited and revised by all authors.

Conflicts of Interest:

The author declares no conflict of interest.

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