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Effect of the Probiotic (*Bacillus* spp.) on Water Quality, Production Performance, Microbial Profile, and Food Safety of the Nile Tilapia and Mint in Recirculating Aquaponic System

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ABSTRACT

A 12-week study was conducted to investigate the effects of the addition of probiotic (*Bacillus* spp.) on water quality, production performance, microbial profile, and food quality of the Nile tilapia (*Oreochromis niloticus*) and mint (*Mentha Spicata)* in a combined aquaponic system. The Nile tilapia fingerlings with an initial body weight of 10g were stocked at 80 fish/$m^{3.}$ Each hydroponic system was composed of 36 mint seedlings with an initial length of 3.65 ± 0.09 cm. Two treatments with three replicates were designed: the 1st with probiotic addition (W/ Pro) and the 2nd without probiotic addition (W/O Pro). Increased dissolved oxygen concentration was observed in the control group (5.43 ± 0.07 mg/l). NH_3 and NO_2 were significantly lower in W/ Pro treatment (0.03 ± 0.00 and 0.46 ± 0.01 mg/l) than the control (0.04 ± 0.00 and 0.51 ± 0.02 mg/l). Final body weight, weight gain, average daily weight gain, and specific growth rate (38.14± 1.41, 28.15 ± 1.41. 0.33 ± 0.01, and 2.34 ± 0.11, respectively) were significantly improved with the W/ Pro treatment. The survival rate% was higher in W/ Pro treatment without significant differences. A better feed conversion ratio was obtained in the W/ Pro treatment (1.41 ± 0.05) compared to (1.65 ± 0.05) in the W/O Pro systems. Plant fresh weight and length at harvest were better in W/Pro, while moisture content was significantly higher in W/O Pro ones. Adding probiotics increased the total bacterial counts in fish, water and mint. Bacteria that are considered a potential risk of seafood-borne infection including *Bacillus cereus* were only identified in fish of the control. Similarly, pathogenic *Aeromonas hydrophila* and *Acinetobacter baumannii* were associated with water and mint from the control. Adding probiotics promoted the growth of beneficial lactic acid bacteria, *Bacillus lecheniformis,* prevented the pathogenic ones and did not affect the presence of *Rahnella aquatilis* bacteria.

INTRODUCTION

The agricultural industry has discovered novel practices to produce local and sustainable food in the possible safest way. Aquaponics system reduces start-up, operating, and infrastructure costs of the aquaculture and horticulture sides by merging the two systems. Additionally, aquaponics reduces water usage and waste discharge to the environment **(Tyson *et al.*, 2011)**. Aquaponics is the symbiotic relationship between aquaculture, horticulture, and microorganisms, either in a coupled or decoupled system

(Rackocy, 2012). In coupled system, water returns to the fish tank following plant irrigation; while in de-coupled system, irrigated water does not return to the fish tank **(Goddek *et al.*, 2015)**. In either situation, aquaponics allow for maximum water and nutrient uptake efficiencies by minimizing the waste of nitrogen resources **(Tyson *et al.*, 2011; Rakocy, 2012; Somerville *et al.*, 2014; Goddek *et al.*, 2015)**. Freshwater fish excretions including nutrients through their gills, urine, and feces are considered essential for plant growth and development. These nutrients are either soluble or bound to solid organic compounds. Soluble nutrients are dissolved in water in an ionic form, which allows plants to uptake them easily **(Goddek *et al.*, 2015)**.

In the last period, the number of small-scale aquaponics facilities has increased, as they create an additional source of income for aquaculture farmers **(Rakocy, 2012; Abarike *et al.*, 2018)**. Recently, commercial aquaponic units have an increased concern for food safety. There is a need for the optimization of commercial-sized aquaponics including operation, productivity, and food safety to discover more sustainable methods of producing safe food and disseminate such promising system.

Probiotics such as *Bacillus* spp. improve aquaculture in many ways. Probiotics have a sporulation capacity that makes them survive in harsh environmental conditions and are considered non-pathogenic and non-toxic (**Chen *et al.* 2017)**. They can produce antimicrobial substances and fight pathogens and fish diseases through several techniques **(Amin *et al.*, 2015; Kuebutornye *et al.*, 2019)**. Such techniques include the suppression of virulence gene expression, competition for adhesion sites, production of lytic enzymes, production of bacteriocins, and production of organic acids **(Kuebutornye *et al.*, 2020)**. Additionally, probiotics are generally recognized as safe (GRAS) for human and animal consumption by the Food and Drug Administration **(Olmos *et al.*, 2020)**.

The objective of this study was to investigate the effects of adding probiotic (*Bacillus* spp.) to a medium-scale aquaponic system (the Nile tilapia and mint) on water quality, fish performance, plant performance, fish and plant composition, microbial growth, microbial community, and the food safety of the associated mint.

MATERIALS AND METHODS

1. Study area and experimental system

The study was conducted in the aquaponics unit affiliated with the Faculty of Fish Resources, Suez University, Suez, Egypt. The study lasted from August to November 2021. Six aquaponics systems were involved; each unit consisted of a 1000L plastic fish tank. A settling tank with a volume of 220L was used for mechanical filtration. Another (220L) plastic tank filled with plastic media bed (bioball) with a specific surface area of $600m^2/ m^3$ was used for biological filtration. Deep-water hydroponic culture unit was 500L, with floating rafts of Styrofoam sheets with holes, fitted by net pots (36 in each unit).

Water exiting from the fish tank was fed by gravity to the mechanical filter and biological filter and then to the hydroponic unit. Water discharge from hydroponic unit was returned to the fish tank through a 120L sump tank, supplied with a submersible pump (VA-MA model Q1CS-400B1, 400 watts*)*. Net pots were filled with gravel for supporting plant roots. Each fish tank was fitted with water and air inlets. The hydroponic unit and biological filter were also aerated through air diffusers from the main air line (air pipes connected to the main blower). Three blowers (Vortex® gas pump1.5 Hp) were used to aerate the whole system; the blowers were working interchangeably.

2. Experimental design

The experimental design consisted of two treatments: 1) with probiotic addition (W/ Pro.) in which the commercial probiotic (Sanolife® Pro-W; $5x10^{10}$ CFU g^{-1}) was added to the culture water every week at a concentration of 100ppm. 2) represents the control without probiotic (W/O Pro.). Each experimental treatment had three randomly assigned replicates.

3. Experimental management

3.1. Fish culture

The Nile tilapia (*Oreochromis niloticus*) fingerlings with an initial body weight of 10g were obtained from a commercial hatchery located in Ismailia Governorate, Egypt. A total of 80 fish were stocked in each fish tank (1000 L) Fish were fed with commercial floating pellets (Grand aqua®, Egypt) containing 30% of crude protein. The daily feeding rate was 1.5 % of the total biomass. Daily feed was distributed at three feedings (09:00 am, 12:00 pm, and 15:00 pm). The feed amount was adjusted every two weeks after fish sampling.

3.2. Hydroponic system

Mint (*Mentha spicata)* seedlings with an initial length of 3.65 ± 0.09 cm were introduced from a private plantation facility in the Suez Governorate, Egypt. Seedlings were involved in the system, while roots were able to access nutrients through the floating rafts. Roots were submerged in the water using net pots. Plants were individually weighed and randomly planted into Styrofoam sheets floating rafts. Each aquaponic unit was implanted with 36 plants, with a spacing distance of 15cm between plants. Nitrate levels were 10mg/ l at the beginning of the experiment, while the dissolved oxygen was above 5.0mg/ l in the deep-water hydroponic unit. Iron in its chelated form (6% Fe-EDDHA) was supplemented to all experimental systems at an application rate of 2mg/ l every two weeks.

3.3. Probiotic addition

Aquaponics units assigned to the probiotic treatment received 100mg/ l of a commercial probiotic (Sanolife PRO-W, INVE Technologies®, Belgium) once a week till the end of the experiment. The calculated amount of probiotics was dissolved in the system water and then added into the sump tank for each unit.

4. measurements

4.1. Water quality

Dissolved oxygen (DO), pH, ammonia (NH_3), nitrite (NO_2), and turbidity were frequently measured and monitored. Water temperature and dissolved oxygen concentration were daily measured using a dissolved oxygen meter (HANNA, HI9146-04). pH was daily measured using a portable pH meter (Milwaukee, MW102). Ammonia (NH_3) and nitrite (NO_2) were measured twice a week using a photometer (HANNA, HI97715, and HI97708, respectively), while turbidity was measured once weekly with a turbidity meter (Lovibond, TB211 IR). The water temperature mean in all experimental tanks during the study period was 26.7 ^{o}C±2.05. There was no water exchange during the experimental period, while clean-aged water was added to all systems to compensate for evaporation.

4.2. Fish performance

Each fish tank was sampled at a 15-day interval to adjust the required feed. All fish were weighed and counted at the end of the experiment for the assessment of growth and feed utilization efficiency. Growth-related parameters were assessed in terms of final weight (FW), final length (FL), condition factor (K) specific growth rate (SGR), weight gain (WG), average daily weight gain (ADWG), total biomass, and biomass increase percentage. Feed utilization efficiency was represented as feed intake (FI), feed conversion ratio (FCR), feed efficiency (FE), and the calculations were conducted according to previous studies **(Tacon *et al*., 2002; Lugert *et al*., 2016; Opiyo *et al*., 2019; Büyükdeveci *et al*., 2022)**.

4.3. Plant performance

Plant performance was measured at the terminal of the experiment. Five plants were randomly selected from each system to be involved in the plant growth assessment. Plant performance was performed based on plant total length (cm), total weight (g), root length (cm), branch length (cm), and several leaves per branch **(Knaus *et al*., 2020; Ogah *et al*., 2020; Kasozi, *et al*., 2021)**. The fresh weight of plants (g) was immediately measured after removing free surface moisture, using an analytical weighing balance (Radwage®) with an accuracy of 0.01g.

4.4. Fish and plant proximate analysis

At the termination of the study period, five fish and five plants from each experimental system were taken for composition analysis. Fish and plant samples were weighed before analysis. Fish and plant samples of each group were homogenized by a mixer and stored at -20^{o}C until analysis. Dry matter was determined for the homogenized plant and fish materials. Chemical methods of analysis were done according to the Association of official analytical chemists **(A.O.A.C., 2000)**. Samples were dried in an oven at 60°C for 24- 36h. Nitrogen was determined by the Kjeldahl method, and crude protein was calculated as N×6.25. Crude lipid content was analyzed following the Soxhlet method, crude fiber by automatic fiber analysis system (FOSS,

Fibertec^{TM} 8000). While ash content was determined by incineration in a muffle furnace at 600°C for 4h.

5. Bacteriological analysis

A total of 15 fish, 100 ml water, and 3 plants (mint) samples were collected separately from each system. Samples were wrapped in sterile bags inside a cool polystyrene box containing packed sterile ice to keep the temperature range at 4- 6°C during transportation **(FDA, 2001)**. Samples were cautiously transported to the laboratories of the Faculty of Fish Resources, Suez University, and analyzed instantly.

5.1 Sample preparation

Fish and plant samples (5 g each) were separately placed in sterile bags and homogenized with 45ml of buffered peptone water (0.1%) (Lab M, UK) for 2min, using a stomacher (Seward Stomacher 400 circulator, UK). While water samples (1 ml) were vortexed for 2min with buffered peptone water (0.1%) in a 1- 10 dilution factor **(FDA, 2001)**.

5.2. Total bacterial count

Serial dilution to tenth folds was done for the total bacterial count. Dilutions up to 10^5 were spread onto plate count agar (PCA, Oxoid, UK). According to **FDA (2001)**, the bacterial count was reported as a log of colony-forming units (log CFU/g) for fish and mint samples and log CFU/ ml for water samples. Experiments were done in duplicates, and results were demonstrated as means ± standard deviations.

Bacterial colonies of diverse morphology were separately selected from plate count agar and overgrew in the Trypticase soy agar slants (TSA, Lab M, UK). Then isolates were biochemically tested with indole (I), Voges-Proskauer (VP), methyl red (MR), and citrate tests **(FDA, 2001)** and confirmed with API 20E strips (BioMérieux, France) **(Al-Harbi & Uddin, 2005; Said & Ahmed, 2022; Thaochan *et al*., 2010)**.

Total coliform was enumerated by pour-platting in violet-red bile agar (Oxoid, UK) with an overlay. Coliform confirmation was done by lactose fermentation and bubble formation in brilliant green lactose bile broth. DeMan Rogosa Sharpe agar (MRS Lab M, UK) plates were used to count lactic acid bacteria by pour-platting technique. MRS was incubated at 30°C for 24h, and spindle-shaped suspected colonies were gram-stained and biochemically identified with I, MR, VP, citrate, catalase, and oxidase tests **(FDA, 2001; Santo *et al*, 2008**; **Dorick *et al*., 2021)**.

5.3 Identification of bacteria by PCR and 16S rDNA gene sequencing

According to **Azwai1 *et al*. (2016)**, DNA extraction was done using a bacterial DNA preparation kit (Jena Bioscience, Thuringia, Germany). Partial 16S rDNA was amplified using the universal oligonucleotides primers forward 5′-GAGTTTGATCCTGGCTTAG-3′ and reverse 5′-GGTTACCTTGTTACGACTT-3′. Briefly, 2µl DNA templates (20 ng/ µl) were added to 12.5µl Master Mix (Qiagen, Hilden, Germany) and 10.5 µl deionized H_2O for a total volume of 25µl. The mixture

was then amplified in a DNA Thermal Cycler (Techne Progene, Marshall Scientific, Hampton, NH) using the following program: one denaturation step at 94°C for 5min; 37 cycles (the 30s at 94ºC, 30s at 51ºC, and 30s at 72ºC), and a final extension for 5min at 72ºC. Agarose gel (1.5%) with Tris-acetate-EDTA (1X, TAE) buffer was used for gel electrophoresis.

5.4 DNA sequencing

Purification of the PCR products was performed using QIAquick Kit (Qiagen, Hilden, Germany). The second PCR was performed using the BigDye Terminator v3.1 cycle sequencing kit. Each reaction (a total of 20µl) contained a terminator-ready reaction mix (8µl), primer (3.2pmol); DNA template quantized according to the PCR product size and deionized water. The thermal profile for Cycle Sequencing PCR was 1min at 96ºC; 25ºC cycles as follows: 10s at 96ºC, 5s at 50ºC, and 4min at 60ºC. After an additional step of purification with CENTRI-SEP Columns (Princeton Separations, Freehold, NJ), DNA sequencing was carried out by 3500 Genetic Analyzer (Applied Biosystems, Massachusetts, USA). The obtained consensus sequences were subjected to NCBI- Blast search through the Mega program (7.0.20). The genetic sequences were sent to NIH-GenBank of the National Library of Medicine database with recorded accession numbers.

6. Statistical analysis

Data were statistically analyzed using IBM SPSS Statistics version 25 (IBM Corporation, NY, USA). An independent sample t-test was used to analyze fish and plant growth performance, fish and plant proximate composition, and bacterial counts. Water quality parameters were compared by two-way repeated-measures ANOVA, with the treatment (with or without probiotic addition) as the main factor and sampling date as the repeated measures factor. Results were expressed as the mean ± SD. Mean differences were compared by Duncan's multiple-range tests. A probability value (*P*) of less than 0.05 was used to indicate statistically significant differences.

RESULTS AND DISCUSSION

1. Water quality

Dissolved oxygen concentration was above 5mg/ l in all experimental units. Significantly ($P < 0.05$) higher dissolved oxygen concentration was observed in the control group (5.43 ± 0.07mg/ l), compared to 5.08 ± 0.06mg/ l in the units with probiotic addition (Table 1).

Table 1. Water quality parameters of aquaponic systems (the Nile tilapia and mint) for a 12-week study with and without probiotic (*Bacillus* spp.) addition

Parameter	W/ Pro.	W/O Pro	Sig.
DO (mg/l)	5.08 ± 0.06	5.43 ± 0.07	0.001
NH_3 (mg/l)	0.035 ± 0.00	0.045 ± 0.00	0.01
NO_2(mg/l)	0.46 ± 0.01	0.51 ± 0.02	0.11
pH	7.16 ± 0.03	7.45 ± 0.02	0.001
Turbidity (NTU)	9.51 ± 0.27	4.9 ± 0.10	0.001

Probability value (*P*) of less than 0.05 was used to indicate statistically significant differences.

Both NH_3 and NO_2 were significantly (P <0.05) lower in the probiotic group (0.03 ± 0.00 and 0.46 ± 0.01mg/ l, respectively) than (0.04 ± 0.00 and 0.51 ± 0.02mg/ l) in the control units. Additionally, the pH level was also influenced significantly by the addition of the *Bacillus*; a lower pH level was recorded in the probiotic-treated units (7.16 ± 0.03) compared to (7.45 ± 0.02) the control systems. A turbidity concentration of 9.51 ± 0.27 NTU was found in the probiotic units, which was significantly higher than the control group (4.9 ± 0.104 NTU).

Water variables NH_3, NO_2, pH, and dissolved oxygen were all maintained within acceptable ranges in both treated and control units as reported for aquaponics **(Somerville *et al*., 2014; Yanes, *et al*., 2020)**. These concentrations allow tilapia fish to grow without bad effects on their health. The superiority of some water quality parameters (NH_3, NO_2, and pH) in the probiotic-treated units agrees with those recorded in the studies of **El-Haroun *et al*. (2006)** and **Elsabagh *et al*. (2018)**, who tested the effect of adding commercial probiotics made from *Bacillus licheniformis* and *B. subtilis* to the diet of the Nile tilapia *Oreochromis niloticus* L. on a 17- week culture. **Lalloo *et al*. (2007)** tested the effect of several strains of *Bacillus* isolated from *Cyprinus carpio* in improving water quality in ornamental fish culture. Their results revealed that three out of nine isolates resulted in lowering ammonia, nitrate, and phosphate concentrations at rates of 74%, 76%, and 72%, respectively. In contrast, **Queiroz and Boyd (1998)** applied a commercial probiotic in catfish (*Ictalurus punctatus*) diets and recorded a significant increase in the survival rate and net fish production in the probiotic-treated fish and detected no significant differences in the water quality parameters such as ammonia, nitrate, and dissolved oxygen between the treated and control treatments. In addition, **Taoka *et al*. (2006)** tested the commercial probiotics including cultures of bacteria and yeast on the survival of Japanese flounder *Paralichthys olivaceus* and water quality in a closed system for 50 days and noted that, water quality parameters were reduced in

probiotics-treated tanks compared to control tanks. Additionally, **Li *et al*. (2006)** studied the effect of a commercial probiotic made from *Bacillus* sp., *Saccharomyces cerevisiae*, *Nitrosomonas* sp., and *Nitrobacter* sp. and recorded that, the probiotic could reduce the levels of inorganic nitrogen from 3.74 to 1.79mg l^{-1} and phosphate from 0.1105 to 0.0364mg l^{-1}. The better water quality recorded as a result of the addition of probiotic strains, especially of the Gram-positive genus *Bacillus* may be due to the efficiency of this bacterial group compared to the Gram-negative in transforming organic matter to CO_2 **(Balcázar *et al*., 2006)**.

2. Fish performance

Growth performance, survival rate, and feed utilization parameters are presented in Table (2).

Table 2. Growth performance, survival rate and feed utilization of the Nile tilapia reared in aquaponic systems after a 12- week rearing period with and without probiotic (*Bacillus* spp.) addition

Parameter	W/ Pro.	W/O Pro	Sig.
Initial weight (g)	10 ± 0.00	10 ± 0.00	1
Final body weight (g)	38.14 ± 1.41	29.76 ± 1.53	0.001
Final Length	10.49 ± 0.18	9.75 ± 0.30	0.049
Condition Factor	3.31 ± 0.09	3.27 ± 0.16	0.824
Weight Gain	28.15 ± 1.41	19.76 ± 1.53	0.001
ADG (g /day)	0.33 ± 0.01	0.23 ± 0.01	0.001
SGR %	1.58 ± 0.04	1.28 ± 0.05	0.001
Biomass (Kg)	3.04 ± 0.29	2.28 ± 0.23	0.120
Biomass increase %	506.05 ± 49.99	380.79 ± 40.11	0.122
Survival rate%	99. 4 ± 0.57	96.6 ± 2.56	0.346
Feed intake	4.3 ± 0.49	3.75 ± 0.28	0.385
Feed Conversion Ratio	1.41 ± 0.05	1.65 ± 0.05	0.037
Feed Efficiency	0.71 ± 0.02	0.60 ± 0.02	0.038

Probability value (*P*) of less than 0.05 was used to indicate statistically significant differences.

Final body weight, weight gain, average daily weight gain and specific growth rate (38.14 ± 1.41, 28.15 ± 1.41, 0.33 ± 0.01 and 1.58 ± 0.04, respectively) were all significantly higher ($P < 0.05$) in tilapia groups reread with the addition of probiotic than those of the control groups (29.76 ± 1.53, 19.76 ± 1.53, 0.23 ± 0.01 and 1.28 ± 0.05, respectively). No significant difference was recorded in the condition factor between the two experimental treatments. Both total biomass and biomass increase percentages did not significantly differ between the experimental groups. The survival rate% was numerically higher in the probiotic-treated systems though without significant difference.

A significantly better feed conversion ratio was obtained in the probiotic treatment (1.41 ± 0.05) when compared to (1.65 ± 0.053) in the control system. Feed efficiency was also significantly higher in the probiotic-added group (0.71 ± 0.02 vs 0.60 ± 0.02).

Higher growth performance and feed utilization efficiency maintained in the probiotic groups agree with previous studies which demonstrated that probiotic administration has beneficial effects on fish growth performance, feed utilization, and survival **(Nayak, 2010)**. Many probiotics have been provided to enhance growth performance. For example, the probiotic *E. faecium* increased the final body weight and average daily weight gain of Nile tilapia **(Wang *et al*., 2008)**. Probiotics composed of *Bacillus amyloliquefaciens* also enhanced the growth, and feed conversion of Nile tilapia **(Ridha and Azad, 2012)**. The commercial product (Organic Green™) *B. pumilus* improved the growth rate of Nile tilapia (**Aly *et al*., 2008a**). The growth enhancement of Nile tilapia has been recorded in tilapia which fed dietary *B. subtilis*-based probiotics **(Kuebutornye *et al*., 2020; Tachibana *et al*., 2021; Ghalwash *et al*., 2022)**.

The higher feed utilization efficiency may be due to the beneficial effect of probiotics on intestinal microbiota that can produce proteolytic, amylolytic, cellulolytic, and lipolytic enzymes, which are important for more efficient digestion and absorption of nutrients **(Gutowska *et al*., 2004)**. **Kasozia *et al*. (2021)** reported that fish treated with the *Bacillus* had higher activity of the digestive enzymes α-amylase, alkaline protease, and alkaline phosphatase than in the control. Previous studies have also revealed that *Bacillus spp.* have exo-enzymatic activities which are very effective in digesting a large variety of carbohydrates, lipids, and proteins, which allows higher growth performance and feed utilization efficiency **(Wu *et al*., 2012; Purwandari and Chen, 2013)**. *Bacillus* species also can produce endospores that can exist in unfavorable environmental conditions **(Nayak, 2010; James *et al*., 2021)**; this can allow them for long-term storage and good survival in the gastrointestinal tract **(Hong *et al*., 2005)**. The improvement of fish growth performance by probiotics may be due to the improved gastrointestinal morphological changes in the epithelium **(Ghalwash *et al*., 2022)**. **Hai *et al*. (2015)** also reported that probiotics may enhance fish appetite and lead to enhanced growth and better feed utilization efficiency **(Del'Duca, *et al*., 2013)**.

The higher survival rates achieved in the probiotic treatment are in agreement with **Villamil *et al*. (2014)** who recorded an increased survival rate of Nile tilapia during a challenge with *A. hydrophila P. fluorescens* when fed diets supplemented with *L. acidophilus* probiotic. Also, **Aly *et al*. (2008b)** recorded that a higher survival rate and body weight gain of Nile tilapia were obtained after feeding for one to two months with a probiotic, either *B. subtilis* or *L. acidophilus* compared to the control.

3. Plant Performance

Plant fresh weight at harvest was significantly ($p < 0.05$) higher in the probiotic-added systems (67.00 ± 7.46 g *vs* 46.51 ± 5.80 g). Significantly higher plant length was also obtained in the probiotic-treated group (88.80 ± 3.32 cm) as compared to (72.41 ± 5.40 cm) in the control system. Insignificant differences were recorded in branch length and root length between the two experimental groups (**Table 3**).

Table 3. Mint growth performance in aquaponic systems (Nile tilapia and mint) after a 12-week rearing period with and without probiotic (*Bacillus* spp.) addition.

Item	W/ Pro.	W/O Pro	**Sig.**
Total length (cm)	88.80 ± 3.32	72.41 ± 5.40	0.01
Total weight (g)	67.00 ± 7.46	46.51 ± 5.80	0.03
Root length (cm)	50.07 ± 1.83	49.27 ± 4.52	0.87
Branch length (cm)	31.40 ± 2.05	27.60 ± 1.07	0.11

Probability value (*p*) of less than 0.05 was used to indicate statistically significant differences

Mint growth performance was significantly higher in the probiotic treatment in the forms of plant weight, length, and number of branches. Better plant performance might be due to the nutrient levels in the probiotic treatments. *Bacillus* spp. was reported as a plant growth promoter and can protect plants from pathogens by mechanisms related to induced systemic resistance **(García *et al*., 2004, Martínez-Viveros *et al*., 2010, Niazi *et al*., 2014, Medina *et al*., 2016)**.

Similar beneficial results of probiotic addition for the plants in the aquaponic system were obtained by **Kasozi *et al*. (2021)** in lettuce. Sprout fresh weight at harvest was 23.8% higher in plants from the probiotic treatment than in the control. Also, the dry weight of the lettuce sprout from the probiotic (*Bacillus*) treatment was 38.2% higher than the control group. **da Silva Cerozi *et al*. (2016)** reported enhanced lettuce plant growth and phosphorus accumulation in aquaponics units treated with *Bacillus*. In addition, *Bacillus* may affect factors that enhance root growth, leading to better nutrient uptake.

4. Fish and plant proximate analysis

The approximate chemical analysis of fish and plant (mint) is shown in **(Table 4)**.

Table 4. Proximate body composition of Nile tilapia and mint produced from aquaponic systems after a 12-week rearing period with and without probiotic (*Bacillus* spp.) addition.

Tilapia	W/ Pro.	W/O Pro	**Sig.**
Moisture %	80.58 ± 0.34	81.27 ± 0.31	0.14
Protein %	13.40 ± 0.19	12.79 ± 0.14	0.01
Lipids %	4.12 ± 0.04	4.10 ± 0.05	0.82
Ash %	2.1 ± 0.08	2.1 ± 0.07	1.0
Mint			
Moisture %	79.03 ± 0.32	80.31 ± 0.22	0.01
Protein %	10.09 ± 0.26	9.05 ± 0.18	0.01
Lipids %	2.27 ± 0.02	2.29 ± 0.03	0.47
Ash %	6.94 ± 0.11	6.54 ± 0.16	0.06
Fiber %	4.57 ± 0.06	4.67 ± 0.05	0.23

Probability value (*p*) of less than 0.05 was used to indicate statistically significant differences

Regarding fish body composition, significantly higher crude protein (13.40 ± 0.19) content was found in the probiotic group. No significant differences ($p > 0.05$) were noted in moisture, lipid, and ash contents between the two treatments.

As for plant proximate composition, significantly higher protein content was reported in the probiotic treatment compared to the control treatment 10.09 ± 0.26 *vs* 9.05 ± 0.18. Significantly higher moisture content was reported in the control group. No significant differences were found neither in the lipid nor in ash and fiber contents of the plants.

Results showed significantly higher protein contents of tilapia reared in aquaponic systems supplied with probiotics when compared to control systems. **Bagheri *et al*. (2008**) reported an increase in the level of protein in *O. niloticus* and *O. mykiss* fed on probiotic-supplemented diets. Similarly, **Reda and Selim (2015)** noted higher protein contents in fish treated with probiotics compared to the control. They also reported that whole-body moisture and ash contents were not affected by the supplementation of *B. amyloliquefaciens* to the diets of Nile tilapia (*Oreochromis niloticus)*. **Opiyo *et al*. (2019)** also reported that *B. subtilis* supplemented diets led to significantly higher protein content

in the Nile tilapia body compared to the control ($P < 0.05$). In contrast, other studies documented that probiotic addition had no significant effect on lipid, protein or ash content **(Merrifield *et al*., 2010; Hassan *et al*., 2018; Kasozia *et al*., 2021)**.

The increased protein content may result from increased nutrient deposition. Also, this might be due to higher proteins secreted by the probiotics in the gastrointestinal tract of Nile tilapia and the more efficient conversion of ingested food into structural protein used in building muscles **(Mehrabi *et al*., 2012; Lara-Flores, & Olvera-Novoa, 2013)**.

5. Bacterial assessment

Total bacterial count in fish, water and plants in aquaponic systems supplemented with probiotics was about one log or higher than samples collected from control (tanks without probiotics). The bacterial load in fish from both probiotic and control tanks did not exceed the maximum level of the Egyptian Organization for Standardization and Quality Control (2005) of fresh chilled fish (< 10^6 CFU/g). While coliform bacteria were associated only with water and plant in control tanks. Lactic acid bacteria (LAB) were recorded in this study with fish and water from tanks supplemented with probiotics **(Table 5).**

Table 5. Total bacteria, coliform, lactic acid bacteria counts and identified bacteria using API 20E isolated from aquaponic systems after a 12-week rearing period with (W/pro) and without probiotic (W/O pro) addition.

	W/O Pro				W/ Pro			
	Total bacteria count	**Total Coliform count**	**Lactic acid bacteria count**	**Identified bacteria**	**Total bacteria count**	**Total Coliform count**	**Lactic acid bacteria count**	**Identified bacteria**
Fish	2.17 ± 0.42	< 1	< 1	-*Brucella spp.* - *Erwinia spp.*	2.93 ± 0.21	< 1	1.2 ± 0.41	*Proteus mirabilis*
Water	2.4 ± 0.01	2.4 ± 0.21	< 1	*Aeromonas hydrophila*	3.9 ± 0.14	< 1	1.5 ± 0.25	*E. coli*
Mint	2.1 ± 0.32	2.5 ± 0.41	< 1	*Aeromonas hydrophila*	2.8 ± 0.30	< 1	< 1	-

Bacterial counts were represented by means ± SD

They are considered beneficial microorganisms due to their abilities to stimulate host gut development, digestive function, mucosal tolerance, stimulate an immune response, and improve disease resistance **(Ringø *et al*., 2018).** LAB are considered potential probiotics, and also showed a broad-spectrum antibacterial activity against aquaculture pathogens such as *Vibrio harveyi*, *V. splendidu* **(Alonso *et al*., 2019).**

Several pathogenic bacteria were identified in control systems. *Brucella spp.* were associated with fish **(Table 5).** It likely came from infected animals' manures that was used as plant fertilizers. Similarly, *Brucella abortus* and *B. suis* have been isolated from surface water and tilapia fish from Volcano Lake in Mexico, as they came from the biological material of the breeding animals common to the region **(Ramos-Ramírez *et al*., 2020).** Besides that, *Erwinia spp.* was identified in this study and was previously documented as heterotrophic bacteria in aquaculture and a possible bacterial pathogen **(Michaud *et al*., 2009).**

Aeromonas hydrophila in this study was identified in water and mint. *Aeromonas hydrophila* is a major waterborne pathogen, isolated from 70% of diseased Nile tilapia fish samples in Egypt **(Saleh *et al*., 2021)**. It poses a food safety concern due to the lack of efficient hurdles to eliminate microbial contaminants from fresh produce. As mint could be used for food consumption without any further heat processing, the occurrence of this bacteria in water raises concern over public health risks that may be associated with the consumption of salad vegetables **(Beuchat, 1996)**. It has been reported in retail ready-to-eat vegetable salads, especially lettuce cause human foodborne illnesses **(Umutoni *et al*., 2020)**.

The addition of *Bacillus spp.* as a probiotic supplement increased the total bacterial load, eliminated coliform and promoted beneficial lactic acid bacteria. It also helped prevent pathogens that developed in control tanks **(Table 5).** Similarly, they were reported in combating diseases against pathogens such as *Aeromonas hydrophila*, *Vibrio spp.* and *Acinetobacter* in the aquaculture industry (**Abriouel *et al*. 2010**; **Santos *et al*. 2018; Olmos *et al*., 2020).** While only non-pathogenic bacteria such as *Proteus mirabilis* and *E. coli* were associated with probiotic treatment. The pathogenicity of *E. coli* was not investigated, and it might exist as a part of the fish commensal waste. *Proteus mirabilis* is considered a fish spoilage bacterium **(Yazgan *et al*., 2019**, therefore good hygienic measures and good post-harvest practices are needed to maintain good fish quality.

Other pathogenic bacteria that are potential causes of fish diseases and foodborne infections were isolated and confirmed by PCR and 16S rDNA gene sequencing. *Rahnella aquatilis* was found in the water of both control and probiotic tanks **(Table 6).**

Table 6. Bacteria were identified using 16S rDNA gene sequencing (NCBI Blast) with their GenBank Accession numbers from aquaponic systems after a 12-week rearing period with (W/pro) and without probiotic (W/O pro) addition.

	W/O Pro		W/ Pro	
	Identified bacteria	**Accession number**	**Identified bacteria**	**Accession number**
Water	*Rahnella aquatilis*	OP546122	*Rahnella aquatilis*	OP546123
	Acinetobacter baumannii	OP548625		
Fish	*Bacillus cereus*	OP546124	*Bacillus lecheniformis*	OP546125
Mint	*Acinetobacter baumannii*	OP548626	-	

It is a rare aquaculture Gram-negative rod-shaped bacterium that causes a fish infection and was first isolated from diseased crucian carp in eastern China **(Lü *et al*., 2017).** Supplemented probiotics did not affect the presence of *Rahnella aquatilis* in the water. Other pathogens such as *Acinetobacter baumannii* were associated with water and mint in control tanks. *Acinetobacter baumannii* is a bacterial pathogen that poses public-health concerns due to its rapidly increasing drug-resistance properties. It emerges from aquaculture as a fish pathogen, which could introduce resistant genes into the food chain **(Xie *et al*., 2020).** The association of this bacterium with mint introduces a food safety concern, as mint is fresh produce usually used without any heat process to control bacterial growth.

Bacillus cereus was confirmed in fish from control tanks. *Bacillus cereus* is one of the most important foodborne pathogenic microorganisms, which causes gastrointestinal and non-gastrointestinal diseases. *B. cereus* was reported in different aquatic food products in China **(Zhang *et al*., 2020).** *B. cereus* is associated with food poisoning, foodborne outbreaks, food spoilage and low-quality food with significantly reduced edibility **(Özdemir and Arslan, 2019).** While beneficial *Bacillus licheniformis* was associated with probiotic tanks. *B. licheniformis* is considered a feed additive worldwide due to the absence of toxigenic potential and its widespread use as a probiotic **(Muras *et al*., 2021).**

CONCLUSION

Probiotic (*Bacillus* spp.*)* addition has many positive effects on the production of Nile Tilapia (*Oreochromis niloticus*) and mint (*Mentha spicata)* in an integrated aquaponic system. Water quality parameters were enhanced in terms of lower NH_3, NO_2, and pH. Tilapia growth performance was significantly improved. Final weight, daily weight gain, weight gain, and specific growth rate% were all significantly higher ($p < 0.05$) with the probiotic addition. Feed utilization increased by about 18% with the addition of (*Bacillus* spp.*).*

Plant final weight was significantly ($p < 0.05$) increased with the probiotic addition. Significantly higher plant length was also obtained in the probiotic-treated group as compared to in the control units. Protein content in fish and plants increased significantly with the probiotic usage. Higher moisture content was reported in the plants and in the control group.

Adding probiotics increased the total bacterial counts associated with fish, water, and mint. Bacteria that are considered a potential risk of seafood-borne infection including *Bacillus cereus* and *Aeromonas hydrophila* were only identified from fish in control tanks and did not exist in tanks with probiotics. Similarly, pathogenic *Acinetobacter baumannii* was associated with control tanks water and mint, while was not identified with probiotics. In addition, probiotics promoted the growth of beneficial lactic acid bacteria, *Bacillus lecheniformis,* and did not affect the presence of *Rahnella aquatilis* bacteria in the water of both treatments.

Generally, probiotics may be involved in the medium-scale aquaponic system (tilapia and mint) with many beneficial effects including water quality, fish performance, plant performance, and fish and plant proximate composition. Besides the benefits which may be gained from the integrated production of the aquaponics, the addition of probiotics could improve the water quality, fish and plant production performance, microbial profile, and food safety of the Nile tilapia and mint.

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