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RESEARCH PAPER

Effect of Water Recirculation Duration and Shading on Lettuce (*Lactuca sativa)* Growth and Leaf Nitrate Content in a Commercial Aquaponic System

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Abstract

Two experiments were conducted to evaluate the effects of three water recirculating duration (daytime (07.00h to18.00h); nighttime (18.00h to 07.00h); and 24 hours control) and shading (30%, 60% and no shading control), respectively, on growth and sap's nitrate content of lettuce in a commercial aquaponic system integrated with red tilapia culture. First experiment showed that water recirculating time did not affect the lettuce growth, thus offering the possibility to increase aquaponics profitability by reducing the operational cost. The second experiment revealed that shading affected nitrate content in the sap of the leaf midribs, but it remained in range specified by EU regulations in all treatments. The highest lettuce growth was observed at the rate of 30% shading whereas 60% shading gave the lowest growth ($P<0.05$).

Keywords: Recirculation duration, aquaponic system, lettuce, nitrate, shading.

Introduction

Unlike hydroponics, which entirely relies on nutrient supplements, aquaponics integrates recirculating aquaculture system (RAS) with hydroponics by using nitrogen-rich fish wastes as an organic fertilizer (Pantanella *et al*., 2010; Blidariu and Grozea, 2011; Love *et al*., 2015). Although commercial aquaponic systems exist, high investment and operation costs of RAS can be a significant constraint for the adoption of this system in developing countries (Rakocy and Bailey, 2003; Martins *et al*., 2010). A higher profitability and reduction of operational costs are needed to make aquaponics an economically viable food production system (Klinger and Naylor, 2012; Goada *et al*., 2015).

Reduction in water recirculation period might be a solution for reducing operational costs. However, a limited research has been conducted to determine how water recirculation time affects plant growth performance, water quality, nutrient removal and the quality and food safety (Cometti *et al*., 2011; Liu and Yang, 2012). Previous studies have shown that nitrate content in cultivated plants is partly determined by culture practices and in some conditions it could reach concentrations deemed hazardous for human consumption. This has become a global concern; for example, the European Union (EU) limits the maximum permissible leaf nitrate concentrations in vegetables to 4,500 mg kg^{-1} NO_3^- fresh weight (FW) for the winter season crops and 2500 mg kg^{-1} NO_3^- fresh weight (FW) for the summer crops (Commission Regulation (EC) No 1881/2006, 2006; Cometti *et al*., 2011; Liu and Yang, 2012). Environmental factors, such as excessive light intensity, have been identified as one of the major factors affecting NO_3^- uptake in vegetables (Liang and Chien, 2013) but limiting light intensity affects the plant growth (Santamaria, 2006) and nutrient uptake.

This paper presents the results of two experiments conducted to evaluate different water recirculation durations and shadings on lettuce growth and nitrate content in the sap. This study also evaluated whether the different culture practices employed in this study maintain the nitrate content of the sap in compliance with food safety regulations.

Materials and Methods

Aquaponic System at Association Réunionnaise pour le Développement De l'Aquaculture (ARDA)

The experiments were conducted in a pilot commercial-scale aquaponics system located in the experimental research station of the Association

Réunionnaise pour le Développement de l'Aquaculture (ARDA), in Étang-Salé-les-Bains, Reunion island (S 21° 17' 7.21", E 55° 22' 41.80"). The aquaponic system was comprised of three components: (1) a fish rearing system with four fish tanks; (2) a filtration system with a mechanical and biological filter; and (3) a hydroponic system with six hydroponic tanks (Figure 1).

Four 5 m^3 tanks were stocked with a hybrid tilapia (*Oreochromis niloticus*, *O. mossambicus* X *O. hornorum* X *O. aureus*; Baroiller and D'Cotta, 2001) at a density of 1000, 1000, 500, and 500 fish per tank, respectively. The initial fish weights in the 4 tanks were 52g, 145g, 168g and 218g, respectively. Initial biomass in all tanks was approximately 500 kg. Fish were fed daily with a commercial pelleted feed containing 34% protein (Nutrima GED 4.5, Reunion, France) using an automatic feeder from 08:00h to 16:00h. Fish tank effluents were allowed to flow by gravity to the filter system, made of two solids removal tanks, eight filtration tanks containing discarded net cages, and one bio-filter tank containing bio-ball (Polymeric, China).

Treated water was pumped to the hydroponic tanks at a rate of 2 $m^3 h^{-1}$. Water level in each tank was kept at 0.3 m in depth maintaining a volume of 6 m^3. Six 3 m^2 Styrofoam floating rafts, each containing 50 lettuce seedlings (16 lettuces m^{-2}) were placed in the hydroponics tanks to cover the whole water surface (except at the inlets and outlets areas, where water samples were collected). Each hydroponic tank had 300 lettuces seedlings. Lettuce plants were approximately 25 cm apart. The seedlings from a locally available variety of lettuce (*Lactuca sativa*) were purchased from a private hydroponic farm in Reunion Island. The plants were three weeks old (four-leaf stage) at the beginning of the experiments. The outflow of hydroponics system was drained to a sump tank via drainage canal and then pumped back to the fish tanks. For the bio-filter to perform well, the system was already in operation 30 days before starting the experiment, as recommended, to enhance bacterial growth (FAO, 2014).

Experimental Design

Both experiments had three treatments including the control. Each treatment was duplicated using a completely random design.

In Experiment 1, three water recirculation duration were tested for 28 days conducted between September to October, 2012), i.e. (i) (T1), daytime water recirculation duration (11 hours from 07:00h to 18:00h); (ii) (T2), nighttime water recirculation duration (13 hours from 18:00h to 07:00h); and (iii) (T3), the control (24 hours water recirculation duration).

In Experiment 2, which was conducted in 2012, three shading rates were tested for 21 days, i.e (i) (S1), 60% shading (ii) (S2), 30% shading; and (iii) (S3) control (no shading). Locally available green agricultural shade nets of 30% and 60% density were used for shading.

Water Quality Sampling and Analysis

In Experiment 1 and 2, water samples were collected in the hydroponic tank's inlets (treated fish tank effluent) and outlets (inside the tank of lettuce at the vicinity of the water outlets). Water temperature, dissolved oxygen (DO Meter ID-100P, Japan), conductivity (Conductivity meter I-1200, USA), pH (HI 9025, Hanna Instruments, USA) and NO_3^- concentration were measured daily at 07:00h and 18:00h. Ammonia nitrogen (NH_3) was determined by using phenate method, nitrite (NO_2^-) by using colorimetric method, nitrate (NO_3^-) by using cadmium reduction method, and orthophosphate (PO_4^{3-}) by using ascorbic acid method (APHA, 1998). The water samples were collected weekly at 08:00h from the inlets and outlets. In addition, in experiment 1, NO_3^- was measured daily at 07:00h and18:00h, and weekly at 08:00h.

Lettuce Growth Measurement

Lettuce height and diameter were measured weekly using a ruler, and the weight was recorded using digital scales. The weight, height and diameter of lettuce in both experiments were measured by randomly sampling 30 plants from each replicate at the end of each experiment.

Data Analysis

Water quality and growth parameters were calculated using the following equations:

Nitrate removal (mg L^{-1}) = NO_3^- concentration of water at the inlet - NO_3^- concentration of the water at the outlet

Percentage of nitrate removal (%) = [NO_3^- concentration of water at the inlet - NO_3^- concentration of the water at the outlet/ NO_3^- concentration of the water at the inlet] x 100

Height: circumference ratio = Diameter/ Height

Time series were subjected to regression and correlation analyses using statistical packages for analyzing the interaction among the treatments. Differences of treatment mean pair were performed using Duncan New Multiple Range Test (DMRT) at P<0.05.

Results and Discussion

Experiment 1

The key water quality parameters of inlet water in Experiment 1 are shown in Table 1. They were found to be satisfactory for growth of lettuce hydroponic plants in all treatments (Rakocy *et al*.,

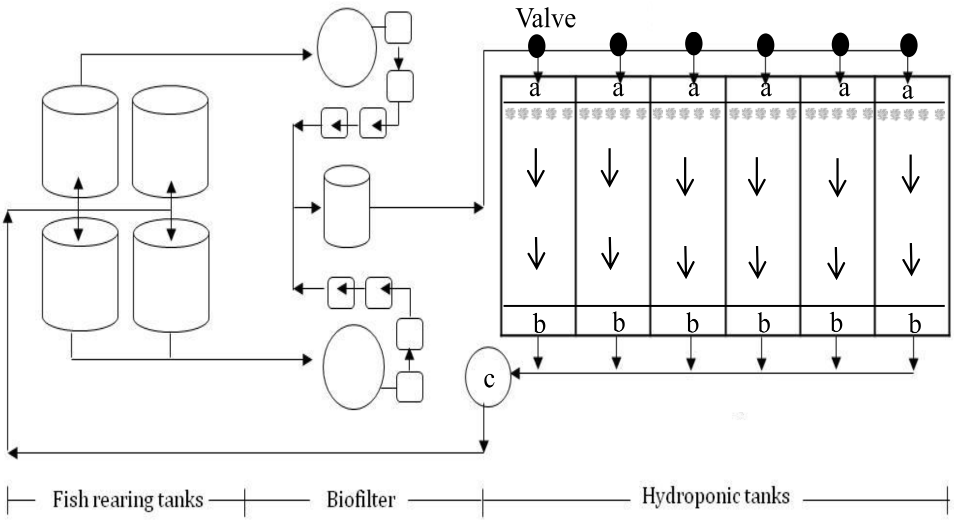

Figure 1. Schematic representation of the ARDA aquaponic system consisting of 3 components. (1) = Four fish rearing tanks (2) = Biofilter; one biofilter tank, two solid removal tanks, eight filter tanks with nets (3) = Hydroponic tanks; in this experiemnet, six hydroponic tanks were used a = Water inlet and sampling point b = Water outlet and sampling point c = Sump ● = Valve.

Table 1. Minimum and maximum temperature, pH, DO and conductivity at the water inlet during Experiment 1

Parameter	Morning (Min-Max)	Evening (Min-Max)
Temperature (^oC)	22.5-24.4	23.4-25.5
DO (mg L^{-1})	6.0-8.3	5.4-8.2
pH	6.5-6.9	6.5-6.9
Conductivity (µS cm^{-1})	630-890	660-910

2006; DeLong *et al*., 2009; FAO, 2014).

Ammonia (NH_3) in the inlet water remained stable during the first and second weeks, and then slightly decreased in the third week. Nitrite (NO_2^-) showed a decreasinged trend throughout the culture period. In contrast, nitrate (NO_3^-) showed an increasing trend. Orthophosphate (PO_4^{3-}) increased during the first week and then slightly decreased throughout the culture period. Nitrite (NO_2^-) increased during the third week then decreased (Table 2).

In this experiment, the weekly average concentrations of NO_3^- in the outlet water showed non-significant differences ($P>0.05$) amongst the treatments. However, NO_3^- content in the outlet water were significantly lower ($P<0.05$) than that of the inlet water (Figure 2). This indirectly shows that some NO_3^- contents in the water in the lettuce tank were absorbed and utilized by the lettuce resulting in lower concentration compared to the inlet water. Meanwhile, similar content in the inlet water might be because the outlet water from each tank was polled before pumping back to the lettuce tank.

Daily NO_3^- concentrations in inlet water in the morning (07:00h) and evening (18:00h) revealed a linear increase until the end of the experiment (R^2 = 0.90 and 0.87, respectively; Figure 3). A positive linear relationship between water NO_3^- contents and time was observed, indicating that accumulation of NO_3^- increased with time. These data implied that the nitrification process properly worked, resulting in the increase of NO_3^-. Lettuce grows well with sufficient uptake of NO_3^- (Chang *et al*., 2013). However, NO_3^- contents in water still increased, possibly due to its continued production from nitrogenous waste excreted by fish throughout the culture period.

Determination of NO_3^- removal can be a way to evaluate NO_3^- uptake by plant. In this study, water recirculation during nighttime showed an increasing trend of NO_3^- (Figure 4) as NO_3^- uptake by plants is at the highest during the night (Navarro *et al*., 1998; Fu *et al*., 2012). Meanwhile, recirculation during daytime showed a nearly stable trend of NO_3^- removal. NO_3^- uptake being lower during the day is possibly due to lettuce efficiently utilizing NO_3^- accumulated during the night and because of photosynthesis. In contrast, a decreasing trend of NO_3^- removal was observed for the recirculation within 24 hours, as a result of the integration of the NO_3^- removal during the day and during night time.

The results showed that the duration of the water recirculation did not significantly affect ($P>0.05$) lettuce growth in terms of average height (Figure 5), diameter (Figure 6), height-to-circumference ratio (Figure 7) and weight (Figure 8), Since water

Table 2. Ammonia (NH_3), nitrite (NO_2^-), nitrate (NO_3^-) and orthophosphate (PO_4^{3-}) concentration at the water inlet during the Experiment 1

Week	NH_3 (mg L^{-1})	NO_2^- (mg L^{-1})	NO_3^- (mg L^{-1})	PO_4^{3-} (mg L^{-1})
0	0.67±0.028	0.60±0.001	44.0±0.070	3.80±0.127
1	0.64±0.014	0.55±0.025	50.7±1.485	7.35±0.106
2	0.66±0.007	0.56±0.028	55.5±1.272	7.33±0.169
3	0.64±0.042	0.52±0.038	107.1±1.414	5.23±0.148

Values are means (± standard deviation).

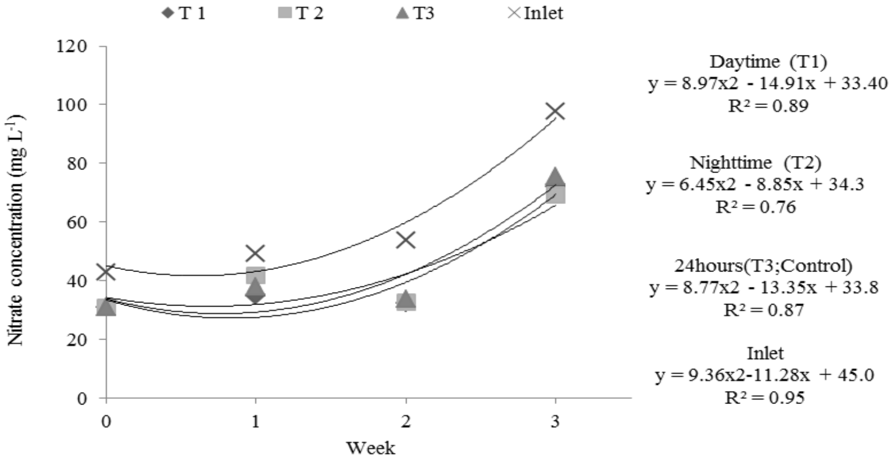

Figure 2. Nitrate concentrations at the inlet and outlet of Treatment 1 (daytime water circulation), Treatment 2 (nighttime water circulation) and Treatment 3 (24 hours water circulation).

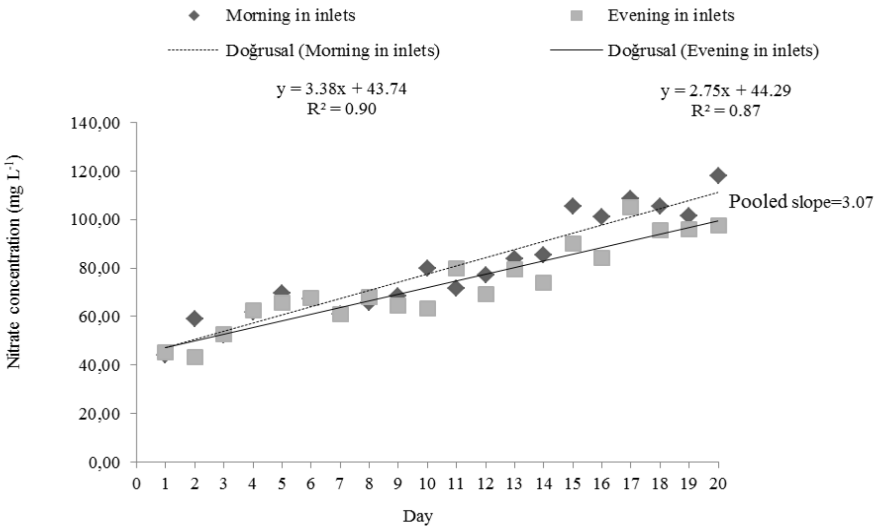

Figure 3. Nitrate (NO_3^-) concentration at the inlet in the morning (07:00h) and in the evening (18.00h) during the experiment.

recirculation duration has no effect on growth and NO_3^- removal, it can be reduced from 24 hours to 11-13 hours. This finding is in accordance with the result of Shete *et al.* (2013) on culturing goldfish with spinach wherein water circulation can be reduced to 12 hours per day for economically effective aquaponics production and may be considered as optimum water circulation period for goldfish production in the aquaponic system.

The nitrate concentration in the sap of the lettuce leaf midribs were 2260, 2440 and 2470 mg kg^{-1} NO_3^- FW for T1, T2, and T3, respectively, and there were no significant differences among treatments (P>0.05). These values met standards of the EU maximum

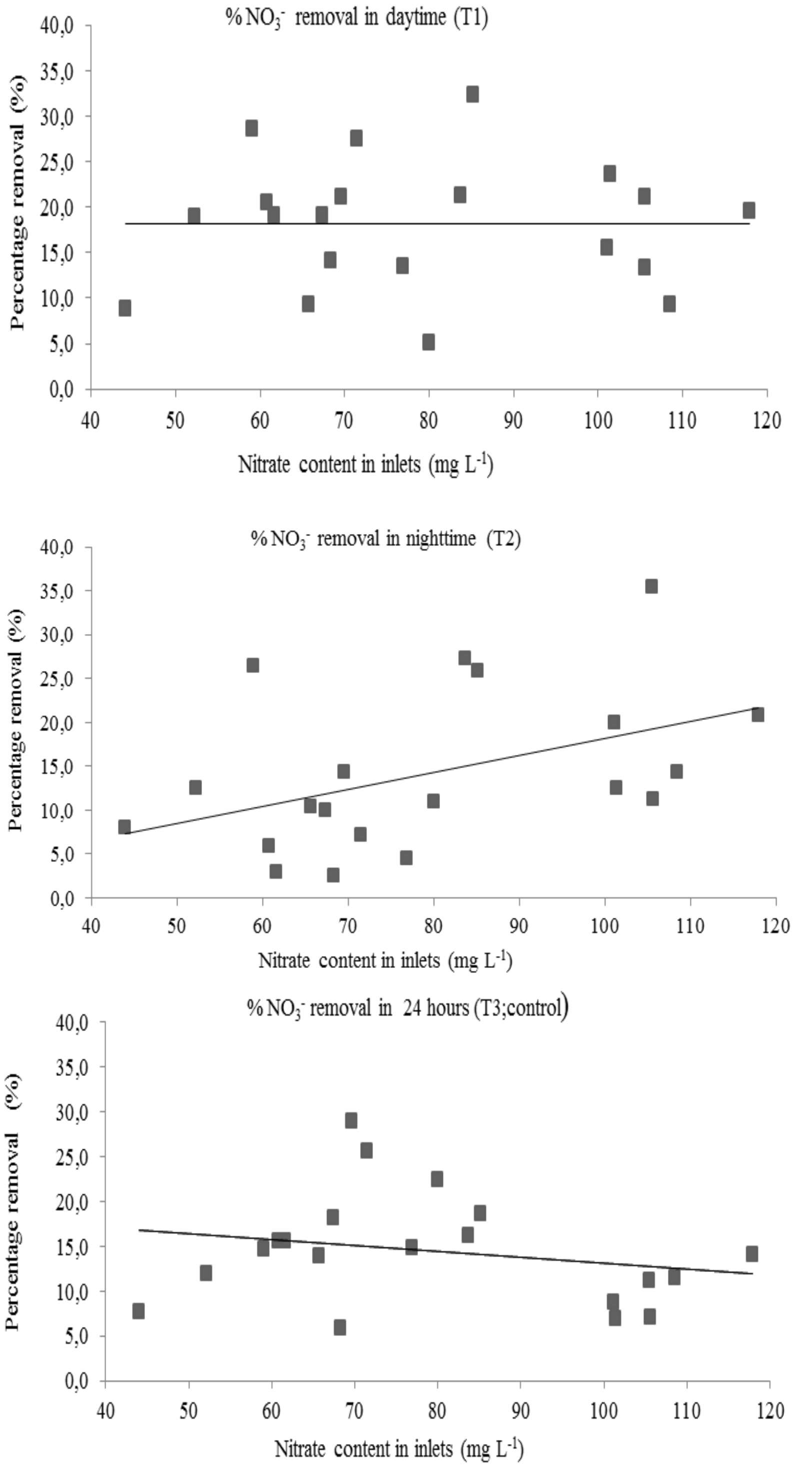

Figure 4. Percentage of NO_3^- removal at the outlet at daytime (T1), nighttime (T2) and 24 hours (T3; control) water recirculating daily during Experiment 1.

permissible leaf nitrate concentrations of vegetable products (Commission Regulation (EC) No 1881/2006, 2006; Cometti *et al*., 2011; Liu and Yang, 2012).

Experiment 2

The key water quality parameters of inlet water in Experiment 2 are shown in Table 3. They were found to be suitable for hydroponic plants in all treatments (Rakocy *et al*., 2006; DeLong *et al*., 2009; FAO, 2014). The concentrations of NH_3, NO_2^- NO_3^- and PO_4^{3-} decreased during the first week, and then increased gradually until the end of experiment (Table 4).

The growth parameters of lettuce are shown in

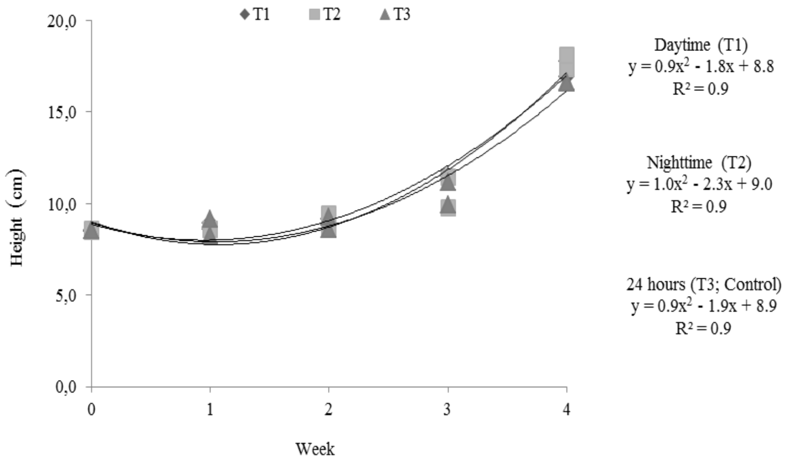

Figure 5. Average height of lettuce in Experiment 1.

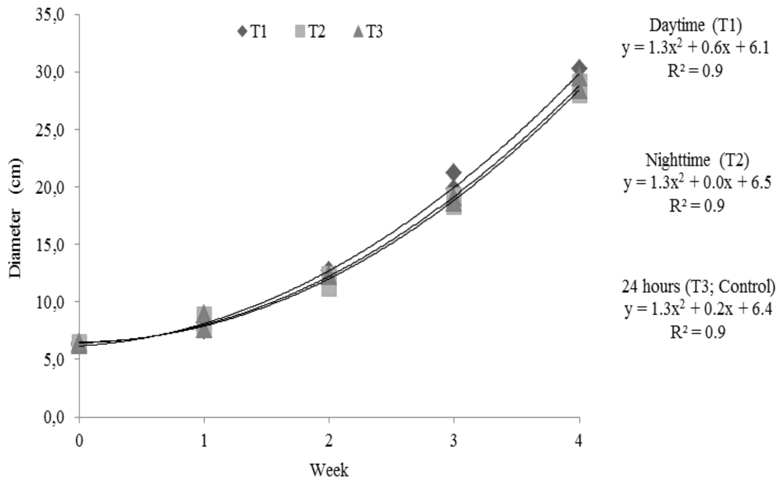

Figure 6. Average diameter of lettuce in Experiment 1.

Table 5. Average weight, diameter and height-to-circumference ratio of lettuce with no shading (S3) and 30% shading (S2) were significantly higher than with 60% shading (S1) ($P<0.05$). The height of lettuce in S1 and S2 was significantly higher than lettuce in S3 ($P<0.05$). In this study, 60% shading showed an inhibitory effect on lettuce growth. Lettuce increased its height to seek the sun light, which increased the height to circumference ratio of the plant. Most farmers use shading to protect crops from excessive temperature and solar radiation (Demšar, 2004; Sikawa, 2010; Fu *et al.*, 2012) that negatively affect plant quality, particularly leafy vegetable, by inducing a scorch appearance (Hunter and Burritt, 2005).

Shading with 60% and 30% increased the nitrate concentrations in the sap of the lettuce leaf midribs significantly. They were 1910±84.9, 1630±70.7 and 1220±82.0 mg kg^{-1} NO_3^- FW for the 60%, 30% and the control no shading treatments, respectively. Similar results of increased NO_3^- content in the plant sap with increased shading were observed by He *et al*. (2011) and Fu *et al*. (2012) but this study showed that the values obtained with all shading rates tested were still in accordance with the EU maximum permissible leaf nitrate concentrations in leaf vegetables (Commission Regulation (EC) No 1881/2006, 2006; Cometti *et al*., 2011; Liu and Yang, 2012).

Conclusions

This study allowed the definition of various technical parameters very important for commercial operation of aquaponic systems:

(1) First of all, it showed that aquaponic technology produced vegetables as safe for human consumption as those produced by more traditional farming systems in terms of nitrate content. Indeed, this nutrient's concentration in the sap of the lettuce leaves midrib in both experiments were always in accordance with the EU maximum permissible leaf

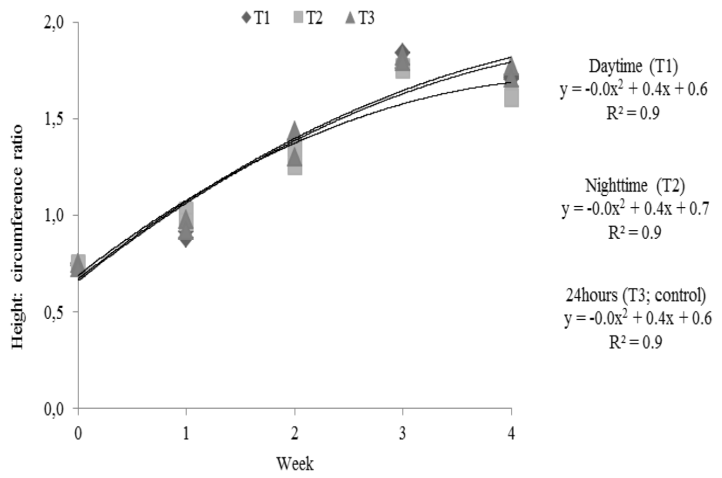

Figure 7. Average height: circumference ratio of lettuce in Experiment 1.

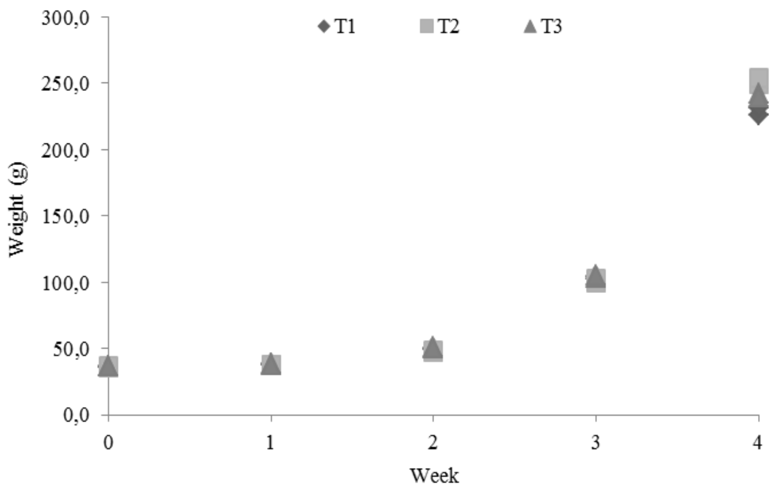

Figure 8. Average weight of lettuce in Experiment 1.

Table 3. Minimum and maximum temperature, pH, DO and conductivity at the water inlet during Experiment 2

Parameter	Morning (Min-Max)	Afternoon (Min-Max)
Temperature (^{o}C)	25.2-29.3	29.3-30.7
DO (mg L^{-1})	6.4-8.5	5.4-8.2
pH	6.5-6.9	6.5-6.9
Conductivity (μS cm^{-1})	630-890	660-910

nitrate concentrations in vegetables for human consumption.

(2) Another major finding was that it is possible to get the same performance when reducing the duration of the pumping for water recirculation to 11 to 13 hours, either during daytime or nighttime. Considering the cost of energy in some areas such as the remote Reunion island, as well as the importance of pumping in the operation costs of aquaponic systems, this open the way to significant economic savings and higher profitability. The choice between daytime or nighttime however depends on the possible differential price of energy during the day and night.

(3) Finally, it allowed to determine that 30% shading was the optimal shading rate for ensuring lettuce leaf quality and consumer safety without affecting its growth. However, shading increased the nitrate concentration in plant, although it always remained safe for human consumption.

Table 4. Ammonia (NH_3), nitrite (NO_2^-), nitrate (NO_3^-) and orthophosphate (PO_4^{3-}) concentration at the inlets during Experiment 2

Week	NH_3 (mg L^{-1})	NO_2^- (mg L^{-1})	NO_3^- (mg L^{-1})	PO_4^{3-} (mg L^{-1})
0	1.62±0.028	0.153±0.004	41.0±0.990	15.26±0.035
1	0.80±0.042	0.093±0.003	24.8±0.949	7.63±0.014
2	0.85±0.085	0.121±0.006	37.2±0.283	14.79±0.559
3	1.05±0.050	0.137±0.005	40.0±0.071	16.33±0.220

Values are means (± standard deviation).

Table 5. Average weight, height, diameter and height-to-circumference ratio of lettuce at the end of Experiment 2

Treatment	Weight (g)	Height (cm)	Diameter (cm)	Height: Circumference ratio
60% shading	136.0 ±2.3^b	18.0±2.7^a	23.9± 0.7^b	1.3±0.0^b
30% shading	248.0 ±1.5^a	16.8± 0.4^a	26.7 ± 1.6^a	1.6±0.0^a
No shading	242.0± 4.9^a	14.3±0.3^b	26.3± 1.9^a	1.8± 0.0^a

Values are means (± standard deviation). Values showing a common superscript are not significantly different (P>0.05).

Acknowledgements

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References

APHA (American Public Health Association). 1998. Standard Methods for Examination of Water and Wastewater, 20th edition. American Public Health Association, America Water Work Association and Water Pollution Control Federation.Washington, DC, USA.

Baroiller, J.F. and D'Cotta, H. 2001. Environment and sex determination in farmed fish. Comparative Biochemistry and Physiology Part C, 130: 399-409.

Blidariu, F. and Grozea, A. 2011. Increasing the economic efficiency and sustainability of indoor fish farming by means of aquaponic-review. Animal Science and Biotechnologies, 44(2): 1-8.

Chang, A.C., Yang, T.Y. and Riskowski, G.L. 2013. Ascorbic acid, nitrate concentration relationship to the 24 hour light/dark cycle for spinach grown in different conditions. Food Chemistry, 138: 382-388.

Cometti, N.N., Martins, M.Q., Bremenkamp, C.A. and Nunes, J.A. 2011. Nitrate concentration in lettuce leaves depending on photosynthetic photon flux and nitrate concentration in the nutrient solution. Horticulture Brasileira, 29: 548-533.

Commission Regulation (EC) No 1881/2006. 2006. Commission Directive 2006/1881/EC of

19 December 2006, setting maximum levels for certain contaminants in food stuffs. Official Journal of the European Communities, L364: 5–24.

DeLong, P.D., Losordo, M.T. and Rakocy, J.E. 2009. Tank Culture of Tilapia. SRAC Publication, No. 282, Texas, USA, 7 pp.

Demšar, J., Osvald, J. and Vodnik, D. 2004. The effect of light-dependent application of nitrate on the growth of aeroponically grown lettuce (*Lactuca sativa* L.). **Journal of the American Society for Horticultural Science**, 129(4): 570-575.

FAO. 2014. Small-scale aquaponic food production. Integrate fish and plant farming. FAO Fisheries and aquaculture technical paper No. 589. Food and Agriculture Organization of the United Nations Rome, Italy, 262 pp.

Fu, W., Li, P., Wu, Y. and Tang, J. 2012. Effects different light intensities on anti-oxidative enzyme activity, quality and biomass in lettuce. Horticultural Science (Prague), 39(3): 129-134.

Goada, A.M.A., Essa, M.A., Hassaan, M.S. and Sharawy, Z. 2015. Bio economic features for aquaponic systems in Egypt. Turkish Journal of Fisheries and Aquatic Sicences, 15 (3): 525-532. doi: 10.4194/1303-2712 ;

He, J., Cheok, L. and Qin, L. 2011. Nitrate accumulation, productivity and photosynthesis of

temperate butter head lettuce under different nitrate availabilities and growth irradiances. The Open Horticulture Journal, 4: 17-24.

Hunter, D.C. and Burritt, D.J. 2005. Light quality influences the polyamine content of lettuce

(*Lactuca sativa* L.) cotyledon explants during shoot production *in vitro*. Plant Growth

Regulation, 45: 53-61. doi: 10.1007/s10725-004-5971.z ;

Klinger, D. and Naylor, R., 2012. Searching for solutions in aquaculture: Charting sustainable course. Annual Review of Environment and Resources, 37: 247-276. doi: 10.1146/annurev-environ-021111-161531 ;

Liang, J. and Chien, Y. 2013. Effects of feeding frequency and photoperiod on water quality and crop production in a tilapia-water spinach raft aquaponic system. International Biodeterioration & Biodegradation, 85: 693-700. doi: 10.1016/j.ibiod. 2013.03.029 ;

Liu, K.W. and Yang, Q.C. 2012. Effect of short-term treatment with various light intensities and hydroponic solution on nitrate concentration of lettuce. Acta Agriculturae Scandinavica, Section B- Soil & Plant Science, 62(2): 109-113. doi: 10.1080/09064710.2011.580366 ;

Love, D.C., Fry. J.P., Li, X., Hill, E.S., Genello, L., Semmens, K. and Thompson, R.E. 2015. Commercial aquaponics production and profitability: Findings from an international survey. Aquaculture, 435: 67-74. doi: 10.1016/j.aquaculture.2014.09.023 ;

Martins, C.I.M., Eding, E.H., Verdegem, M.C.J., Heinsbroek, L.T.N., Schneider, O., Blancheton, J.P., Roque d' Orbcaste, E. and Verreth, J.A.J. 2010. New development in recirculating aquaculture systems in Europe: A perspective on environmental sustainability. Aquaculture Engineering, 43(3): 88-93. doi: 10.1016/ j.aquaeng. 2010. 09.002 ;

Navarro, C.R., Adamowicz , S. and Robin, P. 1998. Diurnal nitrate uptake in young tomato (*Lycopersicon esculentum* Mill.) plants: test of a feedback-based model. Journal of Experiment Botany, 49(321): 721-730.

Pantanella, E., Cardarelli, M., Colla, G., Rea, E. and Marcucci, A. 2010. Aquaponics vs hydroponics: production and quality of lettuce crop. ISHS Acta Horticulturae, 927: 887-893. doi: 10.17660/ActaHortic.2012.927.109 ;

Rakocy, J.E. and Bailey, D.S. 2003. Initial economic analyses of aquaponic systems. In: Aquaculture Europe 2003, Beyond Monoculture, European Aquaculture Society:58-64.

Rakocy, J.E., Masser, M.P. and Losordo, T.M. 2006. Recirculating Aquaculture Tank Production System: Aquaponic-Integrating Fish and Plant Culture. SRAC Publication No. 454, Texas, USA, 16 pp.

Santamaria, P. 2006. Nitrate in vegetables: toxicity, content, intake and EC regulation. Journal of the Science of Food and Agriculture, 86(10): 10-17. doi: 10.1002/jsfa.2351i ;

Shete, A.P., Verma1, A.K., Tandel, R.S., Prakash, C., Tiwari, V.K. and Hussain, T. 2013. Optimization of water circulation period for the culture of goldfish with spinach in aquaponic system. Journal of Agricultural Science, 5(4): 26-30.

Sikawa, D.C. 2010. An evaluation of potential development of a catfish-lettuce aquaponic culture system. PhD thesis. Pathumthani, Thailand: Asian Institute of Technology.