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Utilization of Inland saline underground water for bio-integration of Nile tilapia (*Oreochromis niloticus*) and spinach (*Spinacia oleracea*)

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ABSTRACT

An experimental trial of 45 days was conducted with different Nile tilapia (*Oreochromis niloticus*) to spinach (*Spinacia oleracea)* ratios in a recirculating aquaponics system using inland saline groundwater of salinity 3 g l^{-1}. Different fish to plant ratios, T1 (1:0.7), T2 (1:1), T3 (1:1.3) and C1 (aquaponics without plants), C2 (without hydroponic component) were assigned as treatments and control groups respectively. Total yield of *O. niloticus* (4.69 ± 0.04 Kg m^{-3}), spinach yield (1.42 ± 0.02 Kg/bed) and percentage removal of nitrate (50.43 ± 0.11%), phosphate (47.62 ± 2.20%), and potassium (54.26 ± 1.23%) from the effluent was found to be higher in T3. The daily water requirement of T1, T2, T3 and C1 (1.16–1.29%) was significantly lower compared to C2 (17.67%). Findings of the experiment suggest that aquaponics is feasible with low saline underground water and a ratio of 1:1.3 (Nile tilapia: spinach) is the best among tested ratios for balancing nutrient generation and removal as well as fish and plant production. However, further studies using higher fish to plant ratio could be carried out for optimisation of such systems.

1. Introduction

Salinization and water scarcity are the most severe constraints confronting sustainable agriculture production systems in semi-arid and arid regions. Its intensity has expanded in past decades due to poor management of land and water resources, unprecedented regional as well as global climate change and variability (Wong et al., 2010) and led to an increased pressure on freshwater resources. As the shortage of freshwater is becoming an important issue in the arid and semi-arid zones, concerns about the utilization of poor-quality (saline) underground water has been amplified. The optimal and multiple use of water is the key to resource sustainability in arid and semi-arid regions. In many countries, saline ground water is used as an option for irrigation of saline tolerant agriculture crops (Rhoades, 1984; Grattan and Oster, 2003; Sharma and Minhas, 2005) and also for aquaculture purposes (Boyd and Thunjai, 2003; Allan et al., 2009).

Aquaculture in arid and semi-arid regions has increased economic and social impact through production of food, livelihood security and income generation but confronted with the issue of environment protection (Chithambaran, 2016). However aquaculture is the only way to satisfy the increasing seafood demand in the context of declining ocean fish stock. Considering stringent government regulations and increased awareness of the impact of aquaculture wastewater on receiving waters there is a need for the development of new technologies and innovations to make the aquaculture industry more sustainable and economically viable (Boyd et al., 1998). Aquaponics is one such technology developed to achieve more efficient use of water, maximizing farm production without increasing water consumption, avoiding deposition of aquaculture effluents and supplementing additional fertilizer to the agricultural crops (Mariscal-Lagarda et al., 2012).

Aquaponics is a recirculating aquaculture system that incorporates the production of plants without soil. In Aquaponics, the nutrient rich effluent from the fish tank which contains waste products of the fish metabolism and uneaten feed is used to fertigate hydroponic plant bed, where the nutrients are sequestrated by plants, which in turn is transformed into a valuable resource. The effluent is treated through the process of nitrification and nutrient uptake in the plant component and returned to the fish rearing tanks (Rakocy et al., 2006).Aquaponics forms a closed-loop system where fish, plant, bacteria live and work together to form a mini ecosystem.

Current aquaponics research has mostly focused on integrating freshwater fish and plant species (Rakocy et al., 2004; Enduta et al., 2011; Shete et al., 2013; Hussain et al., 2015; Nuwansi et al., 2016). But, freshwater resource for food production (agriculture and

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aquaculture) is becoming increasingly limited and at the same time salinisation is progressively increasing in many parts of the world (Turcios and Papenbrock, 2014). This has led us to an interest in the use of saline underground water for aquaponics by replacing freshwater and stocking with euryhaline or saltwater fish and salt tolerant plants. Developing a suitable saline aquaponics with salt tolerant plants and fishes can help in sustainable food production in arid and semi-arid lands where the underground water is saline. The selection of compatible plant and fish species, and an optimum fish to plant ratio to maintain nutrient balance is of critical importance regarding suitability for aquaponics production.

Tilapia, which belongs to the family "Cichlidae" is the world's second most important farmed food fish after carps and is also the most preferred fish for experimental as well as commercial aquaponics based production systems (Maucieri et al., 2018; Love et al., 2014). Among tilapia species, *Oreochromis niloticus*, commonly known as Nile tilapia is the most preferred and widely cultured species owing to its faster growth, ability to survive in poor water quality, tolerance to high stocking densities, and adaptability to tank based culture systems and ease of handling (Popma and Lovshin, 1995). Nile tilapia is also known to have wide range of salinity tolerance and has the maximum biomass gain in the salinity range of 0-10 g l^{-1} (Villegas, 1990). Spinach (*Spinacia oleracea*) which belongs to the family Amaranthaceae is one of the perennial leafy vegetable grown throughout the world in greenhouses, polyhouses and hydroponic systems as well. Spinach has an excellent nutritional value and health benefits. Spinach contains high levels of vitamins and minerals viz. Vitamin A, vitamin B, vitamin K, vitamin C, vitamin B2 and B6, phosphorus, iron, potassium, folate, betaine, copper, protein, manganese, zinc, niacin, selenium and omega-3 (Avsar, 2011). Spinach is one of the main vegetables cultured in aquaponics systems because it grows rapidly in response to the high levels of nutrients in aquaculture water (Petrea et al., 2013).

The objective of the present study is to find out the feasibility of bio-integration of Nile tilapia and spinach in inland saline groundwater and to determine the best ratio for such system in terms of production performance and nutrient balancing.

2. Materials and methods

2.1. Experimental design

A 45 days study was conducted at ICAR-Central Institute of Fisheries Education, regional centre, Lahli, Rohtak, India (28° 86′ N, 76° 47′ E). Small-scale aquaponic systems consisting of 15 individual, identical aquaponics unit were installed in a greenhouse (6.10 m × 4.57 m) with polyethylene roofing sheet and knitted shade net of HDPE fabric as side walls supported by GI frames (Fig. 1). The aquaponic system was harboured in a two-floor structure with each individual unit containing a circular fish tanks of 100 l (water volume 75 l) capacity which were placed 0.3 m below the ground level and plant grow bed of 0.52 m^2 (0.93 m × 0.56 m) area placed 0.2 m above the ground level. Hydroponic plant grow beds were filled with cleaned gravels having a size of 1.5–2.0 cm to a thickness of 25–27 cm. Siphon bells were used to create a flood and drain system in plant grow beds. The effluent from the fish tank was pumped to the plant grow bed using 15 W submersible pump (T-KANGO TK-380). Pumping frequency was controlled by automatic timer (SAIA BURGESS CONTROLS) and a flow rate of 250 l $hour^{-1}$ was maintained. The water from the grow bed returned to the fish tank by gravity through a 20 mm PVC drain pipe which was connected to the bell siphon. The aquaponics bed took 7 min for complete drainage and 3 min for filling. Continuous aeration was provided to each fish tank. The rearing tanks were covered with 15 mm plastic net to prevent the fish from jumping out of the tank. Inland saline water with a salinity of 3 g l^{-1} used in the experiment has been made by diluting 10 g l^{-1} borewell water pumped from high saline zone with freshwater. The experimental fish were acclimatized to 3 g l^{-1} by progressively increasing the salinity at the rate of 0.5 g l^{-1} day^{-1} for 6 days.

The experimental design consisted of 3 treatments, each having three assigned replicates. Each treatment was allotted with different fish to plant ratio *viz*, T1, T2, T3 in the ratio 1:0.7, 1:1, 1:1.3, respectively and compared with controls, C1 (aquaponics without plants) and C2 (Fish without aquaponics). In all treatments and controls, the fingerlings were provided with commercial floating feed (32% protein) at a constant feeding rate of 5% of body weight equally divided in two dosages twice a day in the morning (10:00 am) and evening (5:00 pm) hours. Siphoning and water replacement was done on daily basis in C2. The water replacement in C2 and water addition to compensate for evaporative losses in C1, T1, T2 and T3 was done with the water of salinity 3 g l^{-1}.

2.2. Stocking

Initially the system was operated for 12 days with 10 numbers of fish averaging 5 gm weight and fed at 5% of body weight to enhance the ammonia level in the system to promote growth of nitrifying bacteria. Thereafter the fishes were removed and the tanks were stocked with fingerlings of tilapia @ 200 fishes m^{-3}. The mean weight and length of fish at the time of stocking was 5.89 ± 0.04 g and 7.49 ± 0.06 cm, respectively. The stocking density of fish remained the same for all treatments and controls. Seeds of spinach were sown in nursery trays (50 × 25 cm) made of plastic having 120 cavities and filled with coconut husk medium. Plantlets were allowed to grow for 20 days before transplanting to hydroponics tank at varying density (20, 30, 40 m^{-2}). The size of plants at the time of transplanting was 9.60 ± 0.06 cm.

2.3. Sampling

Sampling of fishes was carried out at 15 days interval for assessment of growth (length and weight) and health. The length was measured with the help of a graduated ruler, while the weight was recorded with

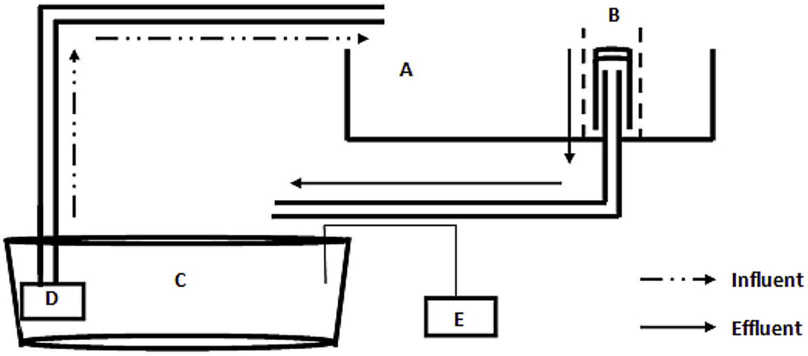

Fig. 1. Schematic diagram of experimental aquaponic system (A. Hydroponics, B.Bell siphon, C. Fish tank, D. Submersible pump, E. Air blower).

the aid of an electronic balance. Growth of plants was observed by measuring height of plants, length and width of leaves. By the end of experiment spinach was harvested.

2.4. Water quality analysis

Water quality parameters were analyzed at an interval of 10 days. The water temperature was measured by thermometer and pH was measured by using hand held pH meter (Hanna HI98130). Salinity was measured using hand-held refractometer (Atago S/Mill-E, Japan). Other parameters such as dissolved oxygen, total hardness, alkalinity, total dissolved solids, ammonia, nitrite, nitrate, and phosphorus were analyzed using the standard methods (APHA, 2005). The Na^{+} and K^{+} levels were analyzed using flame photometry (Microprocessor flame photometer, Model 1382, ESICO, Haryana, India). Calcium and magnesium content of the water was estimated titrimetrically following the standard methods (APHA, 2005).

2.5. Statistical analysis

The data were analyzed using statistical package SPSS version 22 where one-way ANOVA and Tukey's Honest Significant Difference test (Tukey’s HSD) were applied at a significance level of ($P < 0.05$) 95% confidence limit to know the significant difference between the treatments and control means for different parameters.

3. Result and discussion

3.1. Fish growth parameters

Choice of different fish to plant ratio had significant bearing on the growth rates in Nile tilapia. Overall growth performance of Nile tilapia in different treatments is shown in Table 1. The initial mean body weight had insignificant difference among treatments but at the end of experimental trial it was observed that, some of the treatment groups registered a significant difference ($P < 0.05$) in mean body weight gain. By administering the optimal feeding rate (5% of body weight) for tilapia as recommended by Deyab and Hussein (2015), the yield in T3 (4.69 ± 0.04 Kg m^{-3}), T2 (4.57 ± 0.02 Kg m^{-3}) and T1 (4.43 ± 0.04 Kg m^{-3}) were significantly higher than those in C1 (4.26 ± 0.03 Kg m^{-3}) and C2 (3.94 ± 0.03 Kg m^{-3}). The lowest yield observed in control, C2 (3.94 ± 0.03 Kg m^{-3}) can be due to the higher level of ammonia present in the culture water indicating that nitrification was negligible or absent. Nitrifying bacteria require surface for adhesion and prolifeartion which was relatively lesser in C2 than compared to C1 and other treatments. Gravels used in the aquaponics bed provide larger surface for colonisation of nitrifying bacterial population (Crab et al., 2007). The fish growth was found to increase with higher plant density as more number of plant provided better biofiltration and improved the water quality. Atle et al. (2003); Sten et al. (2004) and Lemarie et al. (2004) reported that growth rate of fish decreased when concentrations of ammonia increased. Increased ammonia levels in culture system is known to manifest its effect by lowering feed intake and also by reducing feed conversion efficiency (Schram et al., 2010). At the end of the experiment highest percentage weight gain was observed in T3 (292.88 ± 1.52%) which did not differ significantly from the values observed in T2 (284.61 ± 3.42%) but did differ significantly from that of T1 (273.62 ± 3.34%). A similar trend was observed in Specific Growth Rate (SGR) with values in T3, T2 and T1 being 3.04 ± 0.01% day^{-1}, 2.99 ± 0.02% day^{-1} and 2.92 ± 0.02% day^{-1} respectively. Both SGR and percentage weight gain found in various treatments varied significantly from control groups by the end of experiment. The survival rate of 100% observed in all the treatments indicates that different fish to plant ratio had no influence on survival of fishes. The mean length of fish also did not vary significantly ($P > 0.05$) among any of treatments. FCR of T3 (1.37 ± 0.01), T2 (1.41 ± 0.01) and T1 (1.47 ± 0.02) differed significantly ($P < 0.05$) from C1 (1.56 ± 0.02) and C2 (1.74 ± 0.01). The Feed Efficiency Ratio (FER) values were significantly similar in T3 (0.72 ± 0.01) and T2 (0.70 ± 0.01) but varied significantly from T1 (0.67 ± 0.01), C1 (0.63 ± 0.01) and C2 (0.57 ± 0.01). Protein Efficiency Ratio (PER) also exhibited a similar trend as that of FER with higher values in T3 and T2 (2.27 ± 0.02 and 2.20 ± 0.01) and lowest in C2 (1.78 ± 0.01) which varied significantly. FCR of the present study ranged from 1.37 (T3) to 1.7 (C2) which is in accordance with the result of Al-Hafedh et al. (2008) where FCR for Nile tilapia ranged from 1.0 to 1.7. In the present study the variation in FCR may be attributed to the variation in ammonia concentration in the culture water and also to the non consumption of feed supplied to the fish tank completely in C1 and C2. One of the common behavioural responses to stress in fish is the reduced feed intake and/or disruption of the feeding behaviour. El-Shafai et al. (2004) found that mean feed conversion ratio of *Oreochromis niloticus* decreased as ammonia concentrations increased. The SGR was found to decrease with increase in ammonia concentration. The same was reported by Harris et al. (1998) and Atle et al. (2003) who found that SGR decreases with the increasing concentration of ammonia and it is primarily due to reduced food intake.

3.2. Plant growth parameters

Plant growth is another important criterion to correlate the suitability or efficiency of aquaponic system. Plant plays an important role

Table 1
Fish growth parameters in different treatments.

Parameter	Treatments				
	C1	T1	T2	T3	C2
Mean body weight					
Initial (g)	$5.98^{a} \pm 0.05$	$5.93^{a} \pm 0.05$	$5.94^{a} \pm 0.06$	$5.97^{a} \pm 0.03$	$5.96^{a} \pm 0.08$
Final (g)	$21.30^{c} \pm 0.15$	$22.16^{b} \pm 0.20$	$22.87^{ab} \pm 0.09$	$23.46^{a} \pm 0.24$	$19.70^{d} \pm 0.16$
Final biomass (Kg m^{-3})	$4.26^{c} \pm 0.03$	$4.43^{b} \pm 0.04$	$4.57^{ab} \pm 0.02$	$4.69^{a} \pm 0.04$	$3.94^{d} \pm 0.03$
Mean body length					
Initial (cm)	$7.59^{a} \pm 0.16$	$7.55^{a} \pm 0.23$	$7.53^{a} \pm 0.12$	$7.42^{a} \pm 0.09$	$7.36^{a} \pm 0.11$
Final (cm)	$11.83^{ab} \pm 0.14$	$12.06^{a} \pm 0.23$	$12.06^{a} \pm 0.15$	$12.13^{a} \pm 0.16$	$11.75^{ab} \pm 0.24$
Percentage weight gain	$255.84^{c} \pm 4.31$	$273.62^{b} \pm 3.34$	$284.61^{ab} \pm 3.42$	$292.88^{a} \pm 1.52$	$230.57^{d} \pm 2.49$
Specific growth rate (%day^{-1})	$2.82^{c} \pm 0.02$	$2.92^{b} \pm 0.02$	$2.99^{ab} \pm 0.02$	$3.04^{a} \pm 0.01$	$2.65^{d} \pm 0.01$
Survival rate (%)	$100.0^{a} \pm 0.00$	$100.0^{a} \pm 0.00$	$100.0^{a} \pm 0.00$	$100.0^{a} \pm 0.00$	$100.0^{a} \pm 0.00$
Feed efficiency ratio (FER)	$0.63^{c} \pm 0.01$	$0.67^{b} \pm 0.01$	$0.70^{a} \pm 0.01$	$0.72^{a} \pm 0.01$	$0.57^{d} \pm 0.01$
Protein efficiency ratio (PER)	$1.99^{c} \pm 0.02$	$2.11^{b} \pm 0.02$	$2.20^{a} \pm 0.01$	$2.27^{a} \pm 0.02$	$1.78^{d} \pm 0.01$
Feed conversion ratio (FCR)	$1.56^{b} \pm 0.02$	$1.47^{c} \pm 0.02$	$1.41^{cd} \pm 0.01$	$1.37^{d} \pm 0.01$	$1.74^{a} \pm 0.01$
Daily ration (g day^{-1})	$8.00^{a} \pm 0.00$	$8.00^{a} \pm 0.00$	$8.00^{a} \pm 0.00$	$8.00^{a} \pm 0.00$	$8.00^{a} \pm 0.00$

Mean values (Mean ± S.E) in a row without a common superscript differ significantly ($P < 0.05$) as analysed by one – way ANOVA and Tukey HSD test.

Table 2
Plant growth parameters in different Fish: Plant ratios.

Parameters	Treatments		
	T1	T2	T3
Plant height			
Initial (cm)	9.86^{a} ± 0.88	9.70^{a} ± 0.36	9.78^{a} ± 0.57
Final (cm)	42.89^{a} ± 2.80	41.22^{a} ± 2.18	38.12^{a} ± 3.35
Leaf length			
Initial (cm)	5.63 ^{a} ± 0.15	5.53 ^{a} ± 0.30	5.56 ^{a} ± 0.15
Final (cm)	23.51^{a} ± 1.73	21.49^{a} ± 1.05	19.78^{a} ± 2.60
Leaf Width			
Initial (cm)	2.93 ^{a} ± 0.12	2.89 ^{a} ± 0.13	2.83 ^{a} ± 0.19
Final (cm)	11.37 ^{a} ± 0.72	10.93 ^{a} ± 0.59	10.23 ^{a} ± 1.29
Percentage height gain (%)	334.30^{a} ± 24.73	331.08^{a} ± 38.40	292.98^{a} ± 34.44
Yield (kg 0.5 m^{-2})	0.85^{c} ± 0.01	1.07^{b} ± 0.02	1.42^{a} ± 0.02

Mean values (Mean ± S.E) in a row without a common superscript differ significantly ($p < 0.05$) as analysed by one – way ANOVA and Tukey HSD test.

in nutrient dynamics of aquaponics system as the biofilter component. Spinach (*Spinacia oleracea*) was seen to grow well with the nutrients from tilapia fish culture water, without the addition of extra nutrients. At the end of the growth period (45 days), the plants reached the market size with average height of 38–43 cm (Table 2). Lenard and Lennard (2006) while comparing different systems for growing spinach in an aquaponics system recommended gravel bed or floating raft over the nutrient film technique. Good growth of Spinach even with water of higher salinity and EC value is an interesting observation. Gravel bed technique allows for intermittent submergence and exposure of plant root system to water as compared to the continuous submergence of the roots in nutrient film technique. Intermittent exposure and submergence will reduce the osmotic stress to the plant root and that might have been the reason for the luxurious growth of spinach even in water with high salt content (salinity of 3 g l^{-1} and EC value of 4.4 to 5 ds cm^{-1}). Owing to these reasons Gravel bed system was used in the present study. At the time of harvest the overall height, percentage height gain, leaf length and leaf width of spinach did not show any significant difference ($P > 0.05$) in any of the treatment groups. Similar growth performance of spinach in all the treatments can be due to the presence of excess nutrients in the system. It will not contribute extra to the productivity and quality of the crop, when absorption of nutrients exceeds a threshold (luxury absorption) (Hansen, 1977). But the yield of spinach varied significantly ($P < 0.05$) with increasing number of plant densities and highest yield was recorded in T3 (1.42 ± 0.02 Kg/bed) followed by T2 (1.07 ± 0.02 Kg/bed) and T1 (0.85 ± 0.01 Kg/bed). The results of present study is comparable with results of the Hussain et al. (2014) where fish to plant ratio of 1:1.4 was found to be optimum for spinach - koi carp aquaponics system. However as the plant growth was luxurious even at the highest fish to plant ratio chosen in this experiment, it would be interesting to carry out further trials with higher Fish to plant ratios.

3.3. *Water quality parameters and nutrient dynamics*

All the water quality parameters measured are presented in Table 3. Water temperature is one of the important factors responsible for optimum fish growth, plant growth, and performance of nitrifying bacteria in biofilter. The water temperature was found to be influenced by seasonal changes with higher values at the start of experiment and comparatively lower values by the end of experiment (varied from 25.5-32.0^{0}C) with an average of 28.3^{0}C, but no marked variation was observed in any of the treatment groups. Optimum growth for tilapia is achieved at 27 to 29 °C (Rakocy and Brunson, 1989). Ndakidemi et al. (2009) studied the effect of regulated irrigation water temperature on hydroponics production of Spinach (*Spinacia oleracea*) and optimum growth was recorded at 28 °C. The mean value of salinity of water did not show any significant difference ($p > 0.05$) among treatments and control. pH of the water from different tanks ranged from 7.4 to 7.8 during the study period but no marked variation among the treatments and control and these levels are within the optimum range of 6–9 for the bacteria in the nitrification filter (Wheaton et al., 1994) and within the tolerance range of 5–11 for tilapia (Chervinski, 1982).

Maintenance of adequate Dissolved oxygen (D.O) in culture water is important for fish health and biofilter performance (Hussain et al., 2015). DeLong et al. (2009) recommended Dissolved oxygen (D.O) levels between 5.0 and 7.5 mg l^{-1} for acceptable growth in tilapia and Masser et al. (1999) recommended > 2 mg l^{-1} for efficient performance of nitrifying bacteria. The mean dissolved oxygen content of the present study falls within the recommended limits and ranged between 5.74-5.33 mg l^{-1} with no significant difference in any of the treatments.

The alkalinity and hardness of the culture water was high throughout the experiment since the water used for the study was saline underground water. Higher level of hardness as observed in this case could be explained by high level of Calcium and Magnesium in the borewell water used for the experiment. Total alkalinity values was found significantly similar ($p > 0.05$) in all the treatment groups. Lawson (1995) reported a permissible limit of 50–500 mg l^{-1} as $CaCO_3$ alkalinity in an aquaponic system. The mean value of alkalinity of different treatment groups ranged from 124.20 to 138.73 mg l^{-1} as $CaCO_3$ and was found within the permissible limit. The total hardness was also observed to be significantly similar ($P > 0.05$) in all the groups although it was slightly higher in C1 (854.21 ± 1.48 mg l^{-1}) followed by T1 (848.93 ± 2.59 mg l^{-1}), C2 (847.87 ± 2.67 mg l^{-1}) and T2 (838.27 ± 2.58 mg l^{-1}) due to the accumulation of nutrients whereas; lower hardness was recorded in T3 (831.93 ± 2.42 mg l^{-1}).

Ammonia is the principal nitrogenous waste product of fishes and is normally oxidized first to nitrite and then to nitrate. It is also the main nitrogenous waste material excreted by gills beside urea and amines. The suggested value of ammonia in a recirculating aquaculture system (RAS) should be less than 1.00 mg l^{-1} (Van Rijn and Rivera, 1990; Nijhof and Bovendeur, 1990). In the present study, Total Ammonia Nitrogen (TAN) was significantly higher in C2 (0.61 ± 0.01 mg l^{-1}) but TAN concentration was found to be similar in C1 (0.30 ± 0.01 mg l^{-1}), T1 (0.25 ± 0.02 mg l^{-1}), T2 (0.23 ± 0.01 mg l^{-1}) while T3 (0.18 ± 0.02 mg l^{-1}) had significantly lower concentration of the same. All the values recorded were found within the optimum limit recommended for RAS. Lower levels of TAN in the treatments with more number of plants indicated the efficiency of spinach plants to uptake ammonium ion from the culture wastewater in the aquaponics system. Ammonium (NH_4^+) is one of the major sources of inorganic nitrogen taken up by the roots of higher plants (Vaillant et al., 2004). As plants take up NH_4^+, some of the NH_3 is converted to NH_4^+ to maintain equilibrium. The net result is that the amount of NH_3 decreases (Tyson et al., 2011). During the experiment, Nitrite-Nitrogen did not show any significant difference between treatments and control. Eissa et al. (2015), found optimum range of nitrite 0.02–1.23 mg l^{-1} for aquaponic and nitrite level of the present study was found to be within the limit. The mean Nitrate-Nitrogen concentration showed significant variation ($p < 0.05$) among treatments. The higher Nitrate-Nitrogen level was observed in C1 (47.76 ± 1.79 mg l^{-1}) followed by T1 (33.29 ± 0.39 mg l^{-1}), T2 (27.68 ± 0.26 mg l^{-1}), T3 (23.87 ± 0.29 mg l^{-1}); whereas, lowest Nitrate-Nitrogen level was observed in C2 (1.65 ± 1.89 mg l^{-1}). The nitrate in culture water was found to increase with decreasing plant density. Results of this study coincide with Nair et al. (1985); Rakocy and Hargreaves (1993) and Seawright et al. (1998) who found that NO_3^{-2} accumulated the most in waste water from the fish. According to Watson and Hill (2006), NO_3^{-2} should be maintained below 100 mg l^{-1} in aquaponics and the nitrate level in the present study was within the recommended range. As the plant density increased the nitrate level in culture water decreased. This shows that spinach plants effectively removed nitrates in treatments.

Table 3
Physico-chemical parameters and nutrients dynamics during the experimental period of 45 days for different treatments.

Parameters	Treatments				
	C1	T1	T2	T3	C2
Temperature (^{0}C)	$28.30^{a} \pm 0.21$	$28.31^{a} \pm 0.24$	$28.25^{a} \pm 0.22$	$28.30^{a} \pm 0.21$	$28.23^{a} \pm 0.21$
Salinity (g l^{-1})	$3.2^{a} \pm 0.08$	$3.23^{a} \pm 0.07$	$3.21^{a} \pm 0.07$	$3.23^{a} \pm 0.08$	$3.1^{a} \pm 0.08$
pH	$7.68^{a} \pm 0.19$	$7.54^{a} \pm 0.16$	$7.6^{a} \pm 0.12$	$7.56^{a} \pm 0.15$	$7.6^{a} \pm 0.11$
DO (mg l^{-1})	$5.63^{a} \pm 0.46$	$5.33^{a} \pm 0.50$	$5.74^{a} \pm 0.42$	$5.67^{a} \pm 0.57$	$5.47^{a} \pm 0.35$
Hardness (mg l^{-1})	$854.21^{a} \pm 1.48$	$848.93^{ab} \pm 2.59$	$838.27^{bc} \pm 2.58$	$831.93^{c} \pm 2.42$	$847.87^{ab} \pm 2.67$
Alkalinity (mg l^{-1})	$126.20^{a} \pm 3.48$	$129.01^{a} \pm 3.21$	$130.46^{a} \pm 3.92$	$124.20^{a} \pm 3.97$	$138.73^{a} \pm 3.00$
Ammonia (mgl^{-1})	$0.30^{b} \pm 0.01$	$0.25^{bc} \pm 0.02$	$0.23^{cd} \pm 0.01$	$0.18^{d} \pm 0.02$	$0.61^{a} \pm 0.01$
Nitrite-N (mg l^{-1})	$0.06^{a} \pm 0.08$	$0.05^{a} \pm 0.02$	$0.05^{a} \pm 0.01$	$0.05^{a} \pm 0.02$	$0.04^{a} \pm 0.03$
Nitrate-N (mg l^{-1})	$47.76^{a} \pm 1.79$	$33.29^{b} \pm 0.39$	$27.68^{c} \pm 0.26$	$23.87^{d} \pm 0.29$	$1.65^{e} \pm 1.89$
Phosphate (mg l^{-1})	$0.77^{a} \pm 0.02$	$0.65^{b} \pm 0.01$	$0.59^{c} \pm 0.01$	$0.50^{d} \pm 0.01$	$0.28^{e} \pm 0.01$
Potassium (mg l^{-1})	$12.25^{a} \pm 0.22$	$8.21^{b} \pm 0.43$	$7.02^{bc} \pm 0.69$	$5.38^{c} \pm 0.42$	$6.27^{bc} \pm 0.86$
Calcium (mg l^{-1})	$97.8^{a} \pm 0.93$	$95.30^{b} \pm 1.05$	$93.32^{ab} \pm 0.86$	$90.22^{b} \pm 1.47$	$94.32^{ab} \pm 1.03$
Magnesium (mg l^{-1})	$161.37^{a} \pm 0.59$	$157.24^{b} \pm 0.65$	$153.04^{c} \pm 0.87$	$147.96^{d} \pm 0.56$	$151.91^{c} \pm 1.59$
Sodium (mg l^{-1})	$890.24^{a} \pm 0.39$	$879.45^{b} \pm 0.15$	$868.26^{c} \pm 0.12$	$857.51^{d} \pm 0.18$	$877.43^{b} \pm 0.15$

Mean values (Mean ± S.E) in a row without a common superscript differ significantly ($P < 0.05$) as analysed by one – way ANOVA and Tukey HSD test.

Nitrate-N is relatively harmless and is the preferred form of nitrogen for growing higher plants (Rakocy et al., 2006).

Except calcium all other nutrients like phosphate, potassium, magnesium and sodium showed significantly higher values in C1 compared to other treatments clearly indicates the efficiency of spinach to uptake nutrients. The mean phosphate concentration during the study varied significantly ($p < 0.05$) among all the treatments and control. These nutrients were found to accumulate in control group without plants and decreased with increase in plant density. Adler et al. (1996) found that differences in nutrient removal rates were dependent on plant number and effluent flow rate. da Silva Cerozi and Fitzsimmons (2016) recorded a phosphorus availability of less than 2 mg l^{-1} in aquaponic system. Similarly, in the present study the PO_4-P level ranged between 0.15–1.15 mg l^{-1}. Lower phosphorus level could be due to high calcium level in the culture water. Sheikh et al. (1989) reported a greater avidity of Ca^{2+} towards phosphorus and its consequent precipitation. Hussain et al. (2015) noted a potassium level of 14.2–19.6 mg l^{-1} in aquaponic water without any potassium supplementation; whereas in the present study potassium level in T3 was found to be below the reported range with 5.38 mg l^{-1} which could be due to the low level of potassium in inland saline groundwater in addition to active absorption by plants. The mean concentration of magnesium and sodium varied significantly ($p < 0.05$) between treatments and controls with highest accumulation in C1. Similar results was reported by Shete et al. (2015) where a significantly lower level of potassium, calcium, sodium, magnesium, iron and zinc was found with higher plant to fish component ratio in common carp-mint aquaponics system.

Percentage nutrient removal of nitrate, phosphate, and potassium is depicted in Fig. 2. The result shows that all the treatments T1, T2 and T3 effectively removed the nutrients with highest nutrient removal in T3 with maximum plant density. In the present study nitrate removal was in the range of 42.02–50.43%. Lennard and Leonard (2006) reported 90.9% nitrate removal using gravel bed media in lettuce production. Ghaly et al. (2005) examined the use of hydroponically grown barley for removal of NO_3-N from aquaculture wastewater, and reported NO_3-N reductions ranging from 54.7 to 91.0%. Rana et al. (2011) identified that more than 75% nitrate removal by tomato plants in aquaponics. The lower nitrate removal in the present study could be due to the antagonist nature of Cl^{-} ion with NO_3 in the nutrient absorption process as reported by Fageria et al., 2010. Percentage removal of phosphate was observed to be in the range from 25.09 to 47.62% with maximum removal in T3. Lin et al. (2002) reported that construction of wetlands system receiving aquaculture effluent effectively removed 32 to 71% phosphate. Ghaly et al. (2005) examined the use of hydroponically grown barley for removal of PO_4-P from aquaculture wastewater and reported 91.8 to 93.6% PO_4-P removal. Clarkson and Lane (1991) evaluated the use of the nutrient film technique for PO_4-P removal from aquarium wastewater and observed reduction the PO_4^{3}-P from 4.4 to 0.3 mg l^{-1} using barley in a period of 4 weeks. As tilapia is a fast growing species, the nutrient input into the system in the form of feed is higher and hence further higher number of plants would be required to improve the nitrate and phosphate removal efficiency of the system.

The percentage removal of potassium by the end of the experiment

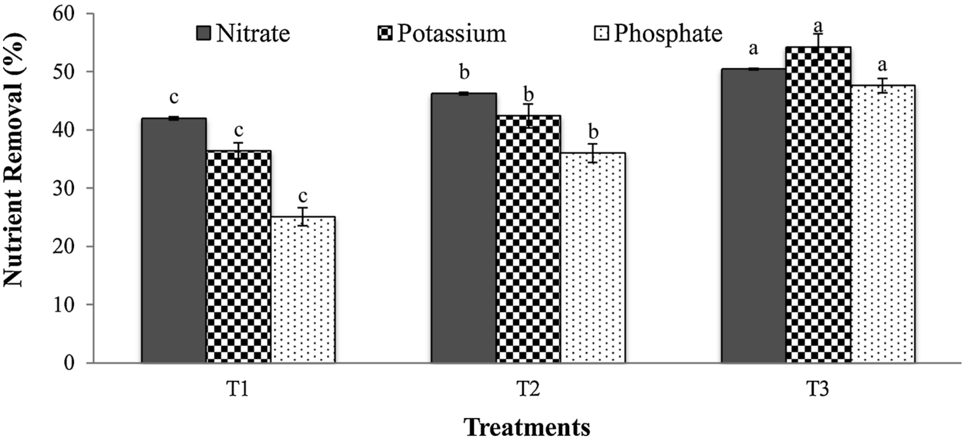

Fig. 2. Nutrient removal during the experimental period of 45 days in different treatments.

Table 4
Daily water requirement and total water consumption during the experimental period.

Parameters	Treatments				
	C1	T1	T2	T3	C2
Daily water requirement (%)	1.16^{b} ± 0.03	1.25^{b} ± 0.03	1.22^{b} ± 0.05	1.29^{b} ± 0.02	17.67^{a} ± 1.45
Total water consumption (l)	39.26 ^{b} ± 1.07	42.30 ^{b} ± 0.89	41.28 ^{b} ± 1.81	43.54 ^{b} ± 0.78	596.25 ^{a} ± 49.03

Mean values (Mean ± S.E) in a row without a common superscript differ significantly ($P < 0.05$) as analysed by one – way ANOVA and Tukey HSD test.

was from 36.39 to 54.26%. Dontje and Clanton (1999) reported 25 to 71% potassium removal in recirculating aquaculture systems using cattails, reed canary grass, and tomatoes grown in sand beds. Mant et al. (2003) achieved 24.9% potassium removal using *Salix viminalis* grown in gravel hydroponic system to treat primary settled sewage. In the present study, potassium was not supplemented from outside source to the aquaponic system. Ground water used for the study contained low level of potassium (3.8–4 $\mathrm{mg\,l^{-1}}$).

3.4. Daily water requirement

The daily water requirement and total water consumption for the entire experimental period is represented in Table 4. The daily water requirement in C2 was 17.67 ± 1.45% followed by T1 (1.25 ± 0.03%), T3 (1.29 ± 0.02%), T2 (1.22 ± 0.05%) and C1 (1.16 ± 0.03%). The daily water requirement of C2 was significantly higher ($p < 0.05$)due to daily water replenishment after siphoning to maintain the water quality; whereas, in all other treatments, the daily water requirement was in the range 1.16–1.29% to compensate evaporation losses. Hence the total water consumption for entire experimental period was highest in C2 (596.25 ± 49.03 l) followed by T3 (43.54 ± 0.78 l), T1 (42.30 ± 0.89 l), T2 (41.28 ± 1.81 l) and C1 (39.26 ± 1.07 l). This was similar to the results of Al-Hafedh et al. (2008) where daily water requirement of 1.4% of the total system water was reported in aquaponics to compensate the evaporation and transpiration losses. Daily water loss in an aquaponics systems is caused by fish sludge removal, evaporation, plant evapotranspiration, and fish splashing during feeding. These losses range from 0.05% (Goda et al., 2015) to 5% (Endut et al., 2014, 2016) of total water. The results showed efficiency of aquaponic system in maintaining water quality for rearing fish without any water exchange. Daily water loss in the aquaponics systems is also influenced by hydroponic surface/fish tank volume ratio and by the plant species used in the hydroponic section (Maucieri et al., 2018).

4. Conclusion

From the study it is concluded that among the fish to plant ratios chosen ratio of 1:1.3 performed best for integration of Nile tilapia and spinach using inland saline groundwater. However this study can only be considered as a pilot study and the authors are interested in further research on optimisation of fish to plant ratio for the same system with wider range of ratios, multiple crop cycle and sufficient fattening period for tilapia. Nile tilapia and spinach was found to tolerate the low saline groundwater used in the experiment. The water quality and nutrient removal was found to be better in treatment with maximum number of plants. The daily water requirement for aquaponic system was found to be extremely low which clearly indicates aquaponics as a water efficient technology.

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