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## Efficacy of fish effluent recycling system for improving growth performance of tilapia (*Oreochromis niloticus L.*) with probiotic supplemented feed

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### Abstract

Aquaculture produces huge quantity of waste water which can be efficiently recycled for plant cultivation and offer an eco-friendly way for increasing economical and sustainable farming. In this study we investigated growth performance of Tilapia fish (*Oreochromis niloticus L.*) and fenugreek (*Trigonella foenum-graecum*) using recycled fish tank effluent. In 30 days experimental period, fishes were fed with probiotic *Lactobacillus delbrueckii* LABT1 supplemented diet and compared with fish grown without probiotic diet and fish reared in aquaculture without recycling of fish effluent was taken as control. Fish cultivated water from each tank were recycled through fenugreek cultivation tray and optimum water quality parameters were maintained throughout the experimental period. Due to the effective utilization of waste water for fish and plant production, significant increased fish length and weight gain were observed using probiotic supplemented diet. Further it showed increased specific growth rate and survival of 100%. Also the fenugreek grown in fish effluent showed significantly higher plant length. Results from this study confirm that fish effluent can be effectively recycled and reused for Tilapia and fenugreek cultivation.

**Keywords:** Tilapia, fish effluent, fenugreek, water quality, probiotic

### 1. Introduction

Tilapias are among the most important warm water fishes used for aquaculture production. The adaptability and tolerance of tilapias to a wide range of environments and intensification of cultivation systems have resulted in a rapid expansion of tilapia farming and introduction of these fish into many subtropical and temperate regions of the world. Among the wide variety of tilapias, Nile tilapia (*Oreochromis niloticus*) is most common in aquaculture. There is a need for a systematic effort to secure and to further improve production because of the increasing demands for healthy food, among the world population. This demand has required assessments of these fish cultured in different system (Santos *et al.*, 2013) [1].

Discharge from tank effluents in aquaculture contain dissolved nutrient such as nitrogen (N) and phosphorus (P), specific organic and inorganic compounds, and total suspended solids (TSS). These constituents originate primarily from uneaten feed and metabolic wastes from the fish. These waste will be accumulated in the culture system and exerting a negative feedback on fish growth and survival. From several types of nitrogen dissolved in the water, ammonia ( $\text{NH}_3$ ) is most dangerous for fish.  $\text{NH}_3$  causes decreasing growth due to reduced appetite and feed intake level. Thus for further expansion of aquaculture activities, development and application of new technologies are required, by which water and nutrients can be recovered during cultivation process, so as to reduce the impact on environment. Recirculation aquaculture system (RAS) is a promising technology in the integration of fish and plant production (Effendi *et al.*, 2017) [2].

Fenugreek (*Trigonella foenum-graecum* L) is a member of the leguminous family which is an annual plants found primarily in Mediterranean countries, the Middle East, and India. Fenugreek is an important spice and its dried seeds have wide application in food and beverages. The leaves of fenugreek plant are edible and often used as a vegetable dish in many parts of India. Fenugreek has medicinal properties such as restorative and nutritive properties (appetite stimulant) with hypocholesterolemic, anti-diabetic, antileukemic and antimicrobial effects. Its medicinal properties are associated with its phytochemicals such as galactomannans, phenolic compounds, alkaloids, proteins, vitamins, volatile oils and bioactive antioxidant substances are also used extensively as an important ingredient in daily food

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preparations and herbal formulations (Wani and Kumar *et al.*, 2018) [3]. No reports are available on the recycling of waste water from Tilapia fish culture system for fenugreek cultivation. Hence this study aimed to analyze the growth performance of Tilapia fish in Recirculating aquaculture system and utilized the fish effluent for Fenugreek cultivation, further evaluating the remarkable variation in growth with probiotic feed.

## 2. Materials and Methods

### 2.1 Experimental fish

All Tilapia fish for studies were caught from local pond in Srivilliputur ( $9^{\circ}51'2N 77.633'E$ ) in Sivakasi, Virudhunagar (Dist.), Tamil Nadu. A total sixteen fishes with an average weight of 2.1 kg ( $\pm 0.12$  S.D.) and length of 1.76 to 1.82 cm ( $\pm 0.1$  S.D.) were caught Jan 2018. Fishes were brought to laboratory in Dept. of Microbiology, SFR College for Women, Sivakasi, Virudhunagar, India.

### 2.2 Experimental design

The fishes were divided randomly into 3 groups to perform three set of experiments. Fresh tap water was stored in rectangular fiberglass tanks for 24 h under aeration in order to dechlorinate the water. Water temperature was maintained at range of 29- 32 °C. Photoperiod was maintained at 12-12 light/dark in both experiments. Control fish (CF) were reared without RAS only in water and were fed with agro wastes based feed. In test experiment (T1) the fish rearing tank were supplemented with only feed blended with agro wastes based feed whereas the T2 experiment was performed with agro wastes based feed supplement with probiotic *Lactobacillus delbrueckii* LABT1 (Radha thirumalaiarasu and Thirumalaiammal, 2019) [4]. Both groups of tanks were connected with vegetable tray (13cm×6cm×7cm) placed above the tank. These plants growing trays were filled with small piece of coco coir and trays were interconnected with pipe. For the plant experiment fenugreek (*Trigonella foenum-graecum*) seeds were cleaned, freed from broken seeds, dust and other foreign materials and then soaked in tap water for 12 h at 37 °C in ratio of 1:5 (w/v). The soaked seeds were rinsed twice in distilled water. All the experiments were conducted for a period of 30 d. Control plants received water only (CP) and after 30 days the fishes were evaluated for growth performance and fenugreek plant were collected and assessed for plant length and total Chlorophyll, phenolics, alkaloids, flavanoid and tannin content (Yaser *et al.*, 2013) [5]. Opening of the one side of a pipe was jointed with the water pump while the other side of the pipe was inserted into a PVC plastic pipe that carries nutrient rich water into the elevated vegetables trays. Water from the trays enters into the fish rearing tank by gravitational force. Rearing tanks of each treatment were interconnected with siphoning pipe.

### 2.3 Fish effluent analysis

The Fish effluents were collected from recirculating aquaculture of both control and Test fish tanks. The microbial load of the water samples and parameters examined were temperature, pH, ammonia, nitrate, nitrite, chemical oxygen demand (COD) and dissolved oxygen (DO). Dissolved oxygen (DO) and chemical oxygen demand (COD) were determined using titration method and pH was measured using pH meter (model No. LI-120). Ammonia, nitrate and nitrite level were measured with standard method (Rajan and Christy, 2011) [6].

## 3. Results and Discussion

Influences of recycled fish effluent on fish production through plant cultivation system were studied. The fish growth was compared in terms of length gain, weight gain, and specific growth rate and survival percentage.

### 3.1 Fish growth study

Results related to length and weight gain, specific growth rate (SGR) and survival of feeding trials of *O. niloticus* with experimental diets are presented in Table 1. The initial and final length of fishes fed with Probiotic diet were  $1.76 \pm 0.05$  and  $3.55 \pm 0.01$  cm, showing a length gain of  $1.79 \pm 0.04$  cm whereas fishes grown without probiotic diet had initial and final length of  $1.82 \pm 0.03$  and  $2.25 \pm 0.05$  cm with length gain of  $0.43 \pm 0.02$  cm. Similarly, the initial and final weights of fishes fed with *Lactobacillus delbrueckii* LABT1 were  $2.1 \pm 0.14$  and  $4.5 \pm 0.027$  g with a weight gain of  $2.4 \pm 0.02$  g was found to be high compared with fishes reared without probiotic diet. The specific growth rate of control fishes and *Lactobacillus delbrueckii* LABT1 fed fishes were  $3.95 \pm 0.64$  and  $7.9 \pm 0.52\%$  with their survival of 100%. Whereas control fish grown with agro based feed only without RAS showed comparatively less growth and survival. The result from this study demonstrate that recirculating aquaculture with fish effluent is an effective ecofriendly method to raise both tilapia and fenugreek production. Discharged fish waste can alter ecosystem dynamics by providing unnatural feeding and unfit environment for survival of living beings (Siikavuopio *et al.*, 2017) [7]. In this study a test experiment were carried out for fish cultivation recycling the fish effluent through fenugreek plant growing. The both experiments performed with recycling fish effluent for plant cultivation the supplemented probiotic feed to fish showed enhanced growth. This relatively increased fish length, weight, specific growth rate and survival. Further SGR of juveniles of *O. niloticus* and *Oreochromis* sp. fed with the probiotic mixture of *Pediococcus parvulus* and yeast to *Candida parapsilosis* ( $2.4 \pm 0.0$  -  $2.6 \pm 0.0\%$ ) was significantly higher than in the control group ( $2.3 \pm 0.0\%$ ) (Gonzalez *et al.*, 2013) [8]. Nile tilapia fed with diets of different levels of *Spirulina platensis* had improved growth performance for the feeding experiment of 75 days (Sahan *et al.*, 2015) [9]. The high consumption of pelleted feed by *Geophagus cf. proximus* influence of the tilapia cage fish farm showed the interference from improved dietary changes and histophysiological response (Kliemann *et al.*, 2018) [10].

**Table 1:** Growth performance of *O. niloticus* reared with fish effluent

Growth performance	CF <sup>a</sup>	T1 <sup>b</sup>	T2 <sup>c</sup>
Initial length of fishes (cm)	$1.80 \pm 0.05$	$1.82 \pm 0.03$	$1.76 \pm 0.05$
Final length of fishes (cm)	$2.12 \pm 0.02$	$2.25 \pm 0.05$	$3.55 \pm 0.01$
Initial weight of fishes (g)	$2.1 \pm 0.02$	$2.1 \pm 0.12$	$2.1 \pm 0.014$
Final weight of fishes (g)	$2.6 \pm 0.01$	$3.3 \pm 0.03$	$4.5 \pm 0.027$
Gain in length (cm)	$0.32 \pm 0.03$	$0.43 \pm 0.02$	$1.79 \pm 0.04$
Gain in weight (g)	$0.5 \pm 0.01$	$1.2 \pm 0.09$	$2.4 \pm 0.02$
Specific growth rate (%)	$2.55 \pm 0.32$	$3.95 \pm 0.64$	$7.9 \pm 0.52$
Survival rate (%)	80	100	100

All the experiments were average of duplicates

<sup>a</sup>Control fish without recirculating aquaculture system

<sup>b</sup>Test experiments in recirculating aquaculture system without probiotic supplement feed

<sup>c</sup>Test experiments in recirculating aquaculture with probiotic supplemented feed

### 3.2 Water quality analysis

In all the three treatments the no significant change in pH was observed whereas temperature showed slight variation. The ammonia, nitrate and nitrite concentration were found to be high for Control fish ( $0.08\pm0.001$  mg L $^{-1}$ ,  $9.2\pm0.56$  mg L $^{-1}$ ,  $0.066\pm0.07$  mg L $^{-1}$ ) where as for T1 ( $0.042\pm0.02$  mg L $^{-1}$ ,  $7.5\pm0.55$  mg L $^{-1}$ ,  $0.15\pm0.01$  mg L $^{-1}$ ) and T2 ( $0.033\pm0.002$  mg L $^{-1}$ ,  $6.64\pm0.24$  mg L $^{-1}$ ,  $0.13\pm0.001$  mg L $^{-1}$ ). The dissolved oxygen was high for T1 and T2 compared with control fish tank. The amount of COD was found to be  $58.4\pm1.89$  mg L $^{-1}$  for control experiment and decrease to  $45.1\pm0.20$  mg L $^{-1}$  for T1 and  $40.6\pm0.31$  for T2 after 30 days (Table 2). Water temperature is one of the most important factors for aquatic organisms which influence other physical, chemical, and biological conditions of a water body. Temperature regulates the growth, reproduction, metabolism and other biological activities as well as feeding intensity of fish (Rahman *et al.*, 2012) [11]. Similarly, during the culture period of 120 days, the water temperature was  $25.08 \pm 0.11$  °C, dissolved oxygen  $7.53 \pm 0.51$  mg L $^{-1}$ , pH  $8.2 \pm 0.08$ , nitrites  $0.19 \pm 0.16$  mg L $^{-1}$ , nitrates  $0.56 \pm 0.09$  mg L $^{-1}$ , and ammonia from  $1.44 \pm 0.72$  mg L $^{-1}$  were observed for *Oreochromis niloticus*. However, the NH<sub>4</sub>/NH<sub>3</sub> concentration was 0.5 mg L $^{-1}$  during the first month of the experiment and NH<sub>4</sub>/NH<sub>3</sub> concentration was reduced to 0 mg L $^{-1}$  later months of the experiment (Khater *et al.*, 2017) [12]. The pH range was varied in between 7 and 8.5 in all the treatments during the experimental period, which is acceptable for aquaculture (Makori *et al.*, 2017) [13]. In our study data interpretation showed that the ammonia, nitrate and nitrite concentration were found to be low for T1, T2 and both pH and temperature were found to be controlled maintained at optimum level.

**Table 2:** The physio-chemical parameters of water from fish cultivation tank

Parameters	Treatments		
	CF <sup>a</sup>	T1 <sup>b</sup>	T2 <sup>c</sup>
pH	$7.72\pm0.26$	$7.69\pm0.24$	$7.71\pm0.22$
Temperature(°C)	$26.13\pm0.73$	$25.78\pm0.95$	$25.72\pm0.69$
Ammonia(mg L $^{-1}$ )	$0.08\pm0.001$	$0.042\pm0.02$	$0.033\pm0.002$
Nitrate(mg L $^{-1}$ )	$9.2\pm0.56$	$7.5\pm0.55$	$6.64\pm0.24$
Nitrite(mg L $^{-1}$ )	$0.066\pm0.07$	$0.15\pm0.01$	$0.13\pm0.001$
DO (mg L $^{-1}$ )	$2.95\pm0.40$	$3.85\pm0.21$	$4.058\pm0.34$
COD (mg L $^{-1}$ )	$58.4\pm1.89$	$45.1\pm0.20$	$40.6\pm0.31$

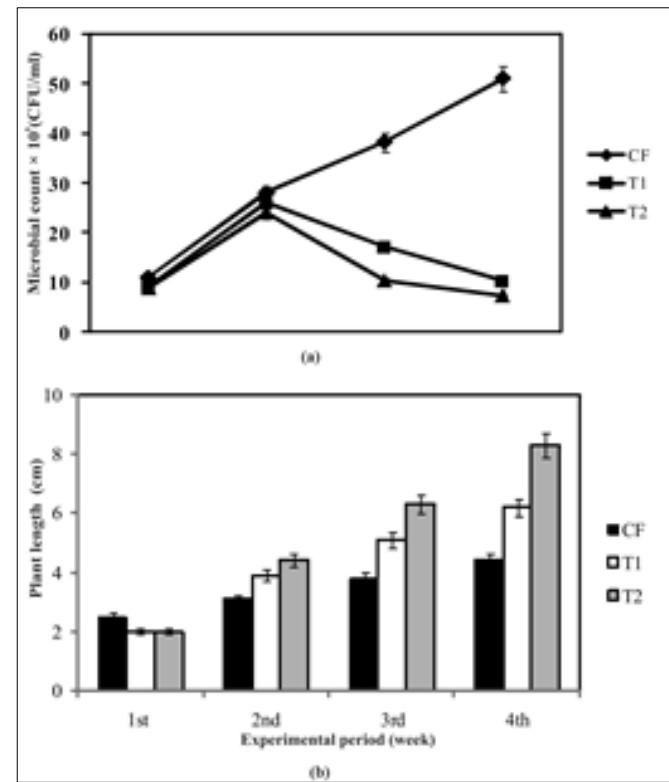
All the experiments were average of duplicates

<sup>a</sup>Control fish, <sup>b,c</sup>Test experiments

### 3.3 Evaluation of biological parameters

Comparable results bacterial count of control and test experiment inferred the presence of minimum microbial load in test experiment suggesting the advantages of recycling fish effluent and additive benefits of probiotic supplemented feed in controlling microbial pathogens. Similarly, comparative analysis study showed that aquaponically grown lettuce had significantly lower concentration of spoilage and fecal microorganisms compared to in-soil grown lettuce (Sirsat and Neal, 2013) [14]. In the second objective of current study Fenugreek (*Trigonella foenum-graecum*) could grow without the addition of extra nutrients, and it only comes from tilapia fish farming waste. During the experiment, fenugreek grew rapidly and showed a positive response to nutrients derived from fish farming waste. Compared with the control the plant length was found to be increased for test experimental plants. The residual water from the intensive tilapia farming provided

higher concentrations of macro and micronutrients in the shoots, higher production of fresh matter of shoots (95.48 g plant $^{-1}$ ) and a larger number of leaves (14.90 %) for Lettuce cultivation (Geisenhoff *et al.*, 2016) [15]. Increased fresh and dry matter yield of leaves of ice lettuce (*L. sativa L.*) was found with fish waste manure (Radziemska *et al.*, 2018) [16]. These results were supported by our earlier work on Molly fish farming and fenugreek cultivation with recycling aquaponic system (Radhathirumalaiaarasu and Thirumalaiammal, 2019) [4].



**Fig 1:** Biological parameters of water from fish cultivation tank with total viable count of microbial population (a) and measurement of Plant length (b) in experimental tank.

### 4. Conclusion

There is a need for alternative of conventional cultivation system rearing fishes with effluent of fish cultivation thereby reduces, reuse and recycle the discharge of wastes. This study shows that fish effluent in recirculating aquaponic system is suitable for both fish and plant growth, without intermediate replacement of the water like in conventional cultivation system. This system is also suitable for rearing fishes with probiotic supplemented diet which might be useful for replacing the commercial antibiotics. Best growth of tilapia was shown in terms of length and weight gain and measurement of specific growth rate proportional to better water quality in this treatment. Fenugreek growth was well recorded in probiotic supplemented fish feed treatment with more chlorophyll content compare to the control. Further research on optimization of the number of plant incorporated in fish culture and scale up of this process is needed with aquaponic system.

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