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Performance of a bio-integrated aquaculture production system in floating net cages with angelfish, aquatic plant and leafy vegetable

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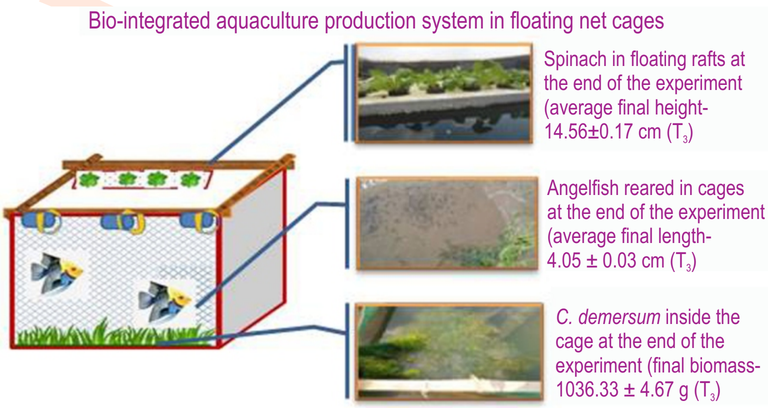
Abstract

Aim: To evaluate the efficiency of an integrated aquaculture system in a reservoir utilizing three components like ornamental fish, ornamental aquatic plant and leafy vegetable in floating net cages.

Methodology: Components used in the study comprised Angelfish (*Pterophyllum scalare*), aquatic plant (*Ceratophyllum demersum*), and leafy vegetable (*Spinacia oleracea*). Angelfish and ornamental aquatic plants were stocked inside the net cages (3x3x3m), while leafy vegetables were grown on rafts floating on the surface of cages. Completely Randomized Design was followed in triplicates for 90 days. Three treatments T_1, T_2 and T_3 were stocked with angelfish (0.24±0.06g) of varying stocking densities 20, 25, 30 m^{-3} respectively, along with uniform stocking densities of *C. demersum* (20 bundles per cage weighing 414.17±5.12 g per cage) and spinach (144 numbers per cage; average height 8.69±0.52 cm), whereas C1 (Angelfish 20/m^3) and C2 (*C. demersum* and spinach) were used to compare the efficiency of the system.



Results: The growth performances of angelfish after 90 days, in terms of average weight, survival rate, feed efficiency ratio and specific growth rate were significantly higher ($p<0.05$) in T3 with 1.60±0.09g, 87.04±0.98%, 0.32±0.001 and 2.11±0.01%/day respectively, whereas Feed Conversion Ratio was significantly lower ($p<0.05$) in T3 (3.15±0.01). There were no significant differences ($p>0.05$) in the biomass production of *C. demersum* and growth of spinach. Digestive and stress enzymes showed no significant difference ($p>0.05$) between the treatments and control.

Interpretation: This study demonstrated the efficiency of the bio-integrated food production system paving a way to enhance the utilization of open water resources benefiting farmers with food and income.

Key words: Angelfish, Bio-integration, Cage culture, *C. demersum*, Spinach

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Introduction

The rising world population is facing challenges to meet the exponentially growing food and livelihood demands. The contribution of agriculture to satisfy the exponentially growing population is inadequate and as a growing industry, aquaculture plays a significant role in serving cheap protein source as well as employment opportunities to the community. Inland open water aquaculture is scarce and one of the resources predominantly available in India is reservoirs, which are underutilized for production (Paliwal *et al.,* 2013). Presently, the total area under reservoirs is 3.51 million ha (Sarkar *et al.,* 2018) and there is an obligation of management in terms of enhancing the production from the reservoirs (Karnatak and Kumar, 2014) by restoring its resources due to inefficient utilization. Adoption of cage culture, among several strategies of enhancement, is the best option of maximizing the utilization of open water resources with the provision of the natural environment to the cultured organisms (Mane *et al.,* 2019). This improves the fish production in inland areas, and thus raises the per capita fish protein availability in the country.

Integrated Agriculture-Aquaculture Systems (IAAS) in cages can be adopted in order to increase the production and utilization of the resource. Integrated Agriculture-Aquaculture Systems serves as linkage between the components directly or indirectly, on-site or off-site, respectively, thus fulfilling the requirements and providing chances for practitioners to produce more (Prein, 2002). It is a way of sustainable intensification which renders more food essentially from the same land area and water without any ecological effects (Godfray *et al.,* 2010) achieving the term “more crop per drop”. It is perceived as an effective way of utilizing water, as it increases water productivity, an important strategy for achieving food security and can assume a significant job and a key role in nourishing the world (Ahmed *et al.,* 2014). Different forms of Integrated Agriculture-Aquaculture Systems exist, such as Freshwater Integrated Multi-Trophic Aquaculture (FIMTA) and aquaponics, nonetheless, both anticipate a similar idea in aquaculture. Aquaponics is a form of Freshwater Integrated Multi-Trophic Aquaculture that combines culture of aquatic animals and plant culture, on a symbiotic relationship via microbial link. This advancement can be adapted to many fish and plant species, under different growing conditions, using three different techniques, namely the floating raft, media-filled bed, and nutrient film technique (Patil *et al.,* 2019).

Ornamental fish culture, an emerging sector of aquaculture in India is contributing a meager 0.32% to the global export, is in high demand in the domestic and international markets. Because of its untapped resources, rearing ornamental fishes in cages can be an enhanced alternative for livelihood improvement of the community people around the reservoirs. An experimental study on rearing ornamental fish in open water resources resulted in faster growth and fascinating colouration as compared to confined water bodies (Gupta *et al.,* 2013). These occupy a significant place in aquaponics next to food fish (Love *et al.,* 2015). Angelfish, *Pterophyllum scalare*, is a commonly sought freshwater ornamental fish, for its attractive body pattern and colouration. As it can be acclimatized easily to natural waters, it will be a suitable species for study in a reservoir.

Besides keeping ornamental fishes in the aquarium, addition of aquatic plants enhances the attractiveness of the tanks. The market of aquatic plants is developing relentlessly as they provide a natural habitat, mimicking the environment; and also serve as biological filters and absorbers when placed inside the aquarium. *Ceratophyllum demersum*, one of the attractive species in the local market grows faster utilizing nutrients. The third component, leafy vegetables namely spinach will be an ideal species for integrating with the aquaculture system since the production would be higher in a shorter time frame with enough nutritional value (Nonnecke, 1989; Rubatzky and Yamaguchi, 2012).

Different components like vegetables can be integrated with fish culture in case of aquaponics, ornamental fishes with aquatic plants in aquaria and so on. The efficiency of different integrated systems in pond has been studied using various combination of fish and vegetables in cages installed in ponds (Mithun *et al.,* 2013; Haque *et al.,* 2015 and Kibria and Haque, 2018). Dimbhe reservoir in the Pune district of Maharashtra, India, has a total area of 1278 ha where there is no standard and appropriate fishing activity to utilize the potential of such a vast area (Mane *et al.,* 2019). Therefore, to utilize water resources efficiently and for improving the livelihood of rural areas adjacent to the reservoir, the present study was undertaken for analyzing the performance of a bio-integrated aquaculture production system in floating net cages with an ornamental fish, *P. scalare*, an aquatic plant *C. demersum* and a leafy vegetable, *Spinacia oleracea* as the candidate species to achieve sustainable resource utilization by vertical integration, a prototype of FIMTA to enhance food and livelihood security to the rural population around the reservoir.

Materials and Methods

Experimental design: The 90-day experiment was carried out in cages (3X3X3 m sized floating net cages) installed in the Dimbhe Reservoir located in Pune, India. The experimental design used in this study was Completely Randomized Design with triplicate. *P. scalare* and *C. demersum* were stocked inside the cages, while spinach was grown on rafts floating on the surface of cages. Three treatments T_1, T_2 and T_3 were stocked with angelfish randomly of varying stocking densities 20, 25, 30 m^{-3}, respectively, along with uniform stocking densities of *C. demersum* (20 bundles per cage) and spinach (144 numbers per cage). Two controls, one with *P. scalare* alone and the other with *C. demersum* (20 bundles per cage) and spinach (144 numbers per cage) were used to compare the efficiency of the system.

Experimental fish, ornamental aquatic plant and leafy vegetable: Angelfish were procured from a private hatchery in

Mumbai. They were acclimatized and reared in glass tanks placed in the ornamental fish rearing unit of the Central Institute of Fisheries Education, Mumbai. Healthy fry of *P. scalare* (1.02 cm and 0.24g) were stocked randomly in each cage after acclimatization of 2 weeks. The fish were fed with a commercial ornamental fish feed (Taiyo grow fish food, 30%CP) throughout the experimental period, as they were commonly in use by the end level consumers. Feeding was done twice a day at 6% body weight (Ribeiro *et al.,* 2012), initially crumbled to match the mouth size of the fish. The quantity of feed was adjusted depending on the biomass in each treatment. Sampling was carried out fortnightly for assessing growth and health performance of fish. Ten pieces of *C. demersum* each measuring 15cm were tied together to form a bundle, weighed and tied with a stone so that it could be placed at the bottom of the cage. The number of bundles stocked was uniform (20 bundles in each cage) in all the treatments (T_1, T_2, and T_3). Spinach seeds were purchased from the local market, which were allowed to germinate and transplanted after 2 weeks. The height of the spinach plantlets were measured before transferring them to the net pots and hydroton clay in the floating rafts. The total area covered by the raft in each cage was 4 m^2 (1mx1m raft with 36 holes of 50mm thickness, *i.e.,* 144 plants per cage). The rafts were tied and fixed to the cages with the help of ropes. Stocking density was uniform in all the treatments. The plants were harvested after 45 days of planting and their heights were measured.

Water quality: Since the experiment was conducted in a reservoir, water quality parameters of different treatments did not significantly differ as compared to control. Even though, the water samples collected from individual cages and water quality were assessed. Water quality parameters like temperature, pH, Dissolved oxygen (Winkler's modified method), free carbon dioxide, total hardness, total alkalinity, Ammonia-nitrogen (NH3-N), Nitrite-nitrogen (NO_2-N), Nitrate-nitrogen (NO_3-N), and Phosphate-phosphorous (PO_4-P) were measured according to the standard procedures of APHA (2017). The mean values of the water quality parameters estimated during the experimental period were as follows: water temperature 25.91±0.15°C, pH7.75±0.04, dissolved oxygen 6.58±0.12 mg l^{-1}, alkalinity 59.13±0.64 mg l^{-1}, hardness 66.37±0.92 mg l^{-1}, free carbon dioxide – nil, ammonia-nitrogen (NH_3-N)0.18±0.42 mg l^{-1}, nitrite-nitrogen (NO_2-N) 0.04±0.8 mg l^{-1}, nitrate-nitrogen (NO_3-N) 0.178±1.0 mg l^{-1} and phosphate-phosphorus 0.05± 0.02 mg l^{-1}.

Growth parameters: At the end of the experiment, the growth parameters such as final length, final weight, mean weight gain, Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), Feed Efficiency Ratio (FER) and survival (%) of fish were calculated (Ribeiro *et al.*, 2012). In aquatic plants, the total biomass of bundles of *C. demersum* per cage was assessed to determine the growth performance (Santana, 2016). Similarly, the plant height was measured and the percent gain in height was calculated to determine the growth of spinach (Shete *et al.*, 2013b).

Physiological parameters: The digestive enzyme and oxidative stress enzyme activities were estimated to assess the physiological response of angelfish. Amylase activity was estimated by dinitro salicylic acid method (Rick and Stegbauer, 1974), protease activity by casein digestion method (Drapeau, 1976) and lipase activity by following the method of Cherry and Crandell (1932). Similarly, liver tissues were used to analyze stress parameters like superoxide dismutase and catalase activity by the method of Misra and Fridovich (1972) and Takahara *et al.* (1960), respectively.

Statistical Analyses: One-way analysis of variance was used to analyze the data and Duncan's Multiple Range Test was performed to determine significant differences between the treatments using SPSS (version 16.0). The results were expressed as mean ± S.E. Statistical significance was made at 5% probability levels.

Results and Discussion

The highest average length and weight was found in Angel fish treated with T_3 treatment. (Table 1). Similar by, increased length and weight of *P. scalare* were obtained in cages when co-cultured with *P. vannamei* in low saline water than pond reared fishes, suggesting that the increase may be due to the confined spaces in cages assuring easy exposure to feeds than open pond environment (Ribeiro *et al.,* 2014). This might be the reason for attaining of higher growth in terms of length and weight in *P. scalare* stocked in T_3 treatment. Contradictory to the results of the present study, Nagata *et al.* (2010) observed in significant difference in the growth of angelfish when they are stocked at different densities. In the present study, T_3 treatment showed the highest specific growth rate, similar to the values obtained by Koca *et al.* (2009). The specific growth rate is almost similar to the study of Wiratama *et al*. (2021). Similarly, the specific growth rate value in the present study was higher than the results of Kumar *et al.* (2005) where the angelfish juveniles were fed with different commercial diets containing 35, 40 and 45% protein levels.

The obtained results predicted the acceptability of integration than other culture techniques adapting the commercial diet to achieve better results. The reason for high specific growth rate in this study was probably due to better utilization of commercial feed as well as natural food organisms available in the reservoir water. The feed conversion ratio obtained in the present study was lower than the results of Kumar *et al.* (2005) the range of 3.42 to 5.81 when angelfish juveniles were fed with different commercial diets containing different protein levels. Feed conversion ratio of 2.32 ± 0.04 was obtained when koi and spinach were grown in aquaponics system (Hussain *et al.,* 2015) and a feed conversion ratio of 4.88 which was higher than the ratio of the present study was reported when integrating *Carassius auratus* and spinach (Shete *et al.,* 2013a). The feed conversion ratio for ornamental fish is generally high compared to fish food, and the obtained results were found within the acceptable range when compared with commercial diet experiments and different integration studies (Santana, 2016).

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Table 1: Growth parameters of *P. scalare* grown in integrated aquaculture of ornamental fish, aquatic plant and leafy vegetable in floating net cages at different stocking densities

Parameters	Control 1	T_1	T_2	T_3	P value
Average initial length (cm)	1.03 ± 0.07	1.00 ± 0.03	1.02 ± 0.19	1.04^{a} ± 0.02	0.349
Average final length (cm)	3.70^{c} ± 0.02	3.71^{c} ± 0.01	3.81^{b} ± 0.01	4.05^{a} ± 0.03	<0.001
Average initial body weight (g)	0.24 ± 0.03	0.24 ± 0.12	0.24 ± 0.07	0.24 ± 0.04	0.157
Average final mean body weight (g)	1.28^{c} ± 0.03	1.30^{c} ± 0.01	1.50^{b} ± 0.01	1.60^{a} ± 0.09	<0.001
MWG	1.04^{c}±0.03	1.06^{c} ± 0.01	1.27^{b} ± 0.02	1.36^{a} ± 0.01	<0.001
SGR (%/day)	1.85^{b} ± 0.04	1.89^{b} ± 0.02	2.05^{a} ± 0.01	2.11^{a} ± 0.01	<0.001
FCR	3.58^{a} ± 0.13	3.57^{a} ± 0.04	3.27^{b} ± 0.03	3.15^{b} ± 0.01	0.005
FER	0.28^{b} ± 0.010	0.28^{b} ± 0.003	0.31^{a} ± 0.04	0.32^{a} ± 0.001	0.003
Survival	83.24^{b} ± 0.40	84.26^{b} ± 0.46	85.26^{ab} ± 0.81	87.04^{a} ± 0.98	0.027

Data expressed as mean ± SE. Mean values in the same row with different superscript differ significantly ($p<0.05$). One way ANOVA was used following Duncan Multiple Range Test in SPSS- 16.0. T_1: *P. scalare*- 20 m^{-3}, *C. demersum*, Spinach; T_2: *P. scalare*- 25 m^{-3}, *C. demersum*, Spinach and T_3: *P. scalare*- 30 m^{-3}, *C. demersum*, Spinach

This justifies the fact that the values obtained in the present study were desirable to advance the culture practice. Feed efficiency ratio varied significantly and the highest efficiency ratio was obtained in T_2 and T_3 treatments. T_3 reported the higher survival rate which was similar to the survival rate of *P. scalare* fed with the mixed protein diet (Ali *et al.,* 2016) and higher than the survival rate of angel fishes reported by Patil *et al.* (2015). Azani and Rasdi (2021) observed the survival rate of angelfish was 66.7% which was lower than the present study (85%). The higher survival rate in the present study may be related to the culture environment. From the above observations made, it is clear that the cage culture of *P. scalare* achieved higher growth, specific growth rate, feed efficiency ratio, survival rate, and lower feed conversion ratio compared to the aquarium reared fishes by utilizing the commercial diet and live food organisms present in the natural environment. The growth of *C. demersum* was found similar ($p>0.05$) in all the treatments and control. *C. demersum*, an ornamental aquatic plant has a high regeneration capacity which has been experimentally proved in a study conducted by Pinowksha (2002), where *C. demersum* grown without snails gained weight in 10 days reporting around 20% increase in their biomass.

The biomass produced in the present study (1029.67g, 1033.33g, 1038.33g, 1036.33g in C_2, T_1, T_2, T_3 respectively) was almost close to the biomass gain produced by *C. demersum* without snails. Santana (2016) reported the growth of *C. demersum* in terms of weight increase and achieved 273.3% growth in 30 days. An increase in the concentration of ammonia and nitrate leads to enhanced growth of *C. demersum*. Since, the present study was conducted in a reservoir, the water quality parameters did not vary significantly between the treatments, leading to similar biomass gain of *C. demersum*, which is in accordance with the study of Kuntz *et al.* (2014). Likewise, the growth performance of spinach showed no significant difference ($p>0.05$) among treatments and control. Spinach, the commonly preferred leafy vegetable in aquaponic set ups, achieved higher growth performance in indoor aquaponics systems. A higher percentage of height gain of 198.28% and 253.40% were obtained after 45 days as reported in previous studies (Shete *et al.*, 2013b; Hussain *et al.*, 2015). The higher growth is attributed to the utilization of biological waste produced from the fish in a land-based system. In contrast to the above findings, the height gain of spinach achieved in the present study (62.99%, 64.54%, 62.90%, 66.91% in C_2, T_1, T_2, T_3 respectively) was optimized due to lower amount of ornamental fish waste in the system as compared with the food fish. Another reason can be possibly attributed to moderate wave action during windy spells being instrumental in displacing nutrients excreted by fish in the natural environment, making it less available to spinach and the natural bolting tendency of spinach due to overexposure to high temperature during hot weather spells or longer photoperiods during the experiment.

There were no significant difference ($p>0.05$) in the activities of amylase, protease, lipase, superoxide dismutase, and catalase among treatments and control (Fig. 1, 2). Depending on the environmental conditions, the digestion of carbohydrates varied between the species of fish, and even between the fishes of the same species (Earle,1995). Bolasina *et al.* (2006) reported that the digestive enzymes exhibited no significant differences between different stocking densities of fish. Similarly, no significant difference in lipase activity was noted in gilthead seabream (Moyano *et al.,* 1996) and proteolytic activities in *Rasbora daniconius* and *Puntius* sp. (Hofer and Schiemer., 1981).

The results of the present study are in agreement with the above reports presumably due to the utilization of commercial ornamental fish feed in all the treatments and control throughout

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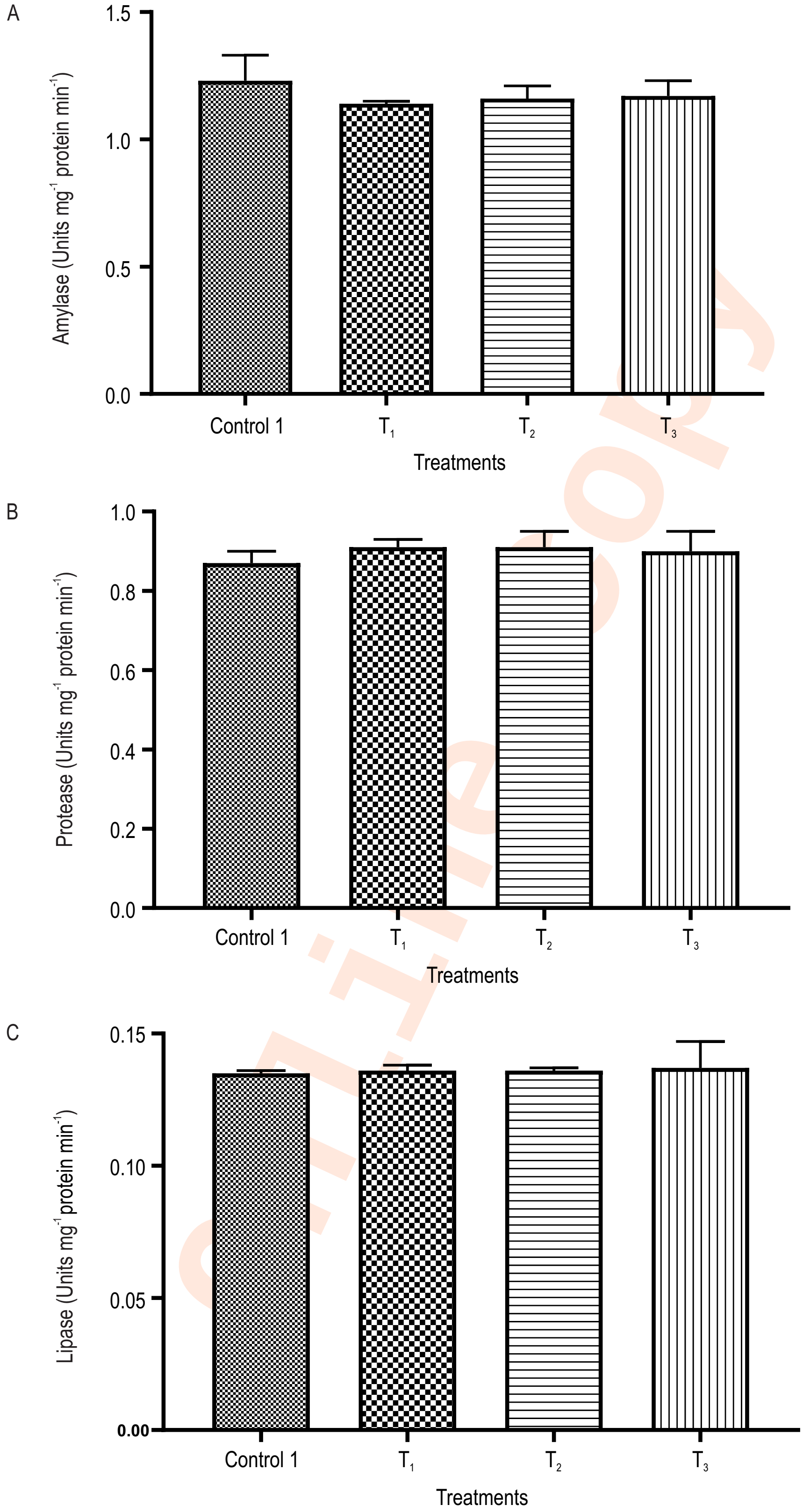

Fig. 1: Digestive enzyme activities of angelfish grown in integrated aquaculture of ornamental fish, aquatic plant and leafy vegetable in floating net cages at different stocking densities: (A) Amylase; (B) Protease and (C) Lipase.

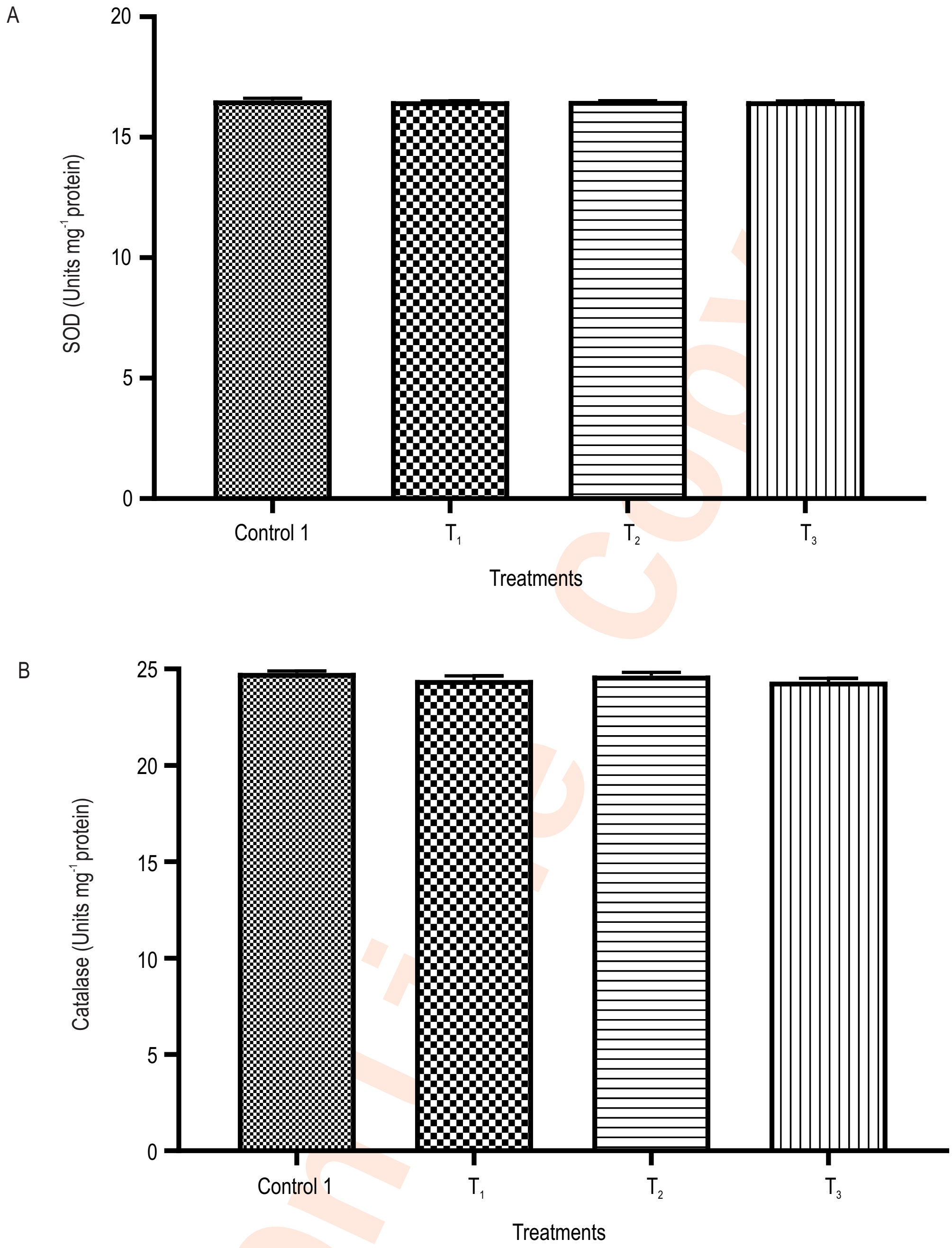

Fig. 2: Stress enzyme activities of angelfish grown in integrated aquaculture of ornamental fish, aquatic plant and leafy vegetable in floating net cages at different stocking densities: (A) Superoxide dismutase and (B) Catalase.

the experimental period. Stress can be due to several factors like stocking density, water quality parameters, environmental conditions, etc. Superoxide dismutase and catalase act as bio-indicators of oxidative stress and immunity in fish (Sagstad *et al.,* 2007). Superoxide dismutase and catalase activities in the present study provided no significant differences among the treatments and control. Braun *et al.* (2010) reported that the stocking density of fish, when reared in cages, does not influence stress enzyme activity. Normal physiology was not affected by the stocking density used in this study due to optimized amount (and not copious amount) of nitrogenous waste produced by ornamental fish. Hence, the results of the present study showed that integration of different species is possible with no significant influence of stocking density of fish and water quality, on the performance of this aquaculture production system in a natural lotic water body. Thus, the present study indicates that treatment T_3 containing *P. scalare* (30 m^{-3}), *C. demersum* and spinach demonstrated the efficiency of integrated system in floating net cages. It improved the growth and health status of fish effectively without any stress and produced more crops by effectively utilizing the same area and resource without any ecological effects, which is the main reason for integrating different components.

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Add-on Information

Authors' contribution: R. Abisha: Carried out the study and prepared the manuscript; **K. Dube:** Conceptualization, guidance and manuscript correction; **S.P. Shukla, B. Sawant Paramita, M. H. Chandrakant:** Co-guidance and manuscript correction; **J. Jane, K.D. Raju:** Assisted during sampling.

Research content: The research content is original and has not been published elsewhere.

Ethical approval: All applicable international, national and institutional guidelines for the care and use of animals were followed. Further, it has been approved by the Institutional Ethical Committee as well as by PME. All the procedures performed in the studies involving animals were in accordance with the ethical standards of the institution.

Conflict of interest: The authors declare that they have no conflict of interest.

Data from other sources: Not applicable.

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