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Effect of co-cultivation of *Wolffia arrhiza* (L.) on water quality parameters in a feed based semi-intensive carp culture system

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Abstract

Aquatic and free-floating water meal (*Wolffia arrhiza* L.) known for its fast reproduction rate was grown in differing restricted zones of supplementary feed based intensified carp polyculture in cement tanks (5m x 4m x 1m) with soil beds. While wolffia could not spread beyond the restricted zone, fishes could consume wolffia as per their desire by entering in the wolffia-zone from below. The main objective of the study was to evaluate the effects of area of wolffia-growing zone as% of total water surface area of the culture system on the overall water quality of the culture system Three levels of wolffia-zones viz. 10% (T_1), 20% (T_2), 30% (T_3) of the total surface area were evaluated against control without any wolffia (C). The culture period was 75 days and stocking density was 2 fish m^{-2}. The seeding rate of wolffia (0.5kg m^{-2}), feeding regime (constant feeding rate @2% d^{-1} offered once a day) and fertilization rate involving cow dung, urea and single super phosphate were the same in all treatments throughout the study period. While no marked differences in the water quality parameters between treatments with wolffia-zone and control (without wolffia zone) were noticed in the early part of the experiment, the concentrations of NH_4-N (range 0.06-0.16 mgL^{-1}), NO_2-N (range 0.01-0.04 mgL^{-1}), NO_3-N (range 0.09-0.44 mgL^{-1}) and PO_4-P (range 0.01-0.02 mgL^{-1}) in the waters showed significantly lower values ($P<0.05$) for T_1-T_3 compared to those in control (C) with values of 0.23, 0.07, 0.75 and 0.02 mgL^{-1}, respectively. The overall mean concentrations of NH_4-N (0.21-0.42 mg L^{-1}), NO_2-N (0.06-0.11 mg L^{-1}), NO_3-N (0.23 -0.7mg L^{-1}) for the whole culture period were also significantly low in treatments with wolffia-zones. However, in general no marked differences were apparent among wolffia-zone treatments.

Keywords: Wolffia, carp polyculture, nutrient stripping, water quality

Introduction

Fertilization, supplementary feeding and higher stocking density employing major carps have been the most important interventions for fish yield enhancement per unit area per unit time in India. However, substantial part of the supplementary feed is released back in the environment as excretory products containing nitrogen and phosphorus and therefore feeding has often been associated with production of effluent that has tremendous impact on not only on aquaculture environmental itself but also the surrounding environment as well. For that matter, only about 20% C, 25% P and 25% N in the form of feed is harvested in the form of fish and remainders are lost to the environment in the form suspended or dissolved forms in case of salmon farming that traditionally utilizes high quality feed ingredients like fish meal (Hakanson, 1986, Kryvi, 1989) [9, 12]. If the nutrient inputs exceed the self-purification potential of the culture system, eutrophication of the water column and underlying sediments may be resulted over a period of time. The resulted eutrophication promotes the algal bloom, increases the biochemical oxygen demand of the ecosystem causing oxygen deficiency in water especially in night and/or cloudy days and anaerobic sediments. Further, other water quality problems like accumulation of ammonia, nitrite and hydrogen sulfide, etc. may also come into existence which may cause mass mortality in extreme cases and may reduce the growth and/or feed utilization even at sub lethal concentrations. Further, resulted poor culture environment may also reduce the flesh quality.

Recent approach towards overcoming the environmental impact of intensified aquaculture has been to utilize excessive nutrient for beneficial purposes employing plants that can be reutilized as a feed source. Floating aquatic macrophytes have been employed to reduce the concentration of noxious phytoplanktons in the effluent from stabilization ponds and to remove nitrogen and phosphorous from the water (Steward, 1970). Similarly, duckweeds have been utilized to treat agricultural and municipal waste waters as it absorb and metabolize pollutants Dasgupta *et al*. (2008) [7], Suppadit *et al*. (2008) [23]. Members of free floating duckweed (Lemnaceae) have shown potential usefulness in the treatment of eutrophicated water (Sutton and Ornes, 1975) [24], Fujita *et al*. (1999) [8], (Appenroth and Augsten, 1996) [2], Bergmann *et al*. (2000) [4]. In particular, *W. arrhiza* appears to be a highly potential candidate in regard to its usefulness. As it grows at very fast rate, relatively high protein content (30-45%) on dry matter basis and its essential amino acid profile is comparable to that of animal protein with substantially high lysine and metheonine as compared to other plant proteins (Porath and Agami, 1986) [19]. More importantly, it is highly preferred food for large number of filter-feeding and herbivorous fishes. It is grown as fresh carp feed in countries like China and Taiwan (Mueller and Lautner, 1954) [16].

Therefore, it appears to be advantageous to grow *Wolffia arrhiza* in part of the aquaculture production in supplementary feed based intensified carp culture systems in integrated manner. It would possibly on one hand stabilize the water quality through uptake of nutrients while on the other would act as live food for carps. On the other hand however, since it floats on surface if its growth outcompetes the feeding pressure of the stocked fishes, it may prevent light penetration and create undesirable situation. Therefore, in such integrated Wolffia-fish production system, it is important to make provisions to restrict Wolffia in specific portion of the culture system. There appears to be no work done on optimizing the Wolffia-growing zone as percentage of total surface area of the culture system. In the present study, effects of incorporating Wolffia in restricted portions of the pond covering 10%, 20% and 30% of the surface area in a semi-intensively managed polyculture carp culture system, and study its impact on water quality parameters.

Materials and Methods

A series of 12 rectangular outlet cement tanks of 20 m^2 at College of Fisheries, Lembucherra were utilized. The bottoms of the ponds were plain with 6 inches soil bed. All the ponds were completely independent having facility of water supply from ground source of water. Fishes were randomly distributed into 4 groups: control (T_0) (no wolffia); T_1 (10% Wolffia zone); T_2 (20% Wolffia zone); and T_3 (30% Wolffia zone) were arranged in triplicates following a completely random design (CRD) design. The tanks were drained and sun dried for one week. Dried tanks were limed (500g $Ca(OH)_2$ $tank^{-1}$) at the rate of 250 kg ha^{-1} and then filled with water from ground water source. All ponds were fertilized about one week after liming with cow dung (4kg/tank). After 1 week of fertilization, tanks were stocked with six species namely rohu (L. rohita, average individual weight 11.64 g), catla (*C. catla*, 38.45 g), mrigal (*Cirrhinus mrigala*, 8.1g), grass carp (*Ctenopharingodon idella*, 9.42), amur carp (*C. carpio*, H. 7.4 g) and puntius (*Puntius gonionotus*, 7.6 g) in all the tanks at the ratio 40:15:15:5:10:15 respectively. All the tanks were stocked at a total stocking density of 2.0 fish m^{-2} (40 fish/tank). Fish were collected from the college farm, College of Fisheries, Lembucherra. Fresh duckweed were supplied to the tanks at the rate of 1kg, 2kg, 3kg in 10%, 20% and 30% Wolffia coverage enclosure area in Wolffia zone, respectively and were available to the fishes for 24 h per day. The live Wolffia was supplied periodically to the Wolffia zone of experimental tanks, whenever there appeared to be no Wolffia in any treatment.

Fertilization

Fertilization of tank was done weekly with urea and single super phosphate at the rate of 60g/tank and fortnightly with organic fertilizer cow dung at the rate of 500g. Loading rates of Nitrogen and Phosphorous through fertilizers were 2kg/ha/day and 0.7 kg/ha/day respectively. Fertilization was performed weekly during 10.00 – 12.00 hrs. Required amount of fertilizers were dissolved and were sprayed over the almost whole surface area. When transparency was less than 2 cm, fertilization was stopped.

Experimental Feed and Feeding

Experimental diet (sinking type) of protein level 21.5% was formulated with locally available feed ingredients viz. wheat flour, masturd oil cake, rice bran and fish meal.

Table 1: Ingredient composition of diets

Ingredient	Rice Bran	MOC	Wheat flour	Fish meal
Percentage (%)	45	20	25	10

The required amounts of ingredients were mixed in mixer. Maida was gelatinized before adding to the mixture of rest of the ingredients. Roughly about 35 to 40% by weight of ingredients, potable water was added to result in semi-moist dough mixture, which was then extruded through a 2 mm dia die. The resulted nodule like strands were broken into pellets and sun dred to moisture level 5-10%.Dried diets were stored in a polythene bag until utilized.

Table 2: Proximate composition of pelleted feed

Moisture (%)	Protein (%)	Fat (%)	Fibre (%)	Ash (%)
5	22.5	6.5	12.1	13.5

Table 3: Proximate composition of Wolffia

Moisture (%)	Protein (%)	Fat (%)	Fibre (%)	Ash (%)	NFE (%)
95	28.6	9.1	15.4	12	34.9

1st one week, only available natural foods formed the basis of nutrition of experimental fish. 2nd week onward similar amount of feed were offered to all the tanks in order to familiarize them with feed. Fish were fed with Experimental feed and covered (wet) to all the treatment tanks. Control treatment was without coverage with same amount of experimental diet. During first 3 week of experimental period, feeding rate was decided 4% body weight of fish in all the tanks. Feeding frequency in experiments was decided as once a day (feeding time 09:00 – 10:00hrs), when abundance of natural food is somewhat lesser. Amount of feed was adjusted after regular sampling of fish during experiment. Then later part of experiment feeding rate was decided 2% body weight of fish.

Effect of wolffia on water quality

1) Temperature (ºC) and Dissolve Oxygen (DO, mgl^{-1}): Biweekly diurnal variation of temperature by Thermometer and DO by Winkler's Method. Readings was taken during dawn, dusk.
2) pH: fortnightly pH variation measured with pH meter Integrated water sample was brought to laboratory and pH was recorded.
3) Transparency: biweekly was measured with Sacchi disk
4) Total Ammonia Nitrogen: Phenate method was used.
5) Phosphate (PO_4-P) :APHA,1998
6) Nitrite Nitrogen (NO_3-N): APHA,1998

7) Nitrate Nitrogen (NO_3-N): APHA, 1998
8) Minerals(Fe, Ca, Mg): APHA, 1998; using atomic absorption spectrophotometer

The mean values of all the parameters were analyzed by one-way analysis of variance (ANOVA). Comparisons made at 5% probability level by using statistical package SPSS, Version 16. Duncan's multiple range tests was used to determine the significant difference between the control and treatment means.

Results and Discussion

Temporal variation in water quality parameters during culture period

During study period, initially mean value of Transparency, DO, Nitrite-Nitrogen, Nitrate Nitrogen, Phosphate, Ammonia-Nitrogen were not significance difference. However, there appeared significant differences between different treatments and as the cultural period progressed. Mean values of transparency in 20% and 30% was significantly increased (13.9cm to 20.5cm and14.6cm to 20.9cm) whereas it decreases in rest of the other treatments towards later part of culture period (Fig.1.). Morning DO and evening DO also showed significant difference between different treatments from 30 days onward. Morning DO in 10% (5 mgl^{-1} to 4.4 mgl^{-1}), 20%(4.8 mgl^{-1} to 4.1 mgl^{-1}) and 30%(4.8 mgl^{-1} to 3.9 mgl^{-1}) were somewhat decreased whereas in control was increased with culture period (Fig.2.). Similarly Evening DO in 10% (7.1 mgl^{-1} to 6.7 mgl^{-1}), 20% (7.4 mgl^{-1} to 6.5 mgl^{-1}) and 30% (7 mgl^{-1} to 6.2 mgl^{-1}) were somewhat decreased, whereas control was increased with culture period (Fig.3.).

Ammonia level decreased in all the treatments, however decrease was more prominent in treatments with Wolffia zones 10% (0.44 $mgl^{-1}$to 0.16 mgl^{-1}), 20% (0.61 mgl^{-1} to 0.18 mgl^{-1}), 30%(0.52 $mgl^{-1}$to 0.06 mgl^{-1}) than the control(0.52 $mgl^{-1}$to 0.23 mgl^{-1})(Fig.4.) Nitrate level in control was (0.73 $mgl^{-1}$to 0.75 mgl^{-1}) whereas in the rest of the other treatments was decreased as the culture period (Fig.5.). Nitrite (NO_2^-) level in control (0.18 $mgl^{-1}$to 0.07 mgl^{-1}), 10% (0.2 $mgl^{-1}$to 0.04 mgl^{-1}), 20% (0.17$mgl^{-1}$to 0.02 mgl^{-1}) and 30% (0.14 $mgl^{-1}$to 0.009 mgl^{-1}) Wolffia productive area was significantly decreased with culture period (Fig.6.). 30 days onwards nitrite, ammonia level were decreased in all the treatment. No significant difference in phosphate levels among the treatments was observed (Fig.7.).

Iron (Fe) level in control (1.31 $mgl^{-1}$to 0.89 mgl^{-1}), 10%(1.32 $mgl^{-1}$to 0.76 mgl^{-1}), 20% (1.26 $mgl^{-1}$to 0.64 mgl^{-1}) and 30% (1.36 $mgl^{-1}$to 0.63 mgl^{-1}) Wolffia productive area was decreased with culture period(Fig.8).Calcium (Ca) level in control (21.2 mgl^{-1} to 17.6 mgl^{-1}), 10%(19.2 mgl^{-1} to 15.8 mgl^{-1}), 20% (21.8 mgl^{-1} to 12.6 mgl^{-1}), 30% (18.6 mgl^{-1} to 11.6 mgl^{-1}) were also decreased with culture period (Fig.9.). The summary and overall mean concentrations during the whole culture period are presented in Table 4.

Table 4: Overall mean (±SD) of water quality parameters in different treatments for the whole culture period

Parameter	Control	10% Wolffia	20% Wolffia	30% Wolffia
Water pH	7.4	7.3	7.6	7.4
Morning Temp (ºC)	20.7±0.115	20.8±0.11	20.7±0.28	20.8±0.14
Evening Temp (ºC)	25.6±0.0	25.4±0.14	25.5±0.39	25.4±0.19
Morning DO (mgl^{-1})	4.9±0.22	4.6±0.42	4.5±0.27	4.3±0.13
Evening DO (mgl^{-1})	7.0±0.17	6.9±0.32	6.9±0.29	6.6±0.08
Transparency (cm)	14.9±1.15	13.4±1.20	17.1±1.32	16.8±2.15
Nitrate(NO3-) (mgl^{-1})	0.7±0.03	0.5±0.03	0.4±0.02	0.2±0.04
Nitrite(NO2-) (mgl^{-1})	0.11±.027	0.09±.03	0.08±0.06	0.06±0.27
Phosphate(PO_4) (mgl^{-1})	0.03±0.012	0.03±.005	0.02±.012	0.02±0.012
Ammonia (mgl^{-1})	0.42±.177	0.29±0.062	0.35±0.067	0.21±0.044

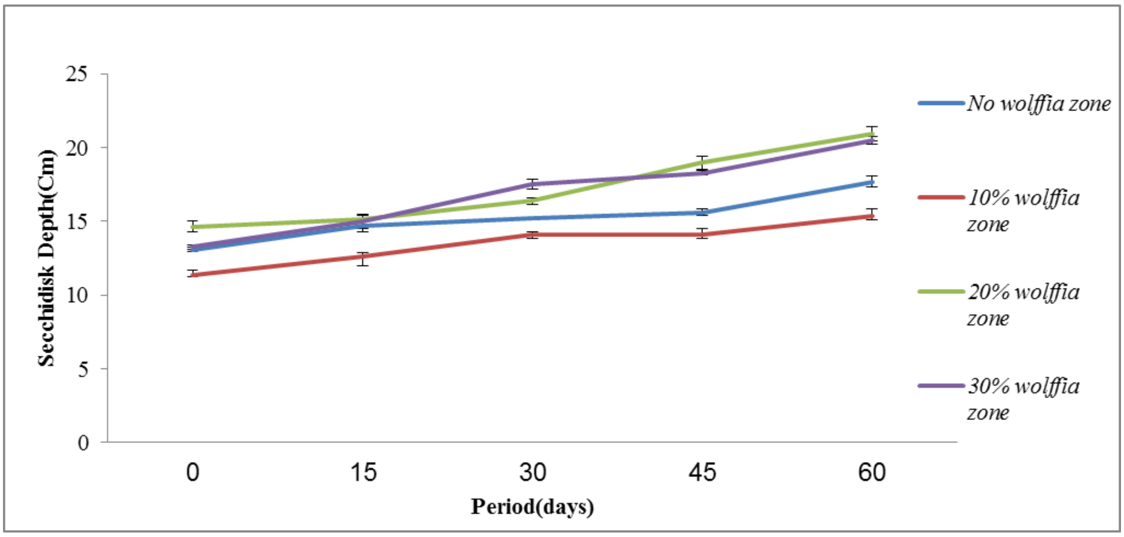

Fig 1: Temporal variation in secchi disk depth in different treatments during the culture period

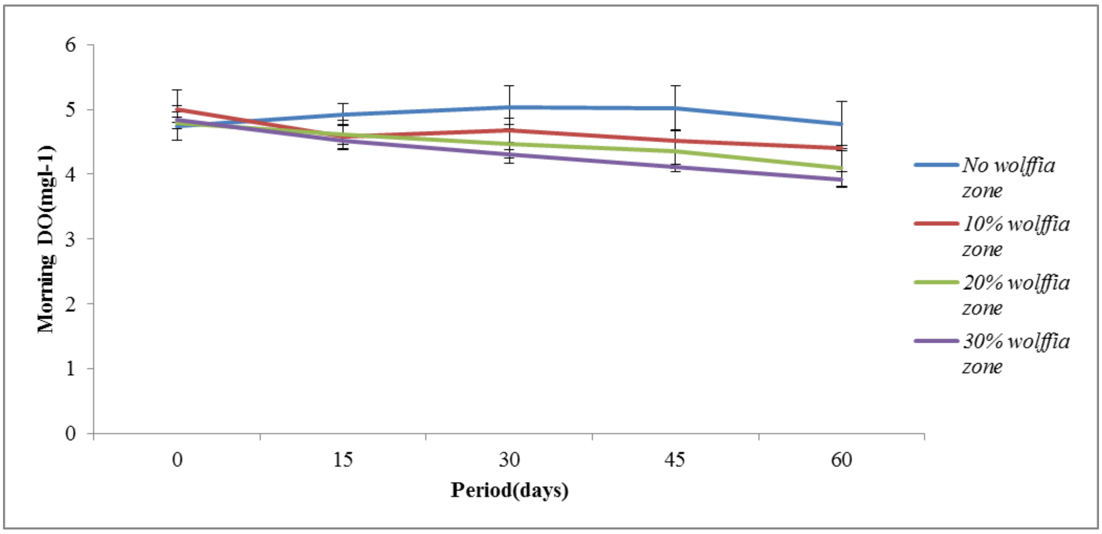

Fig 2: Temporal variation in morning dissolved oxygen of each tank in different treatments during the culture period

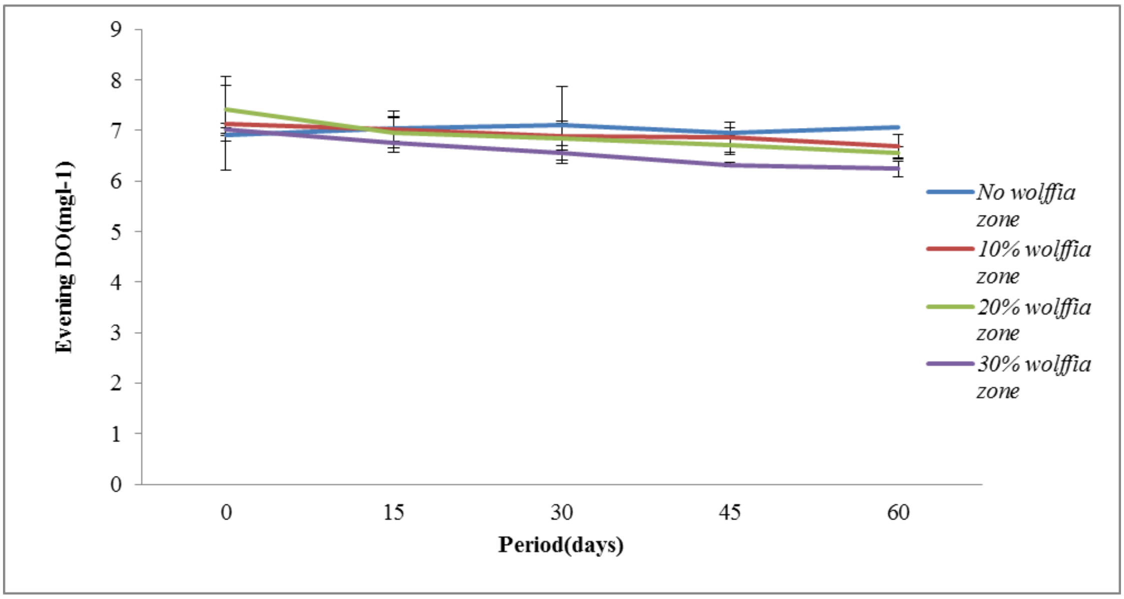

Fig 3: Temporal variation in evening dissovled oxygen in different treatments during the culture period

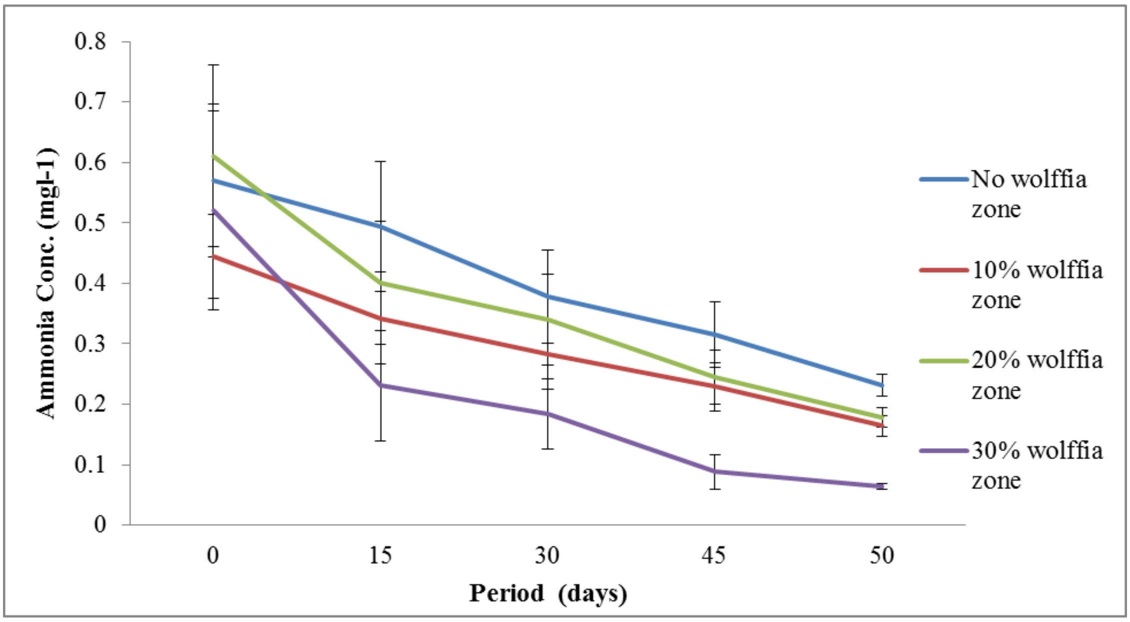

Fig 4: Temporal variation in Ammonia-Nitrogen in different treatments during the culture period

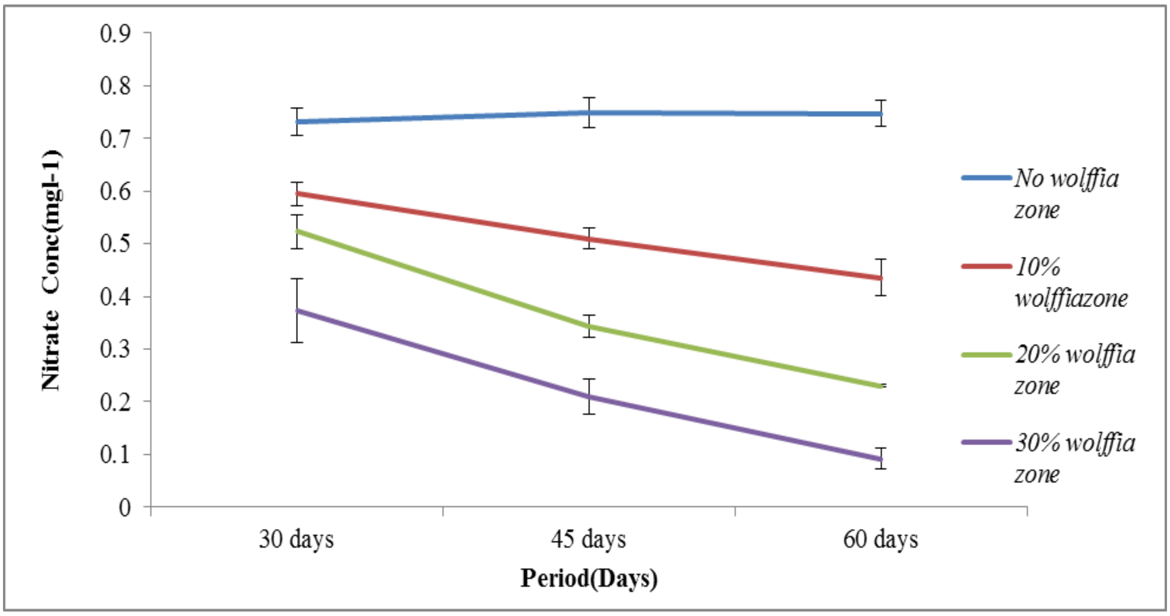

Fig 5: Temporal variation of Nitrate-Nitrogen in different treatments during the cultural period

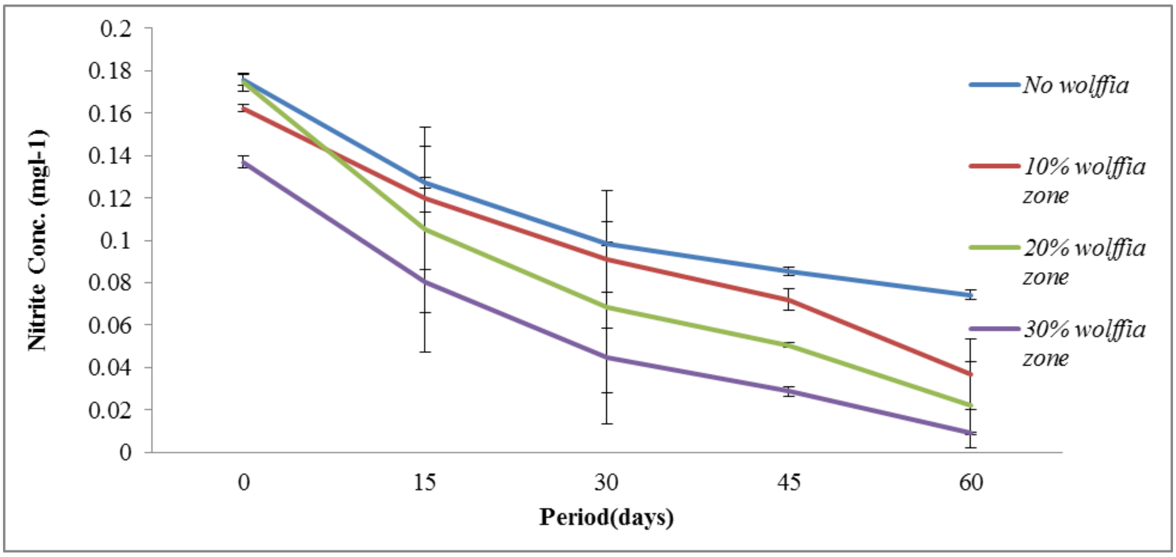

Fig 6: Temporal variation of Nitrite-Nitrogen in different treatments during the culture period

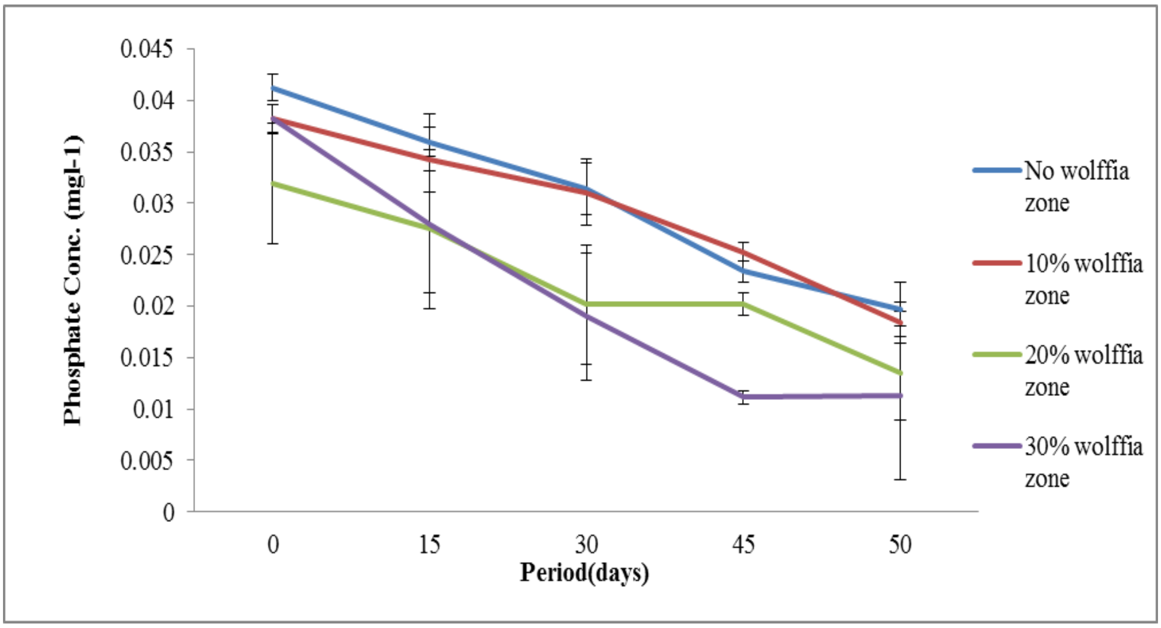

Fig 7: Temporal variation in phosphate in different treatments during the culture period

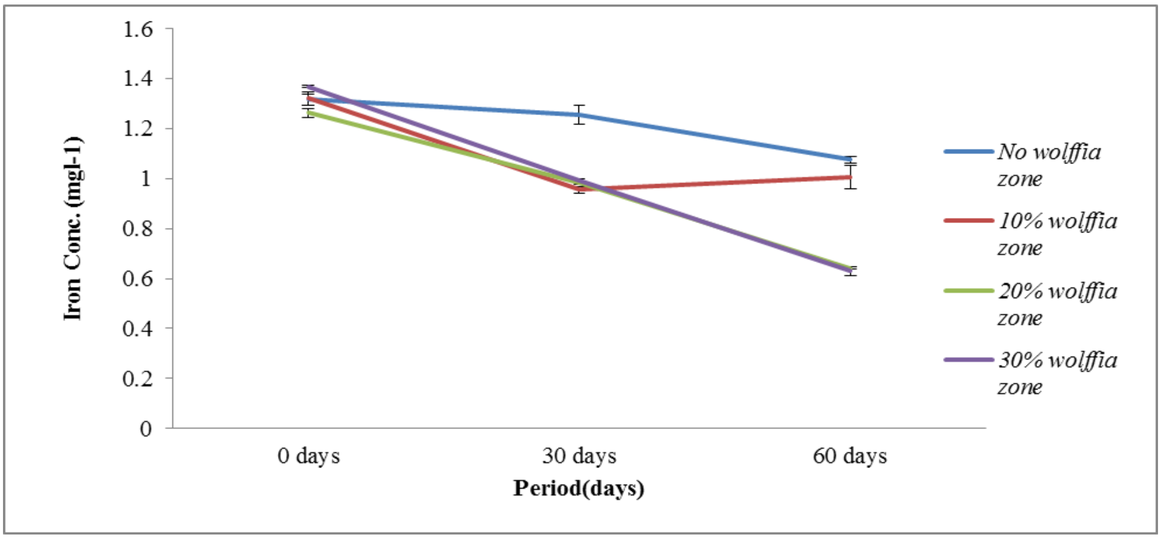

Fig 8: Temporal variation of Iron (Fe) in different treatments during the culture period

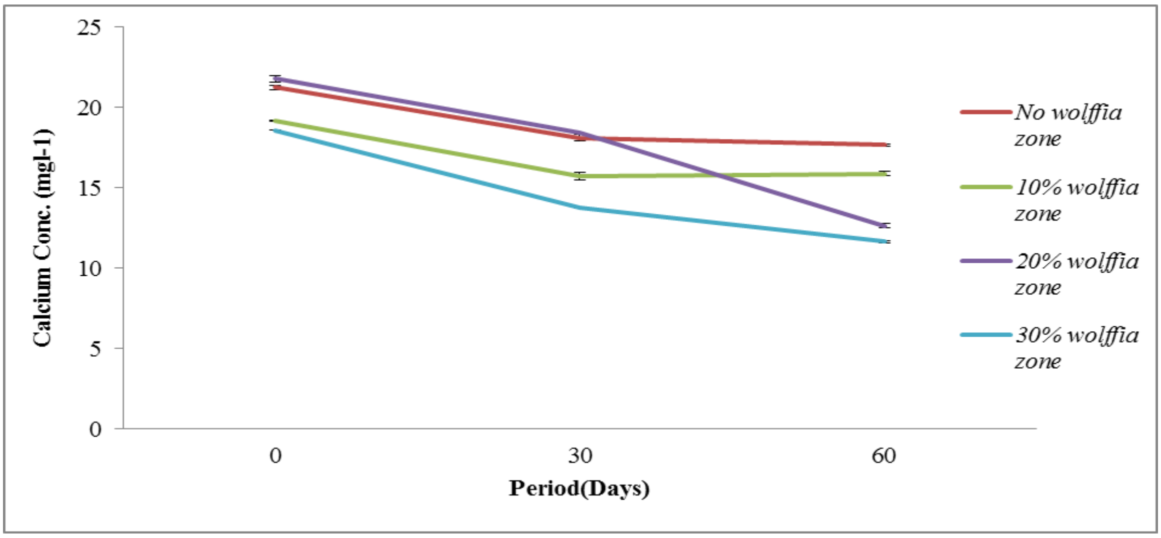

Fig 9: Temporal variation of Calcium in different treatments during cultural period

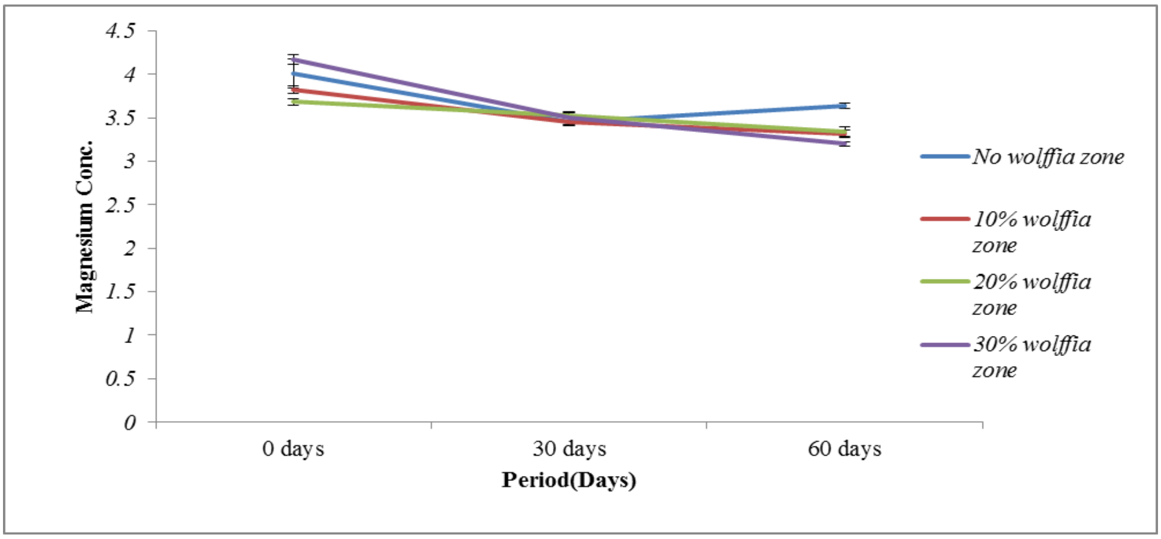

Fig 10: Temporal variation in Magnesium (Mg) in different treatments during culture period

The presence of *Wolffia arrhiza* had significant impact on the water quality parameters of the culture system compared to control. The impact was more evident towards the later part of the experiment. The mean values of parameters including morning and evening DO, ammonia (NH_4-N), nitrite (NO_2^--N), nitrate (NO_3^--N), phosphate (PO_4), iron (Fe), Calcium (Ca), Magnesium (Mg) were significantly low in the treatments with integrated Wolffia-zone in the culture system compared to their respective values in control. There was decline of up to 70-90% reduction in the final concentrations of NH_4-N, NO_2-N and NO_3-N. While decline was about 50% in case of PO_4-P.

The overall physical and chemical water quality parameters of the experimental tanks such as water temperature, water pH, transparency, Dissolved oxygen were found to be within the acceptable range for fish culture. Water pH were neutral to alkaline in all the treatments while morning DO remained above or close to 4.0. The desirable range of DO in cultural tank has been reported to be 4 to 11 (Boyd, 1990) [5]. The morning DO is one of the most important limiting factor in intensified aquaculture system with higher stocking density (Ray *et al*., 2009) [20]. Presence of Wolffia on the water surface could possibly prevent light from penetrating the water thereby halting photosynthesis as well as reduced atmospheric oxygen dissolution by acting as a physical barrier resulting in lower dissolved oxygen in T_2 (20%) and T_3 (30%) with Wolffia zone culture system. However, on the other hand macrophytes absorb the nutrients and limit the algal growth; (Banforth, 1958) [3] reported that water bodies with medium and dense macrophytes cover are characterized by a low concentration of suspended sediments, hence high water transparency and where aquatic macrophytes disappear water transparency is reduced. In the present study transparency was in general increased in the treatments with integrated Wolffia producing zone possibly area may due to high absorption of nutrient.

Application of feed in aquaculture has often been associated with environmental pollution reflected in the form nutrient accumulation and discharge in surrounding environment, particularly in case of intensive culture of carnivorous fishes. Considering that nitrogen and phosphorus are the two most important nutrients in the feed and fertilizers, usually there is increase in the concentrations towards later part of the culture period due to cumulative effects. However, in the present study, in general decreasing trend of NH_4^-N, NO_2^-N, NO_3^-N and PO_4^-P in water was noticed in all the treatments including control with the progress of culture period. It may have happened due to uptake by phytoplankton and/or Wolffia biomass that in turn were consumed by carps. Further, it is also important to note that the application of inorganic fertilizers as well as cow dung was stopped after 30 days of the culture period due to water quality concerns especially reflected in the form of heavy algal bloom. For that matter, Ray *et al*. (2009) [20] observed that in a carp polyculture in lined ponds where fishes were fed on daily basis while fertilization was applied only in the early part of the experiment.

Nevertheless, it was evident that presence of Wolffia in the culture system reduced the concentrations of all the inorganic nitrogen and phosphorus species in the water, especially in the later part of the experiment (Figs 4 to 7). This is in coherence with other studies which have reported that duckweed have high capability to remove phosphorous and nitrogen from waste water (Culley and Epps, 1973; Hillman and Culley, 1978; Oran *et al*. 1986; Landolt and Kandeler, 1987; Leng, 1999) [6, 10, 17, 13, 14]. Further, Alaerts *et al*. (1996) [1] reported that approx. 50% (± 20%) of the total nitrogen load is assimilated by duckweed, and Suppadit *et al*. (2008) [23] observed that NH_4-N in water decreased as the macrophytes biomass increased. Similar trends were also observed for minor nutrients such as Iron and Calcium. (Miretzky, 2006) [15] Reported that duckweed has a high mineral absorption capacity and can tolerate high organic loading as well as high concentrations of micronutrients. Duckweed wastewater treatment systems remove, by bioaccumulation, as much as 99 percent of the nutrients and dissolved solids contained in wastewater Skillicorn *et al*. (1993) [21]. Similarly, Pinto *et al*. 1987 reported that macrophytes have the ability to absorb and accumulate metal ions, organic and inorganic substances.

The overall mean of different water quality parameters for the whole period is summarized in Table 4. As may be apparent, consequent to nutrient absorption impact of Wolffia, mean values of NH_4-N, NO_2-N, NO_3-N were significantly lower ($p<0.05$) in treatments with Wolffia zones (T_{1-3}) compared to control (Table4). Although, there was no statistical difference in PO4-P concentrations in different treatments, values were lower in treatments with Wolffia compared to that in control. (Islam, 1997) [11] Reported that Lemnacae are generally able to absorb 30 to 50% of dissolved phosphorous. Also found that Lemna minor consistently removed the largest amount of ammonia and phosphorus from storm water in 8 weeks.

It is also important to point out that there was an overall significant increase ($p<0.05$) in concentrations of N and P in sediments of the culture system. The nutrients budget in intensive and semi-intensive fish culture ponds reveals that large quantity of these elements is not utilized by fish and often accumulated in pond (Boyd, 1990) [5]. However, unlike in case of dissolved concentrations in water no significant differences were observed between treatments with Wolffia growing zones and control (without Wolffia). It is to be pointed out that while concentration in water is directly a function of degree of absorption in water column, accumulation in sediment is impacted more by deposition of waste material of fish excreta, uneaten feed and death of phytoplankton on one hand and abundance of oxygen in the bottom environment on the other. It may be beyond the scope of this study to elucidate the mechanism of increase of concentrations of these nutrients in sediment.

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