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Effect of Co-cultivation of *Wolffia arrhiza* (L.) on flesh quality and organoleptic quality of Catla, Rohu, Mrigal, Grass carp, Puntius and Amur carp

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Abstract
The main objective of the study was to evaluate the effects of area of *Wolffia*-growing zone as percentage 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 & stocking density was 2 fish m^2 involving Rohu (*Labeo rohita*), Catla (*Catla catla*), Mrigal (*Cirrhina mrigala*), puntius (*Puntius javanicus*), Grass carp (*Ctenopharyngodon idella*) and Amur carp (*Cyprinus carpio haematoperus*). 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. Presence of *Wolffia* in the culture system had a positive influence with regards to somewhat increase in crude protein content with concomitant reduction in crude lipid in case of in Rohu, Catla and Mrigal compared to their counter parts in control. On the other hand, substantial increase ($p<0.05$) in crude lipid content with concomitant decrease in ash content was observed for grass carp and puntius. Further, organoleptic evaluation did not indicate any significant variation in color, odour, gill, eye, body surface of whole fish.

Keywords: *Wolffia*, carp polyculture, proximate composition

Introduction
Macrophytes can be used to grow aquatic macrophytes, which will help to maintain desirable water quality for fish culture; and on the other hand, they will also serve as fish food. The duckweed fronds contain between 92 to 94% water, reports from different authors confirmed that the dry matter of duckweed contains between 35-45% crude protein (Mbagwu and Adeniji, 1988) [18]. Duckweed protein was found to have high concentration of essential amino acids specially lysine and methionine than most of plant protein and closely resembles animal protein in that respect. The plant also has high concentration of trace elements and pigment like carotene and xanthophylls, which makes duckweed a valuable supplement for poultry and other animal feeds (Haustein *et al.* 1992) [14].

Mbagwu *et al.* (1990) [19] have shown that fresh duck weed is a good food source for fish, as it contains about 35-45% crude protein (CP) with good amino acid and mineral profiles. Tilapia and a polyculture of Chinese carp species feed readily on fresh duckweed. The nutritional requirement of fish appears to be met completely in pond receiving only fresh duckweed despite relatively dilute concentration of nutrients in the fresh plant (Cassani & Caton,1983) [5]; Gaighers *et al.* (1984) [12], Moreau *et al.* (1986) [20].

Oran (1994) [23] reported that by cultivating duckweeds the ammonia in ponds for domestic wastewater treatment is converted into valuable protein rich biomass which subsequently can be used animal feed. According to Islam *et al.* (2004) [16] a tiny, fragile, free-floating, aquatic plant, Duckweed could be used as an alternative fish feed as it cited by Fujita *et al.* (1999) [11] *Lemna minor, L. gibba, arrhiza* and *Azolla pinnata* are free floating duckweeds included in *Lemnaceae* family. The vascular aquatic plants known as duckweed which belong to the family *Lemnaceae* have been the subject of great interest during the past decade as a potential source of protein for feed supplement for both aquatic and terrestrial animal stocks, and also possibly for human consumption either directly or indirectly.

The interest is due to the following characteristics of duckweed (Hillman & Culley,1979) [14] high protein content and favourable amino acid pattern Amado *et aI*.(1980) [2]; Rusoff *et al.* (1980) [23]; Culley *et al.* (1981) [7]; Maznah *et al.* (1986) [16]; (Mbagwee,1988) [17] rapid growth and high turnover rates Said *et al.* (1979) [25], response to nutrient enrichment and hence converts various materials into high quality edible tissues (Sutton & Orness, 1975[26]; Culley *et al.* (1978) [6] cloning is the dominant pattern in duckweed and therefore plants with desirable characteristics can be maintained, the plant does not have many pests and therefore can be easily and cheaply maintained. Various studies have indicated that the plant can be used favorably as animal feed Rusoff *et al.* (1977) [24]; Muztar *et al.* (1977) [20]; Truax *et al.* (1978) [27]; Rusoff *et al.* (1980) [23] one species of duckweed, *Wolffia arrhiza,* has even been traditionally consumed by villagers in Burma and Northern Thailand as part of their diet. If duckweed is to be considered an aquacultural crop, research should be carried out to study whether the plant can be grown, managed, harvested and processed economically. In terms of protein production per unit area, duckweed is shown to produce much more protein per hectare than soybean. Estimates by (Culley & Myers,1980) [8] showed that soybean yields about 672 kg crude protein per hectare or only about 8% of the crude protein produced by duckweed. But this estimate was based on small systems (0.04 ha).

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, mustard 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. The resulted nodules like strands were broken into pellets and sun dried 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 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.

Proximate Analysis

Feed ingredients were analyzed before formulating the diet. *Wolffia* & prepared feed were also analyzed. Samples of whole fish were analyzed before and after the experiment. All samples were analyzed in duplicates. Following methods were used.

1) **Moisture content**: By drying the sample (about 5 gm for feed and whole fish carcass) in an oven at 90-105 ºC for 24 hours (AOAC, 2000).
2) **Crude Protein**: Kjeldahl method. Nitrogen content was multiplied by factor 6.25
3) **Crude Lipid:** Lipid of about 2 gm sample was extracted by petroleum ether (Boiling point 60 to 80 ºC) in a soxtec system for 1:30hrs.

4) **Crude Fiber**: Determination by acid and alkali digestion in Fibertec system.(AOAC,2000)
5) **Ash content**: 550 ºC in Muffle furnace for 6 hours (AOAC, 2000).

Study of Organoleptic quality of fish

Initial and final fish sampling was done for estimating sensory quality of fish. Organoleptic quality of fish was evaluated based on Color, Odor, Texture, Gill, Body surface, eye on a scorecard of 10-point Hedonic scale

All statistical analyze was performed using Statistical Package for Social Sciences (SPSS, version 16.0 for windows). Bar diagram and graphs were made in Micro-soft Excel 2007.Analysis of variance (one way - ANOVA) was performed to determine the differences between the mean values were determined by t-Test. Probability levels of 0.05 were used to find out the significance in all cases.

Plate 1. Preparation of tray for feeding Plate 2. Experimental feed

Results

Mean values of moisture, protein content of rohu were significantly different in different treatment (Table 4). The mean values of protein in rohu were higher (73.4%) in 10% *Wolffia* productive treatment followed by other treatments. Mean value of moisture, Ash, protein and fat content of catla were significantly different in all the treatment (Table 5). The mean values of protein in calta were significantly higher (70.7 %) in 20% *Wolffia* productive treatment followed by other treatments. Mean value of Ash, protein and fat content of mrigal were significantly different in all the treatment (Table 6). The mean values of protein were significantly higher (73.2%) in 20% *Wolffia* productive treatment followed by other treatments. The mean values of lipid were significantly higher (17.7%) in 30% *Wolffia* followed by other treatments. The Ash content were significantly lower (12.2%) in 30% *Wolffia* productive treatment followed by other treatment. Mean value of Moisture, Ash, protein and fat content of amur carp were significantly different in different treatment (Table 7). The mean values of protein were significantly higher (79.6%) in 20% *Wolffia* productive treatment followed by other treatments. The mean values of lipid were decrease in 20% & 30% *Wolffia* productive treatment. The Ash content were significantly lower (11.4%) in 30% *Wolffia* productive treatment followed by other treatments. Mean values of proximate composition of puntius were shown in (Table 8). Mean value of protein were significantly increased in treatment 30% *Wolffia* zone. Mean values of proximate composition of grasscarp were shown in (Table 9). Mean value of Ash, fat were significantly different in different treatment. The mean values of lipid 19.7% were significantly higher in 30% *Wolffia* growing treatment followed by other treatment.

Table 4: Mean values (±SD) of final proximate composition of rohu in different treatments

Rohu					
Parameter	**Control**	**10% *Wolffia***	**20%*Wolffia***	**30%*Wolffia***	**Pvalue**
Moisture (%)	78.9^{b}±0.71	77.7 ^{ab} ±1.15	77.2 ^{a}±0.87	76.7 ^{a} ±1.46	0.014
Ash (%)	16.9 ±1.68	14.3 ±5.62	14.7 ±2.63	12.9 ±1.11	0.157
Lipid (%)	14.2 ^{a} ±0.57	14.8 ^{a} ±0.49	15.1 ^{a}±1.01	17.2^{b} ±.68	.000
Protein (%)	65.7 ^{a} ±6.20	73.4^{b}±4.62	65.1 ^{a}±4.67	66.8 ^{a} ±3.37	0.070

Table 5: Mean values (±SD) of final proximate composition of catla in different treatments

Catla					
Parameter	**Control**	**10% *Wolffia***	**20% *Wolffia***	**30%*Wolffia***	**p value**
Moisture	79.1^{b}±1.96	77.2^{ab} ±1.59	76.1 ^{a} ±2.56	75.8 ^{a} ±1.36	.034
Ash	19.2 ^{b} ±1.07	17.8 ^{b} ±1.39	18.9 ^{b} ±0.78	12.5 ^{a} ±0.95	.000
lipid	9.7 ^{a} ±0.36	10.5 ^{a} ±2.01	13.8 ^{b} ±0.61	14.1 ^{b} ±1.83	.000
Protein	61.2 ^{a} ±0.91	67.9 ^{c}±0.39	70.7^{d}±0.69	64.8 ^{b}±3.99	.000

Table 6: Mean values (±SD) of final proximate composition of mrigal in different treatments

Mrigal					
Parameter	**Control**	**10% *Wolffia***	**20% *Wolffia***	**30%*Wolffia***	**p value**
Moisture	79.8 ±3.06	79.4 ±1.57	78.8 ±3.61	76.5 ±2.37	.216
Ash	17.8 ^{c} ±0.83	14.5 ^{b} ±0.73	13.6 ^{b} ±0.71	12.2 ^{a} ±0.64	.000
lipid	12.5 ^{a} ±1.87	12.5 ^{a}±0.48	13.9 ^{a} ±1.10	17.7 ^{b} ±2.10	.000
Protein	58.4 ^{a} ±2.33	66.2 ^{b}±1.05	73.2 ^{c}±4.49	61.6 ^{a} ±3.68	.000

Table 7: Mean values (±SD) of final proximate composition of Amurcarp in different treatments

Amurcarp					
Parameter	**Control**	**10% *Wolffia***	**20% *Wolffia***	**30% *Wolffia***	**P value**
Moisture	78.9^{c} ±0.55	75.6^{b} ±0.99	75.90^{b}±0.86	73.7^{a} ±0.65	.000
Ash	19.1^{c} ±0.81	16.9^{b} ±0.74	15.9^{b} ±0.58	11.4^{a} ±1.41	.000
lipid	19.1^{b} ±0.94	19.7^{b} ±0.94	16.4^{a} ±1.01	15.7^{a} ±2.25	.000
Protein	65.2^{a}±4.93	71.7^{b}±2.14	79.6^{c} ±2.67	67.5^{a} ±1.98	.000

Table 8: Mean values (±SD) of final proximate composition of Silverbarb in different treatments

Silverbarb					
Parameter	**Control**	**10% *Wolffia***	**20% *Wolffia***	**30% *Wolffia***	**P value**
Moisture (%)	75.7 ±1.12	75.3 ±1.66	73.8 ±0.80	72.8 ±1.92	0.147
Ash (%)	19.8^{b} ±1.62	13.9^{ab} ±0.24	13.7^{ab} ±0.79	12.4^{a} ±0.62	0.299
Lipid (%)	19.2^{a} ±2.99	20.2^{ab} ±0.35	24.6^{b} ±1.88	19.9^{a} ±1.16	0.045
Protein (%)	64.3^{a} ±0.56	66.6^{a} ±0.32	66.6^{a} ±2.66	70.8^{b}±1.33	0.09

Table 9: Mean values (±SD) of final proximate composition of Grasscarp in different treatments

Grass carp					
Parameter	**Control**	**10% *Wolffia***	**20% *Wolffia***	**30% *Wolffia***	**P value**
Moisture (%)	83.4^{b}±1.31	81.1^{ab}±1.83	80.6^{ab}±1.83	79.2^{a}±0.99	.037
Ash (%)	17.2^{b}±0.89	16.4^{b}±1.21	16.6^{b}±0.48	13.9^{a}±0.59	.000
Lipid (%)	9.5^{a} ±2.21	15.4^{b}±3.40	16.7^{bc}±3.28	19.7^{c}±2.59	.000
Protein (%)	65.2^{a} ±4.36	65.4^{a}±6.22	67.5^{a} ±2.16	67.4^{a} ±0.61	.493

Organoleptic Quality of Fishes

Mean value of final organoleptic quality of different species in the treatments are presented in Figs.1-6.There were no significant differences among the treatments.

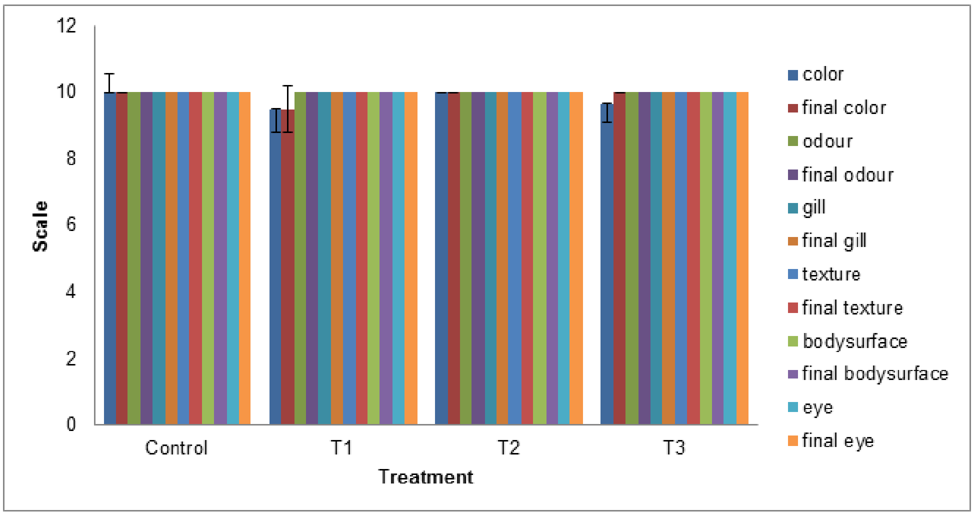

Fig 1: Mean values (±SD) of organoleptic quality of rohu in different treatments

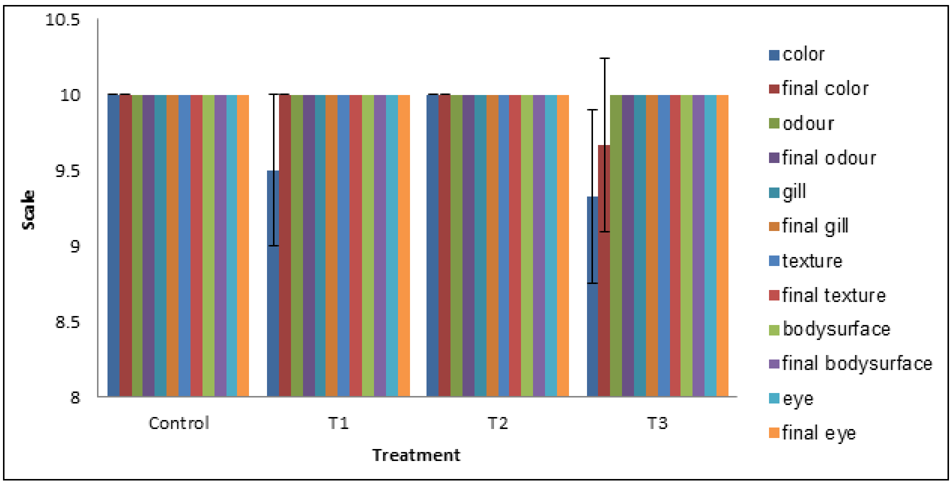

Fig 2: Mean values (±SD) of organoleptic quality of catla in different treatments

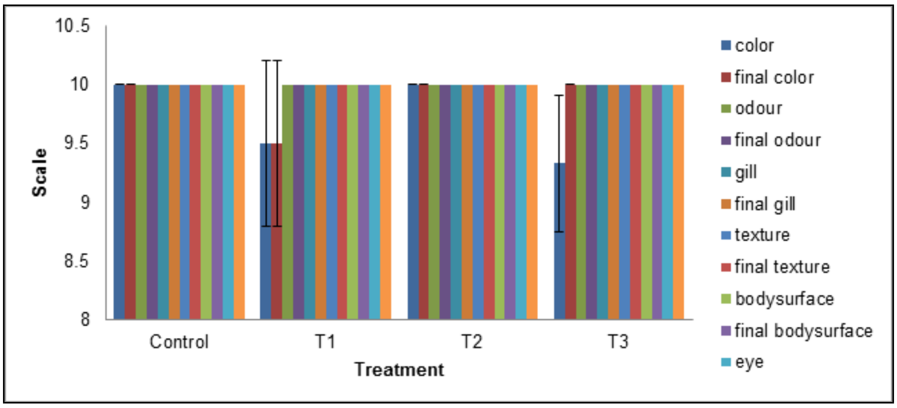

Fig 3: Mean values (±SD) of organoleptic quality of mrigal in different treatments

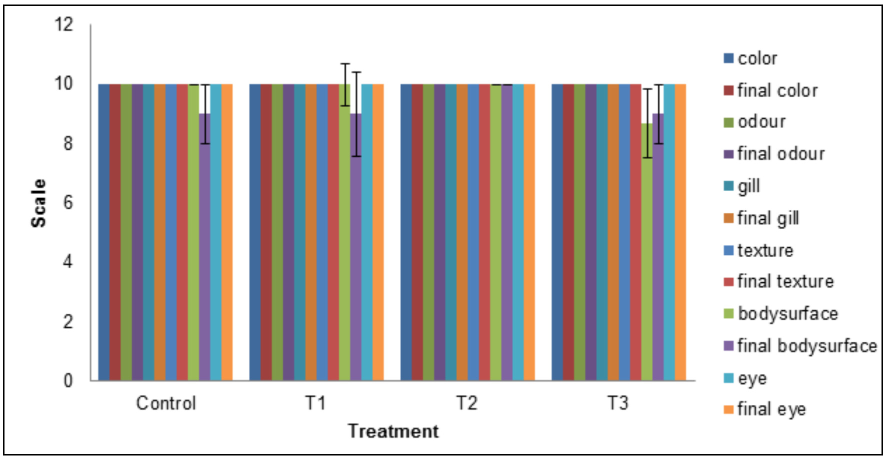

Fig 4: Mean values (±SD) of organoleptic quality of grasscarp in different treatments

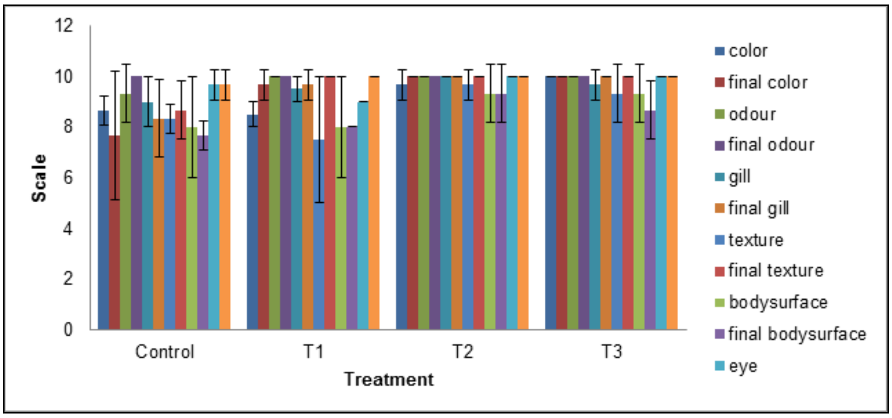

Fig 5: Mean values (±SD) of organoleptic quality of puntius in different treatments

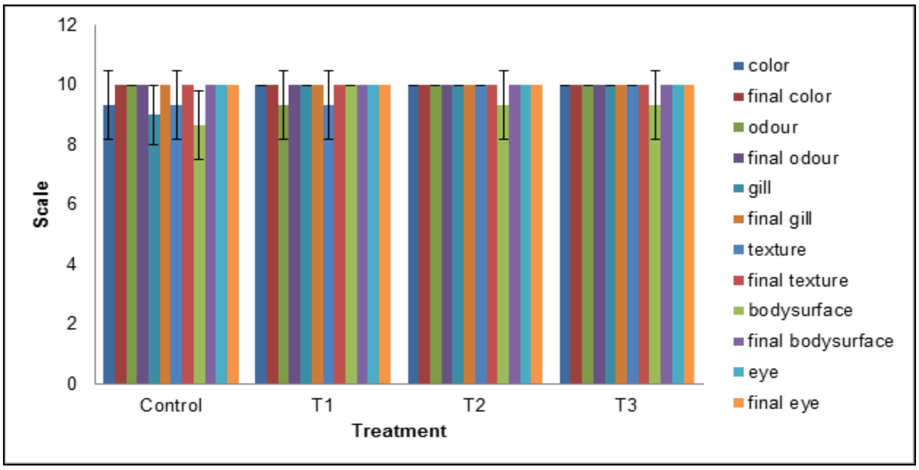

Fig 6: Mean values (±SD) of organoleptic quality of amurcarp in different treatments

Discussion

In general, presence of *Wolffia* in the culture system had positive influence with regards to somewhat increase in crude protein content with concomitant reduction in crude lipid in case of in Rohu, Catla and Mrigal compared to their counter parts in control. On the other hand, substantial increase ($p<0.05$) in crude lipid content with concomitant decrease in ash content was observed for grass carp and puntius.

Significant ($P<0.05$) improvements in proximate contents as reflected by increase in protein (rohu, catla & Amur carp) & fat (grass carp & silver barb), and reduction in moisture (rohu, catla, amur carp & grass carp) & ash contents (catla, mrigal, grass carp & amur carp) were observed in treatments with integrated *Wolffia*-zones compared to that in control viz. without *Wolffia* zone in the culture system. The presence of *Wolffia-zone* in the culture system has positive influence with regards to somewhat increase in phosphorous contents in case of Catla, Mrigal & Amurcarp & increase in iron in case of silver barb compared to that in their counter parts in control, respectively. On the other hand, substantial increase ($p<0.05$) in both iron and phosphorous contents compared to that in control was observed for grasscarp. Mbagwu *et al.* (1990) [18] have shown that fresh duck weed is a good food source for fish, as it contains about 35-45% crude protein (CP) with good amino acid and mineral profiles. Tilapia and a polyculture of Chinese carp species feed readily on fresh duckweed. The nutritional requirement of fish appears to be met completely in pond receiving only fresh duckweed despite relatively dilute concentration of nutrients in the fresh plant (Cassani & Caton,1983) [5]; Gaighers *et al.* (1984) [12], Moreau *et al.* (1986) [19]. (Oran, 1994) [21] reported that by cultivating duckweeds the ammonia in ponds for domestic wastewater treatment is converted into valuable protein rich biomass which subsequently can be used animal feed. According to Islam *et al.* (2004) [15] a tiny, fragile, free-floating, aquatic plant, Duckweed could be used as an alternative fish feed as it cited by Fujita *et al.* (1999) [11] *Lemna minor*, *L. gibba*, *arrhiza* and *Azolla pinnata* are free floating duckweeds included in *Lemnaceae* family.

Further, organoleptic evaluation did not indicate any significant variation in color, odour, gill, eye, body surface of whole fish. It is important to note that organoleptic quality of fresh fish depends on mainly the culture environment, fish feed provided to the cultural organism and since the culture period was pretty short and water quality was within desirable range throughout the experimental period, no significant differences could be apparent.

No significant impact of presence of different levels of *Wolffia*-zone on organoleptic parameters of the harvested fishes was observed.

Conclusion

The final conclusion is that inclusion of *Wolffia arrhiza* (L.) in intensified carp polyculture system appears to improve the flesh quality as well as the fish organoleptic quality.

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