EFFECT OF MICROBIAL BIOFILM IN THE NURSERY PHASE OF MRIGAL, *CIRRHINUS MRIGALA*

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ABSTRACT

The experiment was conducted for 35 days in nine cement tubs (1 x 1 x 1 m) having 15-cm sandy-loam soil base with three treatments in triplicate, *viz.*, cow dung alone at the rate of 1 kg/tub (T_1), cow dung at 1 kg/tub and feed at 10% body wt/d in two meals (T_2), and cow dung at 1 kg and paddy straw at 200 g/tub (T_3). Both manure and substrate were added on dry weight basis. All the tubs were stocked with 10 fry each of mrigal (100,000/ha) of average weight of 0.09 g, seven days after the addition of manure and substrate. The total plate count of bacteria in water did not vary much between the treatments and the mean values were 5.13, 5.49 and 5.85 (CFU x 10^4/ml) in T_1, T_2 and T_3 respectively. The number of phytoplankters and zooplankters in water differed significantly between the treatments. The average number of attached algae (no./cm^2) and fish food organisms (no./cm^3) recorded on the substrate were 145.28 and 70.67, respectively. The mean final weight of mrigal differed significantly ($P < 0.05$) between the treatments with T_3 registering the highest value of 6.93 g followed by T_2 (5.01 g) and T_1 (3.37 g). The specific growth rate and growth increment of fish also followed the same trend as that of weight recorded in the different treatments. Survival was higher in T_2 (83.33%), followed by T_3 (80.00%) and T_1 (76.67%). The study demonstrates that by the introduction of biodegradable substrates like paddy straw into the culture systems, significantly higher growth and survival can be obtained in the nursery rearing of mrigal.

Keywords : Microbial biofilm, nursery, *Cirrhinus mrigala*

INTRODUCTION

The role of microbes as food for fish and shellfish is well documented (Moriarty, 1997). Heterotrophic food production, which is independent of light, has greater potential for increasing aquaculture production compared to autotrophic production (Zhu *et al.,* 1990; Garg and Bhatnagar, 2000). However, there is a limitation for the exploitation of the planktonic microbial cells by fish and fish food organisms because of the small individual cell size. The addition of artificial substrate to promote microbial biofilm favours the aggregation of cells to an easily harvestable biomass by fish. The potential of biodegradable plant substrates such as sugarcane bagasse and paddy straw in enhancing bacterial biofilm and fish production has been well documented (Shankar *et al.,* 1998; Ramesh *et al.,* 1999; Mridula *et al.,* 2003). Grow-out studies conducted employing different substrates yielded varying productions: 47% in *Cyprinus carpio* (Ramesh *et al.,* 1999), 59% in *Oreochromis mossambicus* (Umesh *et al.,* 1999), 77% in *Labeo rohita* (Wahab *et al.,* 1999) and 42% in *Tor khudree* (Keshavanath *et al.,* 2001) higher than the substrate-free control. However, information on the effect of microbial biofilm during the nursery phase of fishes is scarce. Hence, studies were conducted on the effect of paddy straw on biofilm production, and growth and survival of mrigal, *Cirrhinus mrigala,* during its early phase of growth and the results are presented in this paper.

MATERIAL AND METHODS

The experiment was conducted for 35 days in nine cement tubs (1 x 1 x 1 m) having 15-cm sandy-loam soil base. After drying the soil bed for a week, the tubs were filled with water to a depth of 0.8 m. The experiment consisted of three treatments in triplicate: (a) cow dung alone at the rate of 1 kg/tub (T_1), (b) cow dung at 1 kg/ tub and feed and 10% body wt/d in two meals (T_2), and (c) cow dung at 1 kg and paddy straw at 200 g/tub (T_3). Both manure and substrate were added on dry weight basis. Pre-washed and dried paddy straw was tied into 25-30 cm long bundles and suspended in water column from the horizontal bamboo beams placed on tub walls. The substrate remained submerged in water throughout the period of study. Fresh cow dung was made into slurry with water and applied. All the tubs were stocked with 10 fry each of mrigal (100,000/ha) of an average weight of 0.09 g, seven days after the addition of manure and substrate.

Water, plankton and substrates were sampled at weekly intervals. Water was analyzed for dissolved oxygen, total alkalinity, free carbon dioxide, total ammonia, nitrite-nitrogen, nitrate-nitrogen and phosphate-phosphorus by standard methods (APHA, 1995). Water temperature and pH were measured using Horiba (Japan) water quality analyzer (Model U-10). Total plate count of bacteria in water and on substrate was estimated at weekly

intervals (Mridula *et al.*, 2003). Plankton collected by filtering a known volume (10 l) of water through plankton net made of bolting silk (60 µm) was preserved in 4% neutralized formalin. In the laboratory, the plankton composition was determined with the help of a Sedgewick-Rafter cell (Jhingran *et al.*, 1969) and expressed as no./l. Wet and dry weights of plankton were also determined. The enumeration of attached algae on the substrates was done by scraping attached algae and other organisms from a 2 x 2-cm area using a blade and counting with the help of a Sedgewick-Rafter cell, and expressed as no./cm^2.

On the termination of the experiment, all the surviving fishes were harvested, and their length and weight recorded. Survival percentage, growth increment and specific growth rate (%/d) were estimated. Proximate compositions of feed ingredients, formulated feed and biofilm grown on substrate were analyzed for moisture, crude fat, crude protein, crude fibre, nitrogen-free extract and total ash following standard methods (AOAC, 1975). The data obtained were subjected to statistical analyses employing ANOVA and Duncan's multiple range test at $P < 0.05$ (Duncan, 1955; Snedecor and Cochran, 1968).

RESULTS

Different water quality parameters recorded are presented in Table 1. There was no significant difference in water temperature, pH, dissolved oxygen, free carbon dioxide, total alkalinity, nitrite-nitrogen and phosphate-phosphorus between the treatments. Ammonia and nitrate-nitrogen differed significantly between the treatments. All the parameters were within the acceptable range for fish culture.

The values of total bacterial counts in water and on substrate recorded are also given in Table 1. The total plate count of bacteria in water did not vary much between the treatments and the mean values were 5.13, 5.49 and 5.85 (CFU x 10^4/ml) in T_1, T_2 and T_3, respectively. The total plate count of bacteria reached a peak on the 14th day both in water and on substrate in all the treatments. The lower values recorded were on the 0th day (beginning of the experiment) for water and the 35th day for substrate. Mean number of bacteria on substrate in T_3 was 1.34 x 10^6/g.

Phytoplankton number in water differed significantly between the treatments and the mean values (no./l) were 126.11, 141.11 and 130.28 in T_1, T_2 and T_3, respectively (Table 1). Zooplankton number in water was the highest in T_2 (152.5) and differed significantly from T_1 (143.06) and T_3 (145.28). The average numbers of attached algae and attached food organisms on paddy straw (no./cm^2) were 203.00 and 70.67, respectively. Wet weight and dry weight of plankton were higher in T_2 (211.94 and 21.53 mg/100 l, respectively) than in T_1 (209.11 and 21.52 mg/100 l, respectively) and T_3 (201.85 and 20.49 mg/100 l, respectively).

Table 1: Water quality parameters, total plate count and plankton

Parameter	Treatment		
	T_1	T_2	T_3
Water temperature (°C)	26.47[a] (24.80-27.70)	26.40[a] (24.60-27.70)	26.52[a] (24.80-27.80)
pH	7.50[a] (7.06-7.96)	7.63[a] (7.02-7.96)	7.43[a] (7.01-7.99)
Dissolved oxygen (mg/l)	5.34[a] (3.38-6.75)	5.20[a] (3.38-6.38)	5.45[a] (4.50-6.00)
Carbon dioxide (mg/l)	7.88[a] (1.76-14.08)	8.17[a] (0.00-14.00)	8.26[a] (1.76-15.80)
Total alkalinity (mg/l)	44.95[a] (30.00-65.00)	45.22[a] (28.00-68.00)	46.61[a] (30.00-62.00)
Total ammonia (µg at.-N/l)	7.99[a] (3.90-11.40)	9.15[b] (3.68-11.10)	6.55[c] (3.90-8.40)
Nitrite-nitrogen (µg at.-N/l)	1.41[a] (0.50-2.38)	1.51[a] (0.69-2.42)	1.26[a] (0.59-2.13)
Nitrate-nitrogen (µg at.-N/l)	4.90[A] (3.54-5.99)	5.31[B] (4.12-6.51)	6.35[C] (4.45-7.83)
Phosphate-phosphorus (µg at.-P/l)	1.31[a] (0.23-3.61)	1.29[a] (0.27-3.28)	1.12[a] (0.18-2.29)
Total plate count in water (no. x 10^4/ml)	5.13[a] (0.30-13.20)	5.49[a] (0.38-16.40)	5.85[a] (0.36-18.20)
Total plate count in substrate (no. x 10^6/ml)	-	-	1.34 (0.21-3.90)
Phytoplankton in water (no./l)	126.11[a] (20-245)	141.11[c] (35-270)	130.28[b] (25-235)
Attached algae on substrate (no./cm^2)	-	-	203.00 (60-450)
Zooplankton in water (no./l)	143.06[a] (10-275)	152.50[b] (10-275)	145.28[a] (10-300)
Food organisms on substrate (no./cm^2)	-	-	70.67 (15-165)
Wet weight (mg/100 l)	209.11[a] (8.40-1280.00)	211.94[a] (9.20-416.32)	201.85[a] (9.60-398.20)
Dry weight (mg/100 l)	21.52[a] (0.98-42.16)	21.53[a] (1.04-42.60)	20.49[a] (0.90-38.18)

Values are means of three tanks and six sampling days (N = 18).
Values in parentheses indicate range.
Values with the same superscript in each row are not significantly different ($P < 0.05$).

Values of moisture, crude protein, crude fat, ash and nitrogen-free extract of feed ingredients, formulated feed and biofilm are presented in Table 2. The ingredients used for the preparation of feed was of good quality with fish meal having a protein content of 51.83% which is higher than the 50% level recommended by National Research Council (NRC, 1977). The protein contents of formulated feed and biofilm were 26.42% and 23.80%, respectively.

Initial weight, final weight, growth increment, specific growth rate and survival of fish are summarized in Table 3. The mean final weight of mrigal differed significantly ($P < 0.05$) between the treatments with T_3 registering the highest value of 6.93 g followed by T_2 (5.01 g) and T_1 (3.37 g). The specific growth rate and growth increment of fish also followed the same trend as that of weight. Survival was higher in T_2 (83.33%), followed by T_3 (80.00%) and T_1 (76.67%).

DISCUSSION

All the water quality parameters were well within the limits suitable for fish production; pH values were slightly alkaline, indicating good conditions for biological production. Higher ammonia concentration recorded in T_2 could be due to the addition of feed, as protein-rich feed and nitrogenous excretory products contribute to accumulation of ammonia (Mohan, 2001). Treatment with substrate (T_3) resulted in significantly lower ammonia level, which can be attributed to the presence of nitrifying bacteria in the biofilm (Rajesh, 2002). Nitrate-nitrogen concentration was higher in T_3 than in T_1 and T_2. This could be due to the conversion of nitrite to nitrate by *Nitrobacter* sp. present on the substrate (Rajesh, 2002). There was no significant difference in the total plate count of bacteria in water between the treatments. Total plate count recorded on substrate was higher than that in water and the values recorded were comparable to those reported by earlier workers (Ramesh *et al.*, 1999; Umesh *et al.*, 1999; Joice *et al.*, 2002).

The mean phytoplankton in water was the highest in T_2, followed by T_3 and T_1. The major groups of phytoplankton in the order of abundance were green algae, blue-green algae and diatoms. Mean zooplankton density also followed similar trend as that of phytoplankton. The important groups of zooplankton encountered were protozoans, rotifers, ostracods, cladocerans and larval forms. The higher phytoplankton and zooplankton recorded in T_2 may be due to lesser grazing by the fishes as well as the extra nutrients received through the leftover supplementary feed. Attached algae and fish food organisms were higher on substrate compared to water, as reported by earlier workers (Shankar *et al.*, 1998; Ramesh *et al.*, 1999; Mridula *et al.*, 2003). The important groups of attached algae and fish-food organisms on substrate were similar to that recorded in water.

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Table 2: Proximate composition (dry weight basis) of feed ingredients, formulated diet and biofilm grown on substrate

Ingredient/feed	Contribution (%)	Moisture (%)	Crude protein (%)	Crude fat (%)	Ash (%)	Crude fibre (%)	Nitrogen-free extract (%)	Calories (kJ/g)
Rice bran	22.50	8.40 (0.07)	4.92 (0.82)	1.63 (0.05)	17.69 (0.05)	31.80	35.56	7.59
Tapioca flour	21.50	9.99 (0.15)	2.46 (0.00)	0.53 (0.15)	14.68 (0.02)	3.60	81.68	14.38
Groundnut oil cake	27.50	6.58 (0.08)	37.49 (0.72)	6.79 (0.09)	6.50 (0.09)	10.80	31.84	15.73
Fish meal	27.50	6.91 (0.18)	51.83 (1.63)	10.92 (0.16)	25.79 (0.06)	1.90	2.65	15.40
Vitamin-mineral mix	1.00	-	-	-	-	-	-	-
Feed	-	6.15 (0.15)	26.42 (0.44)	6.50 (0.50)	13.37 (0.07)	12.00	35.36	13.89
Biofilm on paddy straw	-	-	23.80	2.15	21.22	25.61	27.22	10.34

Figures in parentheses indicate standard error.

Table 3: Growth, growth increment, specific growth rate and survival of *C. mrigala* in different treatments

Treatment	Tub no.	Initial (0^{th} d)		Final (30^{th} d)		Growth increment (g/d)	SGR (%/d)	Number of fish		Survival (%)
		Length (cm)	Weight (g)	Length (cm)	Weight (g)			Stocked	Harvested	
TI	1	2.40	0.09	6.44	3.21	0.110	0.90	10	8	80
	2	2.10	0.09	6.80	3.56	0.120	1.05	10	7	70
	3	2.30	0.09	6.91	3.34	0.120	0.96	10	8	80
	Av.	2.27	0.09	6.72	3.37	0.120	0.97	10	7.67	76.67
	S.E.	0.09	0.00	0.14	0.10	0.003	0.07	0	0.33	3.33
T2	1	2.40	0.09	7.33	5.09	0.180	1.57	10	8	80
	2	2.20	0.09	7.02	4.88	0.170	1.51	10	8	80
	3	2.30	0.09	7.42	5.05	0.180	1.56	10	9	90
	Av.	2.30	0.09	7.26	5.01	0.180	1.55	10	8.33	83.33
	S.E.	0.06	0.00	0.12	0.11	0.003	0.03	0	0.33	3.33
T3	1	2.50	0.09	8.10	6.85	0.240	2.00	10	7	70
	2	2.20	0.09	8.15	7.25	0.260	2.08	10	8	80
	3	2.10	0.09	7.80	6.70	0.230	1.97	10	9	90
	Av.	2.27	0.09	8.02	6.93	0.240	2.02	10	8.00	80.00
	S.E.	0.12	0.00	0.11	0.28	0.003	0.03	0	0.58	58.00

feed given was 26.42%, whereas the optimum requirement of protein for Indian major carps is around 30% (Renukaradhya and Varghese, 1986). Natural food contributes to substantial protein requirement of fishes (Nandeesha *et al.*, 1994). In the present study, the biofilm protein recorded was 23.8%. The general composition of biofilm is 8-10% ash, 10-30% carbon and 1-10 % nitrogen (cited by Shankar and Mohan, 2001). The present study showed a nitrogen value of 3.81% in the biofilm grown on paddy straw and is comparable with the earlier studies.

The growth of *C. mrigala* in tanks manured with cow dung and provided with paddy straw was significantly high ($P < 0.05$), the percentage increases over treatments provided with manure alone and manure and feed being 105.64 and 48.66, respectively. There was no significant difference in survival between the treatments. The enhanced fish growth observed in substrate-based tanks indicates the effective utilization of the microbial biofilm developed on the substrate by mrigal. Mrigal, a bottom feeder feeds on decayed plant and animal matter, algae, detritus, mud, etc. (Jhingran and Pullin, 1988). The enhanced growth in T_3 can also be attributed to the higher production of attached algae and fish food organisms on the substrate. The better growth recorded in T_2 compared to T_1 may be due to the addition of supplementary feed. This study demonstrates that by introducing biodegradable plant substrates like paddy straw, significantly higher growth can be obtained in the nursery rearing of mrigal.

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