

# On-Farm Evaluation of Indian Major Carp Production with Sugarcane Bagasse as Substrate for Periphyton

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## Abstract

To study the effect of sugarcane bagasse as an artificial substrate for periphyton in fish ponds, an on-farm polyculture experiment was carried out, stocking catla (*Catla catla*), rohu (*Labeo rohita*) and common carp (*Cyprinus carpio*) in an 800 m<sup>2</sup> earthen pond partitioned into eight 100 m<sup>2</sup> compartments with fine meshed nylon netting. Fifty catla and 25 each of common carp and rohu were stocked per compartment and grown for 180 days. Sugarcane bagasse bundles (length 80 cm, diameter 3.3 cm) at densities of 0, 39, 78 and 156 (or 0, 7.0, 13.7 and 28.2 kg 100 m<sup>-2</sup>) were hung in two compartments each as a substrate. Supplemental feed (a 1:1 mixture of groundnut cake and rice bran) was provided in one replicate of each substrate density at a rate of 5% body weight during the first half and 3% throughout the second half of the experiment. Water quality and periphyton biomass were monitored at fortnightly and monthly intervals, respectively.

Bagasse substrate did not adversely affect water quality, dissolved oxygen levels being between 4 and 13 mg·l<sup>-1</sup> throughout the experiment. Only minor differences in periphyton and plankton density were observed between treatments. Marked differences were recorded in fish production. Total fish production of 8076 g·100 m<sup>-2</sup> was obtained without feed and periphyton (control). Feeding alone increased yield compared to controls by 20%. Bagasse substrate alone increased yields by 38, 61 and 62% in the 39, 78 and 156 bagasse bundles per 100 m<sup>2</sup> treatments, respectively, while the combination of feeding and periphyton resulted in 45, 67 and 84% increases in yield in the 39, 78 and 156 bagasse

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bundles per 100 m<sup>2</sup> treatments, respectively. Regression analysis predicted that maximum total fish production would be achieved at bagasse densities of 117 bundles (21 kg) per 100 m<sup>2</sup> without feeding and 156 bundles (28 kg) per 100 m<sup>2</sup> with feeding.

The results demonstrate that sugarcane bagasse can be successfully used as a substrate for periphyton. Further research is needed to assess the practical and economic feasibility of periphyton-based aquaculture in India.

## Introduction

Pond aquaculture of carps in India is largely reliant on inputs of fertilizer and/or supplementary feed. However, external resources are expensive and beyond the reach of resource-poor farmers. Moreover, nutrient use efficiency in ponds is low, with only about 30% of nutrient inputs being converted into harvestable products (Acosta Nassar et al. 1994). Laboratory based experimental studies suggest that some herbivorous fish species are more efficient in grazing periphytic algae than filtering phytoplankton (Dempster et al. 1993). There is thus growing interest in the potential of artificial substrates for periphyton production in ponds, both to reduce costs and to increase nutrient utilization (Beveridge et al. 1998).

Enhancement of pond fish production through provision of substrate for periphyton growth has been demonstrated with various Indian carp species (Shankar et al. 1998, Ramesh et al. 1999, Wahab et al. 1999, Keshavanath et al. 2001). Our earlier studies showed that bamboo poles yield greater periphyton production than PVC pipes and sugarcane bagasse bundles. However, although bagasse bundles (diameter 7.5 cm; density of 784 bundles per 100 m<sup>2</sup> pond area) created serious water quality problems in terms of low dissolved oxygen concentrations, they were much cheaper than bamboo or PVC (Keshavanath et al. unpubl. data). Therefore, the present investigation was undertaken with the objective of studying the effect of sugarcane bagasse as substrate for fish production under farm conditions and at lower densities than in our earlier trials.

## Materials and Methods

### *Pond*

The trial was conducted in an 800 m<sup>2</sup> farmer's pond on sandy loam soil in Chelur Village near Mangalore, Karnataka, India over a period of 180 days (11 October 1999 to 12 April 2000). The pond was dried and quicklime was applied at a rate of 300 kg·ha<sup>-1</sup>. The pond was fertilized at weekly intervals with cow stable washings. The pond was partitioned into eight 100 m<sup>2</sup> compartments by 1 mm nylon mesh netting. Water from a spring was used to maintain the water level at a depth of 80 cm, counteracting evaporation and seepage losses. Dried sugarcane bagasse were made into bundles (diameter - 3.3 cm; length - 80 cm; mean weight - 180.6 g) using nylon twine. Bundles were used at rates of 0 (no sub-

strate), 39 (low density, corresponding to 7.0 kg·100 m<sup>2</sup>), 76 (medium density, 13.7 kg·100 m<sup>2</sup>) and 156 (high density, 28.2 kg·100 m<sup>2</sup>) per compartment, with two replicates per treatment, randomly assigned among compartments. The bundles were suspended at regular intervals from thick nylon ropes tied across the pond.

### ***Fish***

In each compartment, 50 catla (*C. catla*, Cyprinidae; mean weight 1.0 g), 25 rohu (*L. rohita*, Cyprinidae; 3.8 g) and 25 common carp (*C. carpio*, Cyprinidae; 1.1 g) fingerlings were stocked (day 0). Fish in one of each of the four replicate bundle density treatments were fed a 1:1 mixture of groundnut cake and rice bran at a rate of 5% body weight each morning, the remaining four compartments received no feed. Fish biomass in control tanks was monitored each month, and feed ration was adjusted accordingly. Feeding rate was reduced to 3% body weight per day after three months. At harvest, all surviving fish were individually weighed and survival (%) and gross production (g·tank<sup>-1</sup>) were calculated for each compartment.

### ***Water quality monitoring***

Water quality was monitored every 15 days, starting on day 0. Water samples were collected between 0900 and 1000 h. In each compartment, temperature (surface and bottom), dissolved oxygen (surface and bottom), pH, water transparency (Secchi disc depth), total alkalinity, ammonium, nitrate and phosphate were monitored. Temperature and pH were measured using a Horiba (Japan) water quality analyzer (model U10). Dissolved oxygen, total alkalinity, ammonia, nitrate and phosphate were chemically analyzed following standard methods (APHA 1992).

### ***Periphyton and plankton***

Periphyton dry matter, ash, chlorophyll and pheophytin and plankton dry matter and ash were determined every 30 days. First, periphyton was scraped from a 2 cm wide band from three randomly selected bundles in each compartment for determination of chlorophyll-*a* and pheophytin (APHA 1992). The remaining periphyton was then carefully removed from each of the sample bundles for dry matter determination. Samples were dried at 100°C to constant weight and the ash content was determined in a muffle furnace (4 h at 550°C). Ash free dry matter (AFDM) was calculated by subtracting the ash value from the dry matter content. After sampling, the poles were replaced in their respective compartments and marked to avoid further sampling.

Plankton samples were taken by filtering 100 l of water from each compartment through a 60 µm mesh plankton net. The samples were dried and ignited to determine dry matter and ash content.

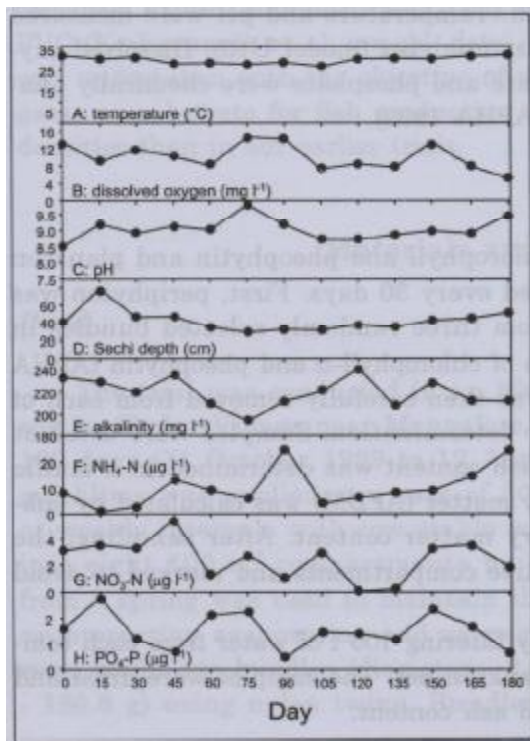
## Statistical analysis

Mean final weights of each species in the eight compartments were compared using the Tukey HSD multiple comparison test ( $\alpha = 0.05$ ). The other fish production parameters (percentage survival and gross yield) were plotted against substrate density for compartments with and without feed, and linear and quadratic regressions were estimated. No statistical tests were done for the comparisons of plankton and periphyton biomass. All statistical computations were made using Systat 5.05 for Windows (SPSS Inc., Chicago, Illinois, USA).

## Results

### Water quality

Due to the exchange of water through the nylon netting, differences in water quality between compartments were small and water quality on a whole pond basis is considered. Generally water quality was good, with temperature, dissolved oxygen and pH values being favorable for fish culture (Fig. 1). Temperature varied from 26 to 33°C. There was a downward trend in dissolved oxygen levels over the course of the trial, decreasing from 13 mg·l<sup>-1</sup> at stocking to around 4 mg·l<sup>-1</sup> at the end of the experiment. Values of pH were high, ranging from 8.2 to 10.2. Secchi disc values decreased from 80 cm on day 15 to a minimum of 20 cm on day 105. Alkalinity fluctuated between 184 and 252 mg·l<sup>-1</sup>. Concentrations of ammonia, nitrite and nitrate were generally low (Fig.1).



### Periphyton and plankton

Mean periphyton biomass was higher in the low density substrate treatment, in terms of both AFDM and pigment content (Table 1). However, this was principally due to the high biom-

Fig. 1. Mean surface water temperature (A °C), surface dissolved oxygen concentration (B mg·l<sup>-1</sup>), pH (C), secchi depth (D cm), alkalinity (E mg·l<sup>-1</sup>), NH<sub>4</sub>-N (F mg·l<sup>-1</sup>), NO<sub>2</sub>-N (G mg·l<sup>-1</sup>) and PO<sub>4</sub>-P (H mg·l<sup>-1</sup>). Figures are means of 8 compartments, except pH (values of 1 compartment).

ass ( $0.2$  to  $0.4$   $\text{mg}\cdot\text{dm}\cdot\text{cm}^{-2}$ ,  $3$  to  $5$   $\mu\text{g}\cdot\text{total pigment}\cdot\text{cm}^{-2}$ ) during the first two months in the low substrate density, fed compartment. After day 60, periphyton biomass remained between  $0.05$  and  $0.1$   $\text{mg}\cdot\text{cm}^{-2}$  in all compartments (Fig. 2). Periphyton dry matter was higher in fed than non-fed treatments, although chlorophyll content did not differ much among treatments (Table 1).

Plankton biomass was generally higher in the treatments with feed, but there were no clear differences between substrate densities. Mean plankton biomass seemed to be higher in the low and medium substrate density treatments with feed, but there was a lot of fluctuation in time (Table 1; Fig. 2).

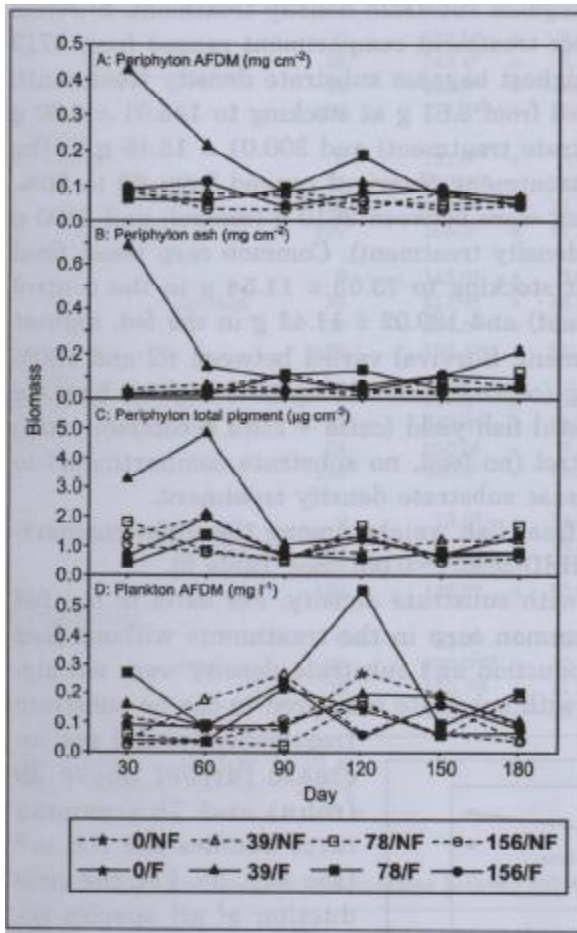


Fig. 2. Periphyton AFDM, ash and total pigment (chlorophyll- $\alpha$  + pheophytin) and plankton AFDM. F = feeding, NF = no feeding. Numbers indicate substrate density (bagasse bundles per  $100 \text{ m}^2$ ).

Table 1. Periphyton and plankton dry matter, AFDM, and periphyton total pigment (chlorophyll- $\alpha$  + pheophytin) content.

Feeding	Substrate (bundles $100\text{-m}^{-2}$ )	Periphyton			Plankton	
		AFDM ( $\text{mg}\cdot\text{cm}^{-2}$ )	Ash ( $\text{mg}\cdot\text{cm}^{-2}$ )	Total pigment ( $\mu\text{g}\cdot\text{cm}^{-2}$ )	AFDM ( $\text{mg}\cdot\text{l}^{-1}$ )	Ash ( $\text{mg}\cdot\text{l}^{-1}$ )
no feed	0	-	-	-	$0.36 \pm 0.18$	$1.00 \pm 0.63$
	39	$0.07 \pm 0.02$	$0.05 \pm 0.03$	$1.19 \pm 0.45$	$0.36 \pm 0.21$	$0.87 \pm 0.73$
	78	$0.06 \pm 0.02$	$0.06 \pm 0.04$	$1.13 \pm 0.64$	$0.29 \pm 0.28$	$0.58 \pm 0.45$
	156	$0.05 \pm 0.02$	$0.02 \pm 0.02$	$0.77 \pm 0.39$	$0.24 \pm 0.16$	$0.44 \pm 0.38$
feed	0	-	-	-	$0.27 \pm 0.08$	$0.65 \pm 0.68$
	39	$0.17 \pm 0.14$	$0.21 \pm 0.24$	$1.98 \pm 1.69$	$0.53 \pm 0.52$	$0.81 \pm 0.53$
	78	$0.09 \pm 0.05$	$0.06 \pm 0.05$	$0.76 \pm 0.32$	$0.69 \pm 0.42$	$1.84 \pm 1.47$
	156	$0.08 \pm 0.02$	$0.06 \pm 0.04$	$1.08 \pm 0.53$	$0.37 \pm 0.13$	$0.80 \pm 0.62$

Figures are means ( $\pm$  sd) of six sampling dates ( $n = 6$ ).

## Fish production

Catla mean weight increased from 1.01 g at stocking to  $112.51 \pm 9.86$  g at harvest in the control (no feed, no substrate treatment) and to  $177.27 \pm 10.15$  g in the no feed, highest bagasse substrate density treatment. Survival ranged from 60 to 68%. Yields per treatment compartment ranged from 3713 g (control) to 5850 g (no feed, highest bagasse substrate density treatment). Rohu mean final weight increased from 3.81 g at stocking to  $145.01 \pm 7.60$  g in the control (no feed, no substrate treatment) and  $300.01 \pm 13.46$  g in the fed, highest bagasse substrate treatment. Survival ranged from 68 to 80%. Yields per treatment compartment were between 2610 g (control) and 5400 g (fed, highest bagasse substrate density treatment). Common carp mean final weight increased from 1.06 g at stocking to  $73.03 \pm 11.54$  g in the control (no feed, no substrate compartment) and  $180.02 \pm 11.42$  g in the fed, highest bagasse substrate density treatment. Survival varied between 92 and 100% and yields were between 1753 g (control) and 4320 g (fed, highest bagasse substrate density treatment). Total fish yield (catla + rohu + common carp) ranged from 8077 g in the control (no feed, no substrate compartment) to 14842 g in the fed, highest bagasse substrate density treatment.

Most of the differences in final fish weight among the eight compartments were significant (Tukey HSD test,  $P < 0.05$ ) (See Table 2).

Fish production increased with substrate density. For catla in the fed treatment, and for rohu and common carp in the treatments without feed the regressions between fish production and substrate density were not significant. Production was higher with substrate compared to the no substrate

treatment, but did not increase further above 39 (rohu) and 78 (common carp) bundles per  $100 \text{ m}^2$  (see Fig. 3). For the production of all species together, the relationship with substrate density was best described using quadratic regressions (Table 3), from which the optimum bagasse density was

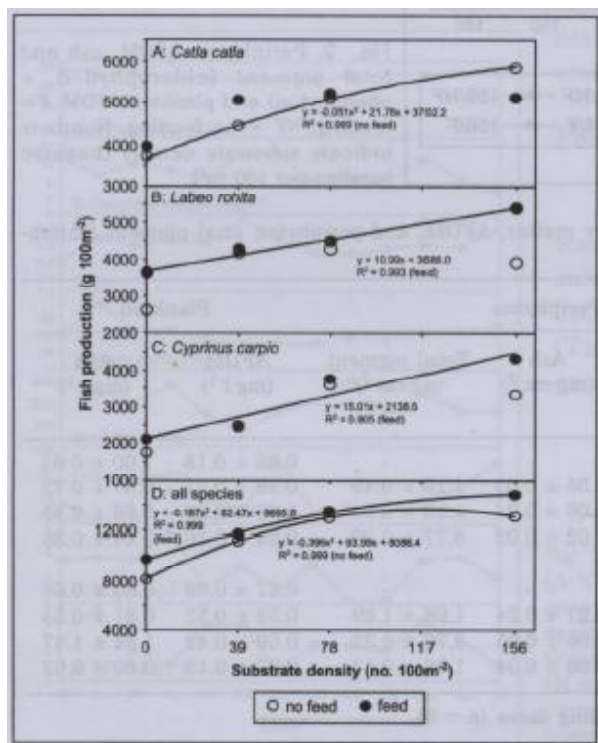


Fig. 3. Fish production in relation to substrate density for catla, rohu and common carp and for all species combined. Substrate densities are in number of sugarcane bagasse bundles per  $100 \text{ m}^2$  (0, 39, 78 and 156, corresponding with 0, 7.0, 13.7 and 28.2 kg/100  $\text{m}^2$ , respectively). Regression lines show the significant (F-test,  $P < 0.05$ ) regressions found.

Table 2. Fish final individual weight, survival percentage and gross yield. Mean weights sharing the same superscript are not significantly different. (Tukey HSD test,  $\alpha = 0.05$ ).

Species	Feeding	Substrate	Final weight (g)			Survival (%)	Gross yield (g-fish 100 m <sup>2</sup> )	
			mean	±	sd			n
Catla* ( <i>C. catla</i> )	no feed	0	112.5 <sup>a</sup>	±	9.9	33	66	3713
		39	143.4 <sup>c</sup>	±	8.1	31	62	4445
		78	150.3 <sup>cd</sup>	±	8.8	34	68	5111
		156	177.3 <sup>gh</sup>	±	10.2	33	66	5850
	feed	0	131.6 <sup>b</sup>	±	9.4	30	60	3948
		39	153.6 <sup>de</sup>	±	10.5	33	66	5068
		78	173.9 <sup>g</sup>	±	11.1	30	60	5216
		156	165.2 <sup>f</sup>	±	11.2	31	62	5122
Rohu** ( <i>L. rohita</i> )	no feed	0	145.0 <sup>a</sup>	±	7.6	18	72	2610
		39	211.4 <sup>c</sup>	±	10.7	20	80	4229
		78	225.0 <sup>de</sup>	±	13.3	19	76	4275
		156	196.1 <sup>b</sup>	±	11.4	20	80	3921
	feed	0	214.3 <sup>cd</sup>	±	11.6	17	68	3643
		39	233.3 <sup>ef</sup>	±	12.5	18	72	4200
		78	250.0 <sup>g</sup>	±	9.3	18	72	4500
		156	300.0 <sup>h</sup>	±	13.5	18	72	5400
Common carp*** ( <i>C. carpio</i> )	no feed	0	73.0 <sup>a</sup>	±	11.5	24	96	1753
		39	100.0 <sup>bc</sup>	±	16.3	25	100	2501
		78	150.0 <sup>f</sup>	±	11.8	24	96	3601
		156	133.3 <sup>e</sup>	±	13.9	25	100	3333
	feed	0	92.0 <sup>b</sup>	±	12.7	23	92	2117
		39	102.0 <sup>bcd</sup>	±	10.2	24	96	2447
		78	150.7 <sup>fg</sup>	±	11.2	25	100	3768
		156	180.0 <sup>h</sup>	±	11.4	24	96	4320

\*Mean weight at stocking = 1.01 g

\*\*Mean weight at stocking = 3.81 g

\*\*\*Mean weight at stocking = 1.06 g

Table 3. Linear and quadratic relationships between substrate density and fish production.

Species	Feeding	Type	a	b	c	R <sup>2</sup>	F
<i>C. catla</i>	no feeding	lin	3858.0**	13.51*		0.969	62.50*
		quad	3702.2**	21.78*	-0.051 <sup>MS</sup>	0.999	882.59*
	feeding	lin	4410.8**	6.23 <sup>NS</sup>		0.485	1.89 <sup>NS</sup>
		quad	4014.7*	27.27 <sup>NS</sup>	-0.130 <sup>NS</sup>	0.949	9.28 <sup>NS</sup>
<i>L. rohita</i>	no feeding	lin	3329.0*	6.30 <sup>NS</sup>		0.288	0.81 <sup>NS</sup>
		quad	2727.5 <sup>MS</sup>	38.25 <sup>NS</sup>	-0.198 <sup>NS</sup>	0.909	5.02 <sup>NS</sup>
	feeding	lin	3686.0***	10.99**		0.993	294.26**
		quad	3672.0*	11.73 <sup>NS</sup>	-0.005 <sup>NS</sup>	0.994	77.96 <sup>NS</sup>
<i>C. carpio</i>	no feeding	lin	2105.2*	10.13 <sup>NS</sup>		0.647	3.66 <sup>NS</sup>
		quad	1656.0 <sup>NS</sup>	33.99 <sup>NS</sup>	-0.148 <sup>NS</sup>	0.947	8.88 <sup>NS</sup>
	feeding	lin	2138.6*	15.01*		0.905	19.15*
		quad	1978.9 <sup>NS</sup>	23.49 <sup>NS</sup>	-0.052 <sup>NS</sup>	0.930	6.61 <sup>NS</sup>
all	no feeding	lin	9292.4*	29.94 <sup>NS</sup>		0.723	5.22 <sup>NS</sup>
		quad	8086.4**	93.99**	-0.396*	1.000	7712.63***
	feeding	lin	10235.2*	32.23*		0.930	28.57*
		quad	9665.8**	62.47*	-0.187 <sup>MS</sup>	0.999	341.53*

\*(P<0.05), \*\*(P<0.01), \*\*\* (P<0.001), MS = marginally significant (P<0.10) NS = not significant (P>0.10)

Superscripts indicate the results of a t-test for the regression coefficients (a, b and c) and an F-test for the whole model.

estimated at 117 bundles (or 21 kg) per 100 m<sup>2</sup> without feeding and 156 bundles (28 kg) per 100 m<sup>2</sup> with feeding (Fig. 3).

Figure 4 shows the increase in gross yield (%) compared to the control (no feed, no substrate). Feeding alone gave a 20% increase in yield, while substrate addition without feeding increased yield by 38, 61 and 62% with densities of 39, 78 and 156 bagasse bundles per 100 m<sup>2</sup>, respectively. The combination of feeding and substrate increased yields by 45, 67 and 84% with densities of 39, 78 and 156 bundles per 100 m<sup>2</sup>, respectively.

## Discussion

Overall, there was little variation in periphyton dry matter with time. Total pigment content (chlorophyll- $\alpha$  + pheophytin) and AFDM levels (0.76 to 1.98  $\mu\text{g}$  total pigment-cm<sup>-2</sup>; 0.02 to 0.17 mg AFDM-cm<sup>-2</sup>) were somewhat lower than that found in a previous study with bagasse as substrate (1.89 to 3.62  $\mu\text{g}$  total pigment-cm<sup>-2</sup>; 0.13 to 0.59 mg AFDM-cm<sup>-2</sup>) (Keshavanath et al. 2001). This may have been because no fish was present in the former study. While periphyton AFDM values were consistently higher in fed than in unfed treatments, there were no such consistent differences in total pigment content. A similar picture was found in an earlier study with the combination of periphyton and feeding (Keshavanath et al., unpubl. data). Periphyton substrates tend

to entrap suspended organic material, which is likely to be more abundant when fish are fed due to uneaten feed and fish feces.

Highest production in the present study due to periphyton alone was around 1300 kg·ha<sup>-1</sup> in 180 days (medium and high substrate densities), comparable to the results from other polyculture studies. Azim et al. (2000) recorded catla and rohu production of 586 kg·ha<sup>-1</sup> in 70 days, us-

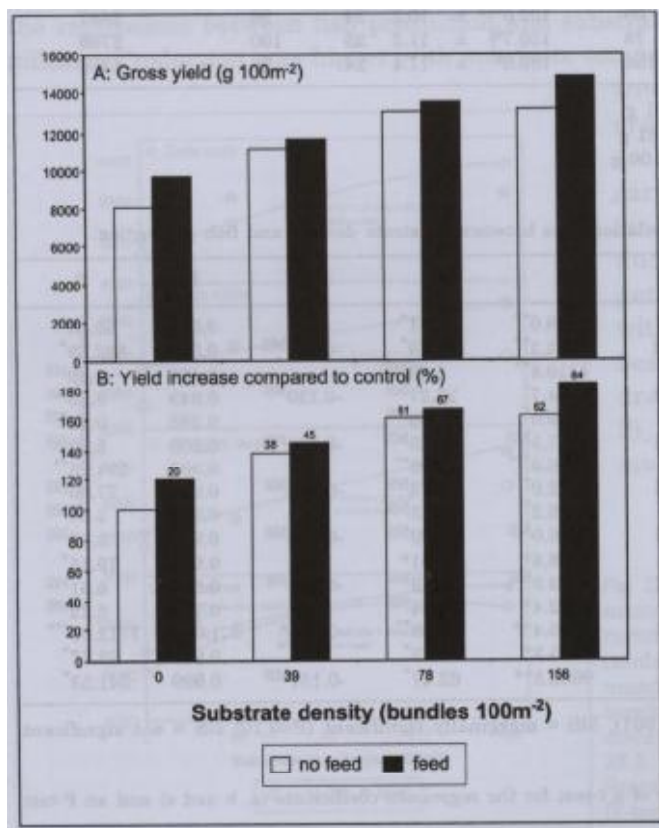


Fig. 4. Gross fish yield and yield increases compared to the no feed, no periphyton (control) treatment.



ing bamboo as substrate, while Ramesh et al. (1999) reported rohu and common carp production of 1235 kg·ha<sup>-1</sup> in 133 days, using sugarcane bagasse as substrate. Increases in yield were also comparable to monocultures of herbivorous fish (*Tor khudree* and *Labeo fimbriatus*) with bamboo substrates: 24 to 55% increase with feed alone, 34 to 71% increase with substrate alone, and 45 to 82% increase using feed and substrate (Keshavanath et al. unpubl. data).

In our previous study, we used the equivalent of 914.2 kg sugarcane bagasse per 100 m<sup>2</sup>, which led to anoxic conditions in the fish tanks and mortality (Keshavanath et al. 2001). The reduced size and density of the bagasse bundles in the present study ensured reasonable water quality, especially in terms of dissolved oxygen which remained in the range of 4 to 13 mg·l<sup>-1</sup> throughout. This shows that sugarcane bagasse can be used as substrate for periphyton in pond culture, provided that the bundle density is not excessive. While bagasse has been shown to be ineffective as a feed ingredient for tilapia (El-Sayed 1991), its use as a fertilizer to ponds led to higher microbial biomass and shrimp growth than feedlot manure (Visscher and Duerr 1991, Freeman et al. 1992). The potential of sugarcane bagasse as a biodegradable substrate for production of bacterial biomass in biofilms was also shown by Shankar et al. (1998) and Ramesh et al. (1999). Its application as a substrate for primary and secondary production that can be subsequently consumed by fish is thus worthy of consideration.

In the present on-farm experiment, the experimental design was constrained by the field conditions. The compartments were separated by nylon mesh, enabling water exchange between compartments, obscuring any effects of treatment on water quality. However, results confirmed the findings of previous on-station experiments that sugarcane bagasse is a suitable substrate for periphyton and that periphyton can be incorporated into carp polyculture. Further research is needed to optimize stocking ratios and to assess the economic feasibility of periphyton-based systems in Indian aquaculture.

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