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Impact of periphyton on makhana (*Euryale ferox* Salisbury) cum fish culture: a study in North Bihar, India

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Abstract – Bihar possesses rich aquatic biodiversity and abundant water resources. Furthermore, the commercial aquatic crop industry in the northern region of Bihar often employs co-cultivation practices. The study was conducted at the ICAR-National Research Centre for Makhana in Darbhanga, Bihar. The objectives of the study were to investigate the impact of periphyton-based aquaculture on the makhana cum fish culture system, the compatibility of Indian major carp (IMC), and assess water quality parameters. The experiment, from December 2021 to November 2022, utilized the '*Swarna Vaidehi*' makhana variety and Indian major carp. The study was conducted in a pond having an area of 800 m^2 with a depth of 1.2 to 1.5 m in triplicates, applying six treatments (MF1 to MF6), including periphyton substrate frame, fertilization, and supplementary feed. Fish fingerlings of various species were stocked during the investigation at the rate of 6000 individuals per hectare in makhana-fish integrated ponds. The stocked species included catla (*Catla catla*), rohu (*Labeo rohita*), and mrigal (*Cirrhinus mrigala*) in a ratio of 2:2:1, respectively. Results indicated significant influences of treatments on fish and makhana yields. Treatment MF4 yielded the highest total fish production, including catla, rohu, and mrigal, with values of 0.98 ± 0.006, 0.98 ± 0.004, and 0.092 ± 0.005 t ha^{-1} yr^{-1}, respectively, while treatment MF5 led to makhana seed production at 1.78 ± 0.077 t ha^{-1} yr^{-1}. The estimated yield in the experiment differed significantly among the treatments, as determined by the ANOVA. Moreover, incorporating periphyton substrate frames improved water quality and natural food availability, enhancing fish production without compromising makhana seed yield. Thus, a periphyton-based makhana cum fish farming system holds the potential to enhance productivity and could be a sustainable farming technique in the northern part of Bihar and elsewhere in India with similar conditions.

Keywords: Bihar / fisheries / integrated aquaculture / makhana / periphyton

1 Introduction

Periphyton is an assemblage of several minute organisms, including algae, plankton, zooplankton, bacteria, and organic matter, attached to submerged objects in aquatic environments (Azim, 2009). It covers many inundated substrates in the littoral zone of freshwater bodies, such as sand, macrophytes, and rocks (James et al., 2000). Periphyton plays a crucial role in the aquatic ecosystem by contributing to primary productivity and providing a habitat for diverse aquatic organisms (Likens, 1985). Periphyton-based fish farming systems offer a cost-effective and simple method for producing natural food for cultured species, making it a potentially sustainable aquaculture approach (David et al., 2022). Many studies have demonstrated that periphyton can serve as a natural food source for fish (Negri et al., 2023; David et al., 2022). Notably, it has been observed that the biomass of periphyton increases subsequently, leading to an increase in fish biomass in periphyton-based aquaculture systems (Azim et al., 2001). Fish production in pond systems has become more expensive due to heavy reliance on external inputs such as feed, fertilizers, medicines, and supplements for natural food production in the ecosystem. Most current production systems and intensive farming methods exhibit inefficiency in nutrient recycling, with only 50–60% of nutrient inputs converting into biomass. The remaining nutrients are wasted in

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sediments, effluent, and atmospheric emissions (Beveridge et al., 1994; Olah et al., 1994; Bhujel, 2012). Intensive fish production systems depend heavily on the environment to disperse and assimilate waste (Beveridge and Phillips, 1993). Considering the need for sustainable farming practices, periphyton-based fish farming is a promising option that could reduce input costs and promote sustainable aquafarming. This farming technique could benefit economically underprivileged regions like Bihar, India, providing a new direction for aqua-entrepreneurs. Periphyton is an ideal natural food material for many fish species, including Indian major carp and tilapia, due to its proximate composition with approximately 27.19% crude protein, 18% lipid and 52% carbohydrates (Abwao et al., 2013). Several studies have reported that periphyton grown on artificial substrates like bamboo poles can enhance fish production by 20–100%, depending on the quantity and quality of periphyton, fish species, stocking density and culture system (Azim et al., 2001). For instance, Faruk-ul-Islam (1996) reported 10% higher tilapia production in the presence of periphyton compared to its absence. Hem and Avit (1994) also documented the increased output of approximately 8 t ha^{-1} $year^{-1}$ of blackchin tilapia from bamboo-based periphyton farming. The specific growth rate (SGR) of fish was also higher in periphyton-based aquaculture systems (Sukumaran et al., 2017).

Euryale ferox Salisbury, commonly known as a gorgon nut, prickly water lily, or makhana, is an annual hydrophyte that thrives in perennial water bodies such as ponds, oxbow lakes, swamps and ditches (Raut et al., 2020). Makhana seeds hold significant importance due to their multifaceted benefits spanning livelihood support, medicinal values, industrial applications and nutritional values. Culturally entrenched in North Bihar, India, these seeds contribute to local economies and culinary traditions (Singh et al., 2023; Raut et al., 2020, 2024; Jha and Kumari, 2016; Mandal et al., 2010). They emerge as a highly nutritious food source after processing from a black hard nut to a white puff. Rich in essential nutrients and bioactive compounds such as carbohydrates, protein, fibre, vitamins, minerals, and polyphenols, makhana seeds offer superior food qualities (Kapoor et al., 2022). Notably, they boast a high amino acid index ranging from 89% to 93% and exhibit favourable ratios of arginine to lysine/proline (Nath and Chakraborty, 1985). Moreover, makhana seeds have demonstrated medicinal properties, offering potential therapeutic effects against various ailments, including diabetes, digestive issues, cardiac conditions, renal problems, and reproductive disorders (Das et al., 2006; Jha and Barat, 2003). Furthermore, the consumption of roasted makhana pops presents a nutrient-rich and low Glycemic Index (GI) option, with a GI of only 37%, making them suitable for inclusion in healthy diets and beneficial for individuals with metabolic disorders such as obesity and diabetes (Liaquat et al., 2022). North Bihar has a wide geographic range and is an important aquatic cash crop cultivated in natural water bodies and agricultural fields. Makhana seed production of approximately 60,000 tonnes has been recorded from the 35,000-ha area of north Bihar, accounting for >85% production of makhana seeds produced in India (Directorate of Horticulture, 2022). The makhana cum fish culture system has emerged as one of the prominent livelihood options among fishermen communities in north Bihar, India. A pilot survey of makhana ponds revealed challenges related to using a wild variety of makhana, farming practices, weed occupancy, humic turbidity, brownish water colour, suspended matter, hypoxic conditions, low primary productivity, vegetation occupancy, and selection and availability of candidate fish species. These factors have been affecting the growth and aquaculture production of culturable fishes. In this context, the environmentally friendly periphyton-based developed makhana variety with cum fish farming system (also called a makhana-fish-periphyton system) can hold the potential to achieve increased overall fish and makhana production. It could be a sustainable farming technique in the northern part of Bihar. Moreover, Bihar has rich aquatic biodiversity and supports small-scale fisheries and aquatic crop farming in wetland areas. Culturing fish species with aquatic crops based on species complementarity and compatibility in a farm setting can optimize resource use and enhance overall aquaculture production. This shift from monoculture to integrating aquaculture systems by harnessing species diversity is widely recognized as a fundamental agroecological principle for designing sustainable aquaculture systems in the future. Hence, the present study aims (1) to investigate the impact of periphyton-based aquaculture on the productivity of the makhana-fish culture system, (2) to evaluate the compatibility of Indian major carp (IMC) within the periphyton-based aquaculture system, and (3) to assess the physico-chemical properties of water within the periphyton-based system.

Table 1. Experimental treatments with combinations.

Treatment	Factors
MF1	Makhana and fish
MF2	Makhana, fish, and periphyton substrate
MF3	Makhana, fish, periphyton substrate, and fertilizer
MF4	Makhana, fish, periphyton substrate, fertilizer, and supplementary feed
MF5	Makhana, fish, fertilizer, and supplementary feed
MF6	Makhana, fish, and supplementary feed

2 Materials and methods

2.1 Experimental design and pond preparation

The experiment was conducted at the ICAR – National Research Centre for Makhana in Darbhanga, Bihar, India (26.189°N & 85.905°E) over the period from December 2021 to November 2022. The makhana plants utilized in the experiment, specifically the "*Swarna Vaidehi*" variety developed by ICAR-NRC for Makhana, Darbhanga, were selected from natural water bodies prevalent in north Bihar. The experiment comprised six treatments denoted as MF1 to MF6, each with a distinct combination of factors. These treatments included variations in the incorporation of makhana, fish, periphyton substrate, fertilizer, and supplementary feed. Each treatment was replicated thrice, resulting in 18 experimental units ($n = 18$) (Tab. 1). The inclusion or exclusion of each factor or group of factors was designed to identify its significant contribution to yield. Comparative analysis of fish and makhana seed production was conducted across various treatments using a standardized protocol under general conditions. The experimental ponds, each measuring 800 m^2 in area and 1.2 m in depth, featured a silt-clay loamy soil texture with a pH of 7.8, organic carbon 0.3%, available nitrogen 200 kg ha^{-1}, available phosphorus 15.6 kg ha^{-1} and available potassium 208 kg ha^{-1}. This systematic approach allowed for a comprehensive evaluation of the impact of different treatments on fish and makhana seed production in an experimental pond setting.

2.2 Makhana cultivation

2.2.1 Nursery raising

Makhana cultivation is generally practiced by two primary methods: the pond system method (PSM) and the field system method (FSM). The PSM starts the cultivation process by sowing makhana seeds in a nursery and then transplanting them as saplings into main well-prepared ponds with a water depth of around 1 meter.

As an aquatic crop, makhana thrives well in high water-retentive clayey soil, rich in organic matter (Kumar et al., 2017). The makhana seeds were broadcasted in December in a separate nursery pond. 20 kg of healthy seeds were uniformly broadcasted over a 500 m^2 nursery area. The water depth in the nursery pond was maintained at about 0.30 m throughout the growing period of the seedlings from December to March, and these seedlings/saplings were used in all treatment ponds.

2.2.2 Makhana pond preparation

Makhana ponds were prepared (i.e., ploughing and puddling) during the first week of December to the second week of March. Before transplantation of the sapling, to retain a sufficient water level between 0.3 to 0.5 m depth in an earthen pond, manuring, and fertilizer application are essential to provide the proper nourishment. Being an aquatic crop with large and heavy leaf sizes, the requirement for nutrients for the makhana crop is very high. The integrated nutrient package (INP) for the makhana cultivation consisting of the ratio of nitrogen, phosphorus, and potassium (N: P: K) at the rate of 100:60:40 kg ha^{-1} is recommended in the study area. Therefore, recommended doses of N (through urea), P (through single super phosphate), K (through muriate of potash) were applied to ponds along with organic manure @10 t ha^{-1} (Singh et al., 2020; Kumar et al., 2020). Half of the total recommended dose of NPK was applied as a basal dose. The entire dose of organic fertilizer, in the form of cow dung manure, was also applied as a basal dose and thoroughly incorporated into the soil before planting. The remaining doses were applied in the early vegetative stage and flowering stage of makhana.

2.2.3 Transplanting

In March, healthy saplings were uprooted from the nursery area and immediately transplanted in the well-prepared ponds (i.e., PMS method). The saplings were spaced one meter apart, both between rows and between plants (Kumar et al., 2020). Generally, there are two designs for integrating makhana with fish during transplantation: central vacant space design, where 10-15% of the entire water spread area is left at the centre, and peripheral vacant space design, where the space is left in the peripheral portion of the pond (Mishra et al., 2003). For this study, a peripheral vacant space design was adopted for all ponds. This design allows solar radiation to penetrate up to the bottom of the pond, providing enough natural food for fish. It also makes managing sprawling makhana leaves in the peripheral region easier. After two months of transplantation (i.e., in May), bright purple and solitary flowers appeared on the makhana plants in an unsynchronized manner (Fig. 1).

2.2.4 Makhana seed harvesting

Flowering and fruiting commence in May and extend through October/November. The maturation of fruits takes approximately 40–45 days post-flowering. Subsequently, the fruits undergo rupturing, and the seeds, enveloped in a pinkish cover, float on the water surface. Within 2–3 days, they settle on the sediment at the bottom of the pond. However, in our research period, makhana seeds were harvested during November by using a lightweight bamboo (*Bambusa* sp.) structure locally referred to as a '*Gaanja*'. This funnel-like sieve device is easily immersed into the pond, reaching the bottom to separate makhana seeds from the sediment. The makhana seeds are collected after vigorous shaking and filtering of muddy water. Afterward, harvested makhana seeds are used in the food processing plant (Kumar et al., 2020).

2.2.5 Periphyton substrates

The periphyton substrate frames were installed in MF2, MF3, and MF4 treatments in the first week of May. The substrate frames were made from locally available bamboo and were inserted vertically into the bottom of experimental ponds (two frames per square meter), ensuring they remained submerged and away from direct sunlight. Successional assemblages of periphyton appeared within a month, forming a slime/biofilm structure on the substrate surfaces (Fig. 2).

2.2.6 Stocking of fish fingerlings

IMC fingerlings were procured from commercial vendors and quarantined by using $KMnO_4$ (2 ppm) (Arthur et al., 2000). The fish were kept in a separate tank for acclimation for 15 days before being released into the treatment ponds at a stocking density of 6000 no. ha^{-1}. The stocked species included catla (*Catla catla,* Hamilton 1822), rohu (*Labeo rohita*, Hamilton 1822), and mrigal (*Cirrhinus mrigala,* Hamilton 1822), in the ratio of 2:2:1, respectively. The fish fingerlings were stocked in the pond during the first week of June to coincide with the well-developed vegetative growth stage and optimum water depth (Fig. 1). Furthermore, feeding included treatment ponds such as MF4, MF5, and MF6 were provided supplementary feed during the culture period.

2.2.7 Supplementary feed

The conventional supplementary feed was applied according to their body mass, initially used at 3 to 5% for two months and 1 to 2% for the remaining month. The mixed composition

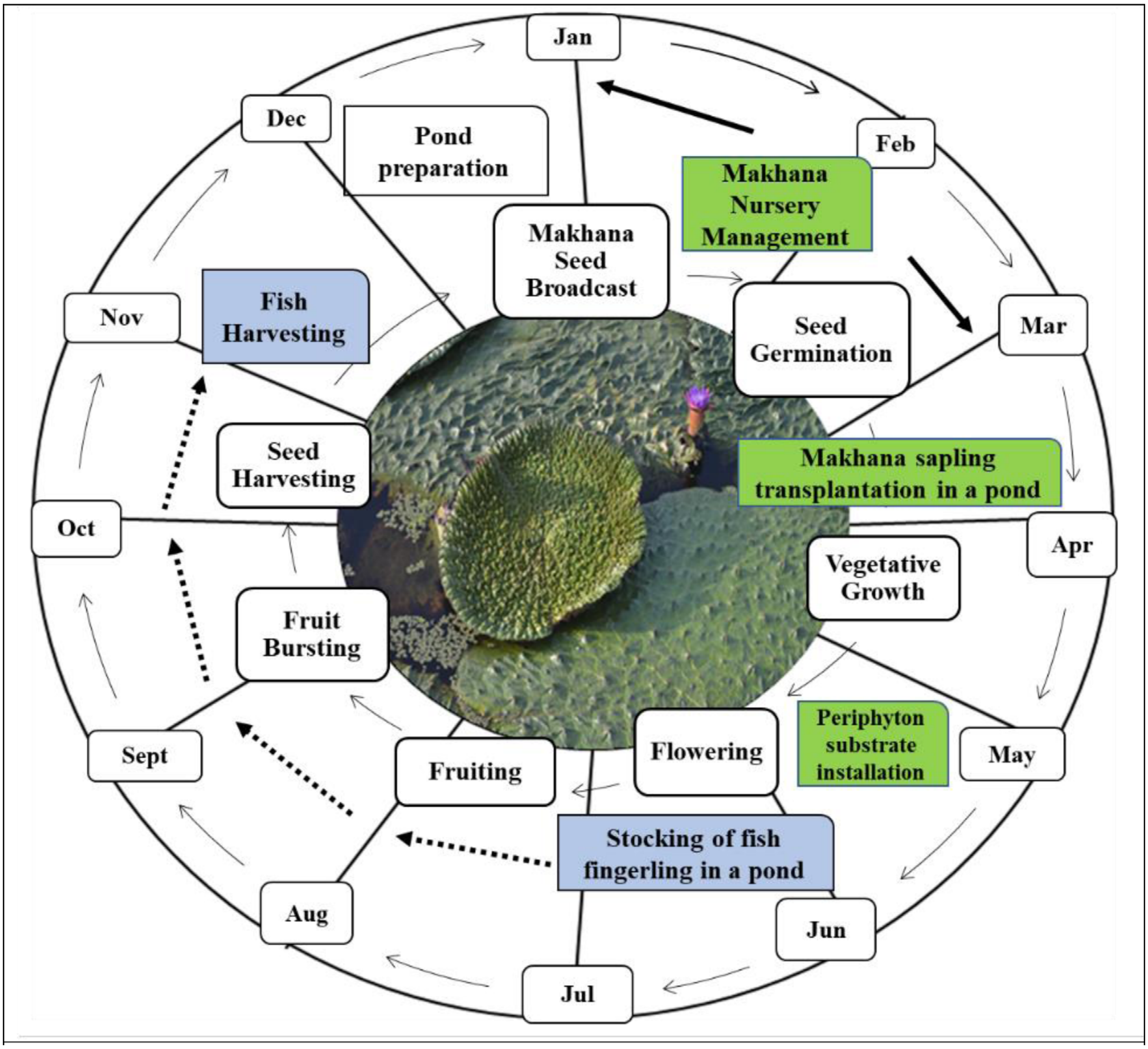

Fig. 1. Life cycle of the makhana plant and culture practices.

of wheat/rice bran and mustard oil cake (MOC) in a ratio of 2:1 was applied twice a day (Paul et al., 2017).

3 Periphyton assessment

3.1 Periphyton density

Fortnightly, the periphyton substrate frame from each of treatment was carefully scraped 2×2 cm^2 surface area with a scalpel blade to remove all periphyton visually without affecting the substrate. After sampling, the substrate was placed in its original position and marked to exclude it from subsequent sampling. The samples of each substrate were re-suspended in 50 ml distilled water and preserved in 5% formalin in a sealed container. After vigorous shaking, 1 ml of sub-sample was transferred to a Sedgewick-Rafter cell (S-R cell) divided into 1000 squares, upon which the number of colonies was counted in 1000 squares field of the chamber under the optical microscope (LYNX microscope, Lawrence and Mayo, India) (Letourneau et al., 2011; Nahiduzzaman et al., 2023). Microscopic periphyton assemblages were identified by using online sources and standard literature (Dutta et al., 2018; Baluni et al., 2018; Kaviyarasan and Athithan, 2019; Anix et al., 2017; Das et al., 2017; APHA, 2017). The periphyton sample densities were calculated using the following formula as given by Azim et al. (2002) and Anix et al. (2020):

$$N = \frac{P \times C \times 100}{S}$$

where, N = number of periphyton cells per cm^2 surface area; P = number of periphytic units counted in ten fields; C = volume of final concentrate of the sample (ml); S = area of scraped surface (cm^2).

3.2 Physicochemical parameters of water

Water samples from each treatment were collected fortnightly in a clean bottle (500 ml) during the morning

Fig. 2. Makhana plant and periphyton substrate in the pond ecosystem.

hours (7:00 AM to 9:00 AM) and chemically analysed in the laboratory using standard methods. In-situ water quality parameters, i.e., temperature (°C), pH, electric conductivity (mS cm^{-1}), and total dissolved solids/TDS, were measured by using a standard portable instrument (Waco, model: WA-2015), and transparency (cm) by secchi disc. Other parameters, such as ammonia-nitrogen (NH_3-N) in the water samples, were estimated colorimetrically using the Indo-phenol blue method. Dissolved oxygen (DO) was measured using the winkler method, total alkalinity and **free carbon dioxide** were measured by the titrimetric method (Apollos et al., 2016; APHA, 2017).

4 Statistical analysis

For data analysis and interpretation, the collected data were organized into tables and tested for normality using the Shapiro–Wilk test. This test is essential for determining whether the data follow a normal distribution, which is important for the reliability of subsequent parametric tests, such as ANOVA. A one-way ANOVA was then conducted to identify significant differences in seasonal data related to water quality, periphyton abundance, fish growth metrics, and biomass, using a 5% significance level. After normality test, Duncan's multiple range test (DMRT) was employed to determine specific group differences, such as variations between seasons, yield, or different treatment groups. All statistical parameters were analyzed by IBM SPSS software (Statistical Package for the Social Sciences, version 20.0).

5 Results

5.1 The life cycle of the makhana plant

The Makhana plant followed a distinctive life cycle characterized by several stages observed throughout the growth period during the present research (Fig. 1). It begins with seed germination, typically from December, marked by the emergence of small red-coloured leaves. As the season progresses into March and April, vegetative growth sets in, with the leaves transforming into vibrant green orbs, sizable in diameter, floating gracefully on the water's surface, and exhibiting a corrugated texture above and pink or deep purple hue beneath, supported by robust, porous, and prickly ribs. Following this phase, flowering and fruiting transpire from May to October/November, with violet-blue or dark pink blossoms adorning the plant, each yielding 15–20 fruits. The subsequent stage involves fruit bursting, where each fruit yields 15–45 seeds encapsulated within a thick sheath around the white edible kernel, which continues intermittently upon fruit maturation. Finally, the cycle concludes with seed sinking, occurring within 2–3 days after fruit bursting, as the seeds settle at the bottom of the pond, preparing for the regeneration of this aquatic crop.

5.2 Periphyton

The variations in periphyton counts (unit cm^{-2}) among the treatments and their replicates are illustrated in Figure 3. There was a gradually decreasing periphyton count from June to

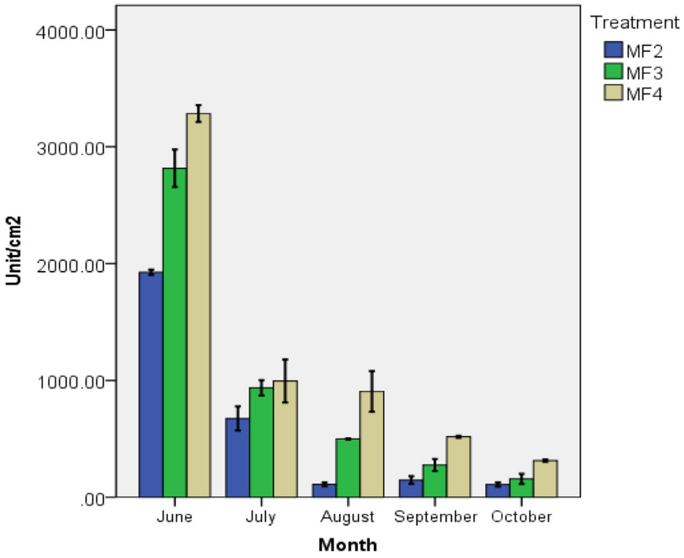

Fig. 3. Dynamics of periphyton count (unit cm^{-2}) with treatments.

September, followed by a slight decline in October. Overall, there were the highest periphyton counts recorded in the treatment MF4 in the range of 312 ± 5.14 to 3283.34 ± 36 unit cm^{-2}, and the lowest count was recorded from the treatment of MF2 between 108 ± 9 to 1925 ± 71unit cm^{-2}. A total of 20 different genera of periphyton were recorded (Tab. 2), with Bacillariophyceae being the most abundant (33-40%), followed by Cyanophyceae (29–38%), Chlorophyceae (10–13%), Cladocera (6–9%), Copepoda (3–4%) and Ostracoda (1–2%).

5.3 Fish and makhana seed yield

The impact of different treatments on the growth parameters and yield of three fish species, *Catla catla*, *Labeo rohita*, and *Cirrhinus mrigala*, as well as makhana seed yield, are shown in Table 3 and Figure 4. It was assessed using a one-way variance analysis (ANOVA) and DMRT to look at differences among the seasons and treatments. In the evaluation of *Catla catla*, significant differences were not observed in the initial mean weight and specific growth rate among treatments ($p < 0.05$). However, the final mean weight exhibited significant differences among treatments ($p < 0.05$). Remarkably, the harvested fish count and highest survival rates also showed significant variations among treatments with MF4 and MF5. Furthermore, the catla fish yield per hectare per year exhibited significant differences among treatments ($p < 0.05$), with MF4 and MF5 yielding the highest fish production. Overall, these findings suggest that treatments MF4 and MF5 had a substantial positive impact on the growth, survival, and yield of *Catla catla* compared to other treatments in the study. In the context of *Labeo rohita*, significant variations were observed across different treatments. Among the growth parameters. The final mean weight significant differences were identified among treatments ($p < 0.05$), and MF4 demonstrated the highest final mean weight. Moreover, the specific growth rate displayed significant differences among treatments, with MF4 and MF6 showcasing the highest growth rates. The harvested fish count also showed significant differences among treatments ($p < 0.05$), with MF4 displaying the highest count. Survival rates demonstrated significant differences among treatments, and MF4 exhibited the highest survival rate. Finally, the fish yield per hectare per year exhibited significant differences among treatments ($p < 0.05$), with MF4 standing out as the treatment with the highest fish yield, significantly different from MF1, MF2, MF3, and MF5. These findings suggest that MF4 played a crucial role in enhancing the growth, survival, and yield of *Labeo rohita* compared to other treatments in the study. In the assessment of *Cirrhinus mrigala*, the final mean weight exhibited significant differences among treatments ($p < 0.05$), and MF4 emerged with the highest final mean weight. Moving on to the specific growth rate, MF5 and MF6 showcased the highest growth rates. The harvested fish count displayed significant differences among treatments ($p < 0.05$), with MF5 exhibiting the highest count. Survival rates also demonstrated significant differences among treatments ($p < 0.05$), and MF4 and MF5 exhibited the highest survival rates. Moreover, the fish yield per hectare per year exhibited significant differences among treatments ($p < 0.05$), with MF5 standing out as the treatment associated with the highest fish yield, significantly different from MF1, MF2, and MF3. Overall, these results highlight the significant impact of MF4 and MF5 on the growth, survival, and yield of *Cirrhinus mrigala* compared to other treatments in the study. Significant differences were observed in both total fish yield ($\mathrm{t\,ha^{-1}\ yr^{-1}}$) and makhana seed yield ($\mathrm{t\,ha^{-1}\ yr^{-1}}$) among treatments ($p < 0.05$). Specifically, MF4 and MF5 emerged with the highest total fish yield, standing out as significantly different from others (Fig. 4). Similarly, for makhana seed yield, significant differences were found among treatments ($p < 0.05$), with MF3, MF4, and MF5 displaying the highest yields, significantly differing from others (Fig. 4). Treatments MF4 and MF5 consistently exhibited superior performance across all growth parameters and fish species, resulting in higher total fish yield and makhana seed yield compared to other treatments.

Table 2. Composition of periphyton assemblages on substrates.

Order	Genus	Order	Genus
Chlorophyceae	*Closterium*	Cladocera	*Daphnia*
	Cosmarium		*Moina*
	Oedogonium		*Bosmina*
	Spirogyra		*Ceriodaphnia*
Bacilariophyceae	*Gomphonema*	Rotifera	*Brachionus*
	Gyrosigma		*Lecane, Filinia*
	Navicula	Testacida	*Difflugia*
	Nitzchia	Copepoda	*Cyclops*
	Hannaea	Ostracoda	*Cypris sp.*
Cyanophyceae	*Chroococcales*		
Zygnematales	*Mougeotia*		

Table 3. The fish growth parameters and yield.

Fish	Growth parameter	Treatments					
		MF1	MF2	MF3	MF4	MF5	MF6
Catla catla	Initial mean weight (g fish^{-1})	5.41 ± 0.28^{a}	4.78 ± 0.67^{a}	4.81 ± 0.75^{a}	4.07 ± 0.22^{a}	5.03 ± 0.15^{a}	5.20 ± 0.17^{a}
	Final mean weight (g fish^{-1})	477.60 ± 2.51^{a}	479.66 ± 1.52^{a}	487 ± 2.64^{b}	571.66 ± 2.88^{c}	574 ± 1.73^{c}	571.65 ± 2.88^{c}
	Specific growth rate (%)	2.97 ± 0.04^{a}	3.03 ± 0.10^{a}	3.04 ± 0.11^{a}	2.99 ± 0.03^{a}	3.11 ± 0.02^{a}	3.04 ± 0.07^{a}
	Harvested fish count (no.)	958.66 ± 3.21^{a}	980 ± 9.16^{b}	1026 ± 21.6^{c}	1729.33 ± 1.15^{e}	1726.67 ± 4.16^{e}	1682 ± 2.64^{d}
	Survival (%)	39 ± 0.57^{a}	41.43 ± 0.83^{b}	42.33 ± 0.57^{b}	71.66 ± 0.57^{d}	72.33 ± 0.57^{d}	70.06 ± 0.11^{c}
	Fish yield (t ha^{-1} yr^{-1})	0.45 ± 0.006^{a}	0.45 ± 0.018^{a}	0.49 ± 0.006^{b}	0.98 ± 0.006^{d}	0.99 ± 0.004^{d}	0.95 ± 0.01^{c}
Labeo rohita	Initial mean weight (g fish^{-1})	5.16 ± 0.69^{a}	4.19 ± 0.08^{a}	3.99 ± 0.38^{a}	4.90 ± 0.05^{a}	4.66 ± 0.57^{a}	4.4 ± 0.20^{a}
	Final mean weight (g fish^{-1})	509.77 ± 9.40^{a}	508.33 ± 7.63^{a}	564.33 ± 5.1^{b}	631.66 ± 2.88^{e}	602.3 ± 2.51^{c}	613.7 ± 2.30^{d}
	Specific growth rate (%)	3.02 ± 0.08^{b}	2.89 ± 0.02^{a}	2.99 ± 0.05^{ab}	3.07 ± 0.006^{b}	3.20 ± 0.08^{c}	3.24 ± 0.03^{c}
	Harvested fish count (no.)	1076 ± 25.16^{a}	1121.33 ± 7.09^{b}	1411.66 ± 10.21^{c}	1560.67 ± 0.57^{e}	1461 ± 34.65^{d}	1488.6 ± 1.15^{d}
	Survival (%)	45.3 ± 0.57^{a}	46.28 ± 0.63^{a}	59.66 ± 0.57^{bc}	64 ± 1.73^{d}	59.34 ± 1.15^{b}	61.3 ± 1.20^{c}
	Fish yield (t ha^{-1} yr^{-1})	0.5508 ± 0.076^{a}	0.575 ± 0.005^{b}	0.79 ± 0.002^{c}	0.98 ± 0.004^{f}	0.8702 ± 0.006^{d}	0.91 ± 0.005^{e}
Cirrhinus mrigala	Initial mean weight (g fish^{-1})	2.2 ± 0.72^{a}	1.83 ± 0.05^{a}	2.06 ± 0.06^{a}	2.9 ± 0.34^{a}	1.9 ± 0.1^{a}	1.56 ± 0.05^{a}
	Final mean weight (g fish^{-1})	93.66 ± 5.5^{a}	91.6 ± 2.88^{a}	119.66 ± 1.52^{b}	161.7 ± 2.88^{cd}	155 ± 0.57^{c}	163.2 ± 5.77^{d}
	Specific growth rate (%)	2.48 ± 0.21^{a}	2.57 ± 0.03^{a}	2.67 ± 0.01^{a}	2.64 ± 0.09^{a}	2.89 ± 0.03^{b}	3.05 ± 0.02^{b}
	Harvested fish count (no.)	384.3 ± 4.50^{a}	409 ± 3.60^{b}	482.33 ± 2.51^{c}	578.3 ± 2.08^{d}	599 ± 2.64^{e}	567.30 ± 2.51^{f}
	Survival (%)	31.66 ± 0.57^{a}	34.45 ± 0.47^{b}	40.5 ± 0.31^{c}	48.26 ± 0.20^{d}	51 ± 12^{e}	47.3 ± 0.21^{d}
	Fish yield (t ha^{-1} yr^{-1})	0.034 ± 0.004^{a}	0.069 ± 0.004^{b}	0.0579 ± 0.003^{c}	0.092 ± 0.005^{de}	0.093 ± 0.002^{e}	0.091 ± 0.006^{d}

Values are given as mean ± SD of six treatments of each fish species. Values share a common superscript (a,b,c,d, and e) and differ significantly at $p<0.05$ post-hoc Duncan's multiple range test. The specific growth rate was calculated as SGR = 100 × (ln final weight − ln initial weight)/days and Survival (%) = (Number of fish harvested/Number of fish stocked) × 100.

6 Water quality analysis

The seasonal variation in physicochemical parameters of treatment ponds (MF1 to MF6) is presented in Table 4, with results expressed as mean ± SD. The parameters analyzed include temperature (Temp), pH, electrical conductivity (EC), total dissolved solids (TDS), dissolved oxygen (DO), chemical oxygen demand Free carbon dioxide (CO), ammonia-nitrogen (NH), alkalinity (AL), and transparency (TR). The data is categorized based on different seasons: winter (December to April), summer (April to June), monsoon (June to September), and post-monsoon (October to December). Each treatment's performance was observed across seasons, revealing distinct variations in the physicochemical conditions of the ponds. Superscript letters were employed to signify statistically significant differences between seasons within each treatment; however, there were no significant differences among the treatments. During the winter season, significant variations were observed compared to other seasons, influenced by parameters such as temperature, AL, and TR. In MF1, the winter season exhibited a temperature of 21.2 ± 3.86 °C, pH of 8.32 ± 0.09, EC of 230.75 ± 54 µS cm^{-1}, TDS of 115.62 ± 26.6 mg l^{-1}, DO of 6.42 ± 1.70 mg l^{-1}, CO of 2.02 ± 1.58 mg l^{-1}, NH of 0.073 ± 0.02 mg l^{-1}, AL of 69 ± 29.2 mg l^{-1}, and TR of 42.3 ± 8.33 cm. The winter season showed significant variations in temperature, pH, EC, TDS, DO, and NH. In summer, water temperature increases to 27.5 ± 0.70 °C, accompanied by a decrease in pH to 7.7 ± 0.84, and significant changes were found in NH and TR. During the monsoon, a further temperature rise (31 ± 1.82 °C) was

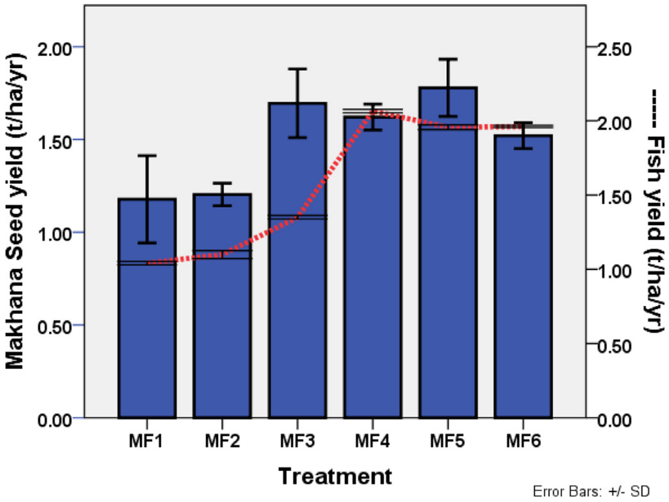

Fig. 4. Fish and makhana seed yield.

observed, accompanied by increases in EC, TDS, and CO. Significant values were noted in temperature, EC, TDS, DO, CO, NH, and AL. The post-monsoon period followed a pattern like the monsoon, with decreased temperature variations shown among the EC, TDS, and DO. MF2 to MF6 exhibited a consistent seasonal pattern like MF1, revealing variations in key physicochemical parameters such as temperature, pH, EC, TDS, DO, CO, NH, AL, and TR. During winter, these treatments demonstrated significant variations in temperature, pH, EC, TDS, DO, and NH. Moving to summer, a distinct shift was observed, marked by significant temperature, EC, and DO values. Monsoon seasons for these treatments showed similar trends with significant values in temperature, EC, TDS, DO, CO, NH, and AL. The post-monsoon period, resembling the monsoon, showcased significant EC, TDS, and DO values. Overall, the collective trends across the treatment underscored a coherent response to seasonal changes, reinforcing the influence of environmental factors on water quality dynamics.

7 Discussion

The research provides valuable insight into the dynamics of periphyton counts, fish and makhana seed yield, and water quality parameters across different treatments in the experimental setup. Periphyton-based fish culture systems offer a new direction in nutrient utilization by effectively supporting the growth of fish species that thrive low in the food chain (Milstein et al., 2003, Levy et al., 2017). Several researchers have utilized bamboo poles or similar structures to promote the development of periphyton assemblages in aquaculture ponds (Keshavanath, 2014). Intensive farming systems designed for high-yield species, typically monocultures heavily reliant on agrochemicals and formulated feed, have resulted in environmental challenges. These issues encompass declining biodiversity, food chain contamination, and deteriorating water and soil quality, ultimately leading to diminished long-term yields. (Letourneau et al., 2011; Isbell et al., 2017). Therefore, shifting from monoculture to polyculture/composite culture and integrating aquaculture systems by leveraging species diversity is widely recognized as a fundamental agro-ecological principle for redesigning sustainable aquaculture systems in the future (Nicholls and Altieri, 2016; Altieri et al., 2017). Henceforth, polyculture systems were introduced into the different treatments for evaluating the compatible fish species and their production performance. In the present study, the variations in periphyton counts among treatments suggest distinct responses to the experimental conditions. The decreasing trend from June to October was consistent across treatments, indicating a seasonal influence and grazing pressure on periphyton growth. Treatments exhibited a fluctuating pattern, with the highest counts in June, suggesting a potential relationship between treatment composition and periphyton development. The counts were reported high in MF3 and MF4 compared to MF2, which might be due to nutrient availability from the applied fertilizers. The study highlights the influence of treatment choice on both fish and makhana seed yields. Treatment MF4 stands out with the highest average fish yield, which may be possible due to the inclusion of periphyton substrates, pond fertilization, and supplementary feed combinations. These factors can influence the yields of fish. Additionally, the average fish yield was high in MF4 to MF6 compared to MF1 to MF3 because of the application of supplementary feed. However, in MF3, the fish yield was recorded >1 t ha^{-1} due to the presence of periphyton substrates and applied fertilizers. while the treatment MF3 to MF5 led to higher makhana seed yield due to applied fertilizer, which enhanced the yield by 35–40% compared to MF1 and MF2, where fertilizer application was absent. In the field system method, makhana seed yield of the same variety was recorded at around 2.8 t ha^{-1} (Kumar et al., 2011). The ANOVA test underscores the significance ($p < 0.05$) of treatment selection, with treatment MF4 displaying the highest total fish yield. After harvesting all fish from each treatment, the weights of the fish species vary across different treatments; Table 3 and Figure 4 provide a share of fish in the total production across different treatments and allow for initial observations regarding variations and trends.Overall, catla and rohu recorded significant weight compared to mrigal.

The observed variations in fish species weights among treatments further emphasize the impact of experimental conditions on fish growth. The weight of catla might have been increased due to the benefits of applied supplementary feed and the presence of periphyton counts. Moreover, rohu's average weight might have been increased due to the sufficient availability of periphyton counts on the substrates. The rohu exhibited a greater diet extent where the treatments with bamboo substrate were present. The fish might have preferred plankton as its primary food resource but later shifted its preference to plankton and periphyton. This indicates that the fish adapted its feeding behaviour to take advantage of the available food resources (Saikia et al., 2013). The water quality analysis reveals substantial variations in temperature, pH, EC, TDS, DO, CO, NH, AL, and TR across treatments and seasons. The fluctuations in these parameters highlight the complexity of the aquatic environment and its responsiveness to different treatments. Across all treatments, temperature exhibited significant seasonal variability. The range extended from 21.1 ± 4°C to 31.2 ± 1.5 °C. This observation aligns with typical seasonal temperature patterns in aquatic ecosystems. Most fish species thrive within a pH range of 6.5 to 9.5 (USEPA, 2023). In the present investigation, the pH values

Table 4. Seasonal variation in the physico-chemical water parameters (mean ± SD).

Treatment	Season	Temp (°C)	pH	EC (μS cm^{-1})	TDS (ppm)	DO (ppm)	CO (ppm)	NH (ppm)	AL (ppm)	TR (cm)
MF1	Winter	21.2 ± 3.86^{a}	8.32 ± 0.09^{a}	230.75 ± 54^{a}	115.62 ± 26.6^{a}	6.42 ± 1.70^{a}	2.02 ± 1.58^{a}	0.073 ± 0.02^{b}	69 ± 29.2^{a}	42.3 ± 8.33^{c}
	Summer	27.5 ± 0.70^{b}	7.7 ± 0.84^{a}	207.5 ± 24^{a}	104.5 ± 14^{a}	5.20 ± 0.31^{a}	2.92 ± 0.31^{a}	0.039 ± 0.02^{ab}	85.5 ± 0.7^{a}	24.75 ± 4.6^{ab}
	Monsoon	31 ± 1.82^{b}	8.03 ± 0.36^{a}	192.25 ± 23.2^{a}	92.25 ± 9.60^{a}	6.71 ± 1.5^{a}	10.55 ± 2.8^{b}	0.023 ± 0.01^{a}	107.5 ± 11.9^{a}	18 ± 2.16^{a}
	Post-monsoon	30.2 ± 3.88^{b}	8.35 ± 0.35^{a}	229.75 ± 1.76^{a}	107.25 ± 8.83^{a}	6.55 ± 2.19^{a}	3.25 ± 1.06^{a}	0.052 ± 0.03^{ab}	67.25 ± 14.9^{a}	32.2 ± 1.34^{bc}
MF2	Winter	21.2 ± 3.86^{a}	8.38 ± 0.10^{a}	240.75 ± 34.8^{a}	115.62 ± 26.6^{a}	6.42 ± 1.70^{a}	1.52 ± 1.06^{a}	0.073 ± 0.02^{a}	64.5 ± 17.9^{a}	42.8 ± 9^{b}
	Summer	27.5 ± 0.70^{b}	7.7 ± 0.84^{a}	202.5 ± 17.69^{a}	102 ± 11.31^{a}	5.2 ± 0.56^{a}	2.92 ± 0.31^{a}	0.039 ± 0.02^{a}	83 ± 4.2^{ab}	24.75 ± 4.6^{a}
	Monsoon	31.2 ± 1.5^{b}	8.05 ± 0.64^{a}	234 ± 37.88^{a}	117.25 ± 2^{a}	6 ± 1.50^{a}	1.22 ± 1.2^{a}	0.050 ± 0.01^{a}	87.75 ± 11.8^{ab}	31.3 ± 10^{ab}
	Post-monsoon	29.2 ± 2.47^{b}	8.42 ± 0.24^{a}	213 ± 25.45^{a}	99.5 ± 2.12^{a}	5.9 ± 1.27^{a}	1.75 ± 1.06^{a}	0.042 ± 0.17^{a}	100.4 ± 6.5^{b}	18.62 ± 5.1^{a}
MF3	Winter	21.1 ± 4^{a}	8.38 ± 0.10^{a}	230.75 ± 54^{a}	115.62 ± 27^{a}	6.17 ± 2.08^{a}	2.25 ± 1.18^{a}	0.073 ± 0.02^{a}	69 ± 29.2^{a}	42.3 ± 8.33^{b}
	Summer	27.5 ± 0.70^{b}	7.7 ± 0.84^{a}	202.5 ± 17.67^{a}	102 ± 11.31^{a}	5.2 ± 0.56^{a}	2.92 ± 0.31^{a}	0.039 ± 0.03^{a}	83 ± 4.2^{a}	24.75 ± 4.6^{ab}
	Monsoon	31.2 ± 1.5^{b}	8.05 ± 0.64^{a}	234 ± 37.88^{a}	117.25 ± 18^{a}	6 ± 1.5^{a}	1.9 ± 1.37^{a}	0.050 ± 0.02^{a}	87.75 ± 11.8^{a}	31.81 ± 9.84^{a}
	Post-monsoon	29.2 ± 2.47^{b}	8.42 ± 0.24^{a}	213 ± 25.45^{a}	99.5 ± 2.12^{a}	5.9 ± 1.27^{a}	2.25 ± 0.35^{a}	0.037 ± 0.01^{a}	75 ± 8.8^{a}	20.12 ± 7.24^{a}
MF4	Winter	21.2 ± 3.86^{a}	8.38 ± 0.10^{a}	231.25 ± 54.4^{a}	115.62 ± 26^{a}	6.45 ± 1.70^{a}	2.02 ± 1.5^{a}	0.072 ± 0.02^{b}	69 ± 29.2^{a}	42.3 ± 8.33^{b}
	Summer	27.5 ± 0.70^{b}	7.7 ± 0.84^{a}	202.5 ± 17.6^{a}	102 ± 11.31^{a}	5.2 ± 0.56^{a}	2.92 ± 0.31^{a}	0.039 ± 0.01^{ab}	83 ± 4.2^{a}	24.75 ± 4.6^{a}
	Monsoon	31 ± 1.82^{b}	8.03 ± 0.36^{a}	192 ± 22.8^{a}	92.25 ± 9.60^{a}	6.71 ± 1.52^{a}	2.55 ± 2.71^{a}	0.024 ± 0.01^{a}	97.25 ± 3^{a}	18 ± 2.16^{a}
	Post-monsoon	30.2 ± 3.88^{b}	8.35 ± 0.35^{a}	229.9 ± 1.6^{a}	107.25 ± 8.8^{a}	6.5 ± 2.12^{a}	3.25 ± 1.06^{a}	0.051 ± 0.01^{ab}	65.75 ± 16^{a}	25.18 ± 1.45^{a}
MF5	Winter	21.2 ± 3.86^{a}	7.85 ± 0.10^{a}	230.75 ± 54^{a}	115.62 ± 27^{a}	6.42 ± 1.70^{a}	2.025 ± 1.5^{a}	0.07 ± 0.01^{b}	69 ± 29.2^{a}	42.3 ± 8.33^{c}
	Summer	30.7 ± 1.70^{b}	8.03 ± 0.36^{a}	192 ± 22.8^{a}	92.25 ± 9.60^{a}	6.71 ± 1.5^{a}	2.55 ± 2.81^{a}	0.023 ± 0.01^{a}	96.85 ± 22^{a}	18 ± 2.16^{ab}
	Monsoon	30.7 ± 1.70^{b}	7.03 ± 0.36^{a}	192 ± 22.8^{a}	92.25 ± 10^{a}	6.72 ± 1.51^{a}	3.51 ± 2.8^{a}	0.023 ± 0.01^{a}	97.25 ± 28^{a}	18 ± 2.7^{a}
	Post-monsoon	30.2 ± 1.70^{b}	8.35 ± 0.36^{a}	229.75 ± 22.8^{a}	107.25 ± 9.60^{a}	6.5 ± 1.5^{a}	3.25 ± 3^{a}	0.057 ± 0.01^{b}	58.75 ± 2^{a}	33.2 ± 2.7^{bc}
MF6	Winter	23.5 ± 4.65^{a}	8.35 ± 0.09^{a}	246.5 ± 24.3^{a}	124 ± 10.8^{a}	6.67 ± 1.4^{a}	1.86 ± 1.3^{a}	0.064 ± 0.02^{b}	80.25 ± 22^{a}	38.92 ± 11.5^{b}
	Summer	29 ± 1.42^{b}	7.67 ± 0.82^{a}	192.5 ± 3.53^{a}	96 ± 2.82^{a}	5.8 ± 1.5^{a}	1.35 ± 6.57^{a}	0.027 ± 0.04^{ab}	92.5 ± 17^{a}	18.25 ± 4.6^{a}
	Monsoon	31.7 ± 1.8^{b}	8 ± 0.31^{a}	200.37 ± 29.4^{a}	95.87 ± 15^{a}	7.01 ± 1.6^{a}	2.55 ± 4.02^{a}	0.025 ± 0.01^{a}	89.95 ± 29^{a}	20.25 ± 2.70^{a}
	Post-monsoon	23.2 ± 6^{b}	8.52 ± 0.10^{a}	191.5 ± 55.8^{a}	88.7 ± 17.32^{a}	4.85 ± 0.35^{a}	3.15 ± 0.91^{a}	0.075 ± 0.02^{ab}	38.35 ± 3.7^{a}	42.87 ± 0.88^{ab}

Temp: Temperature, EC: Electric conductivity, TDS: Total Dissolve Solid, DO: Dissolved oxygen, CO: free Carbon dioxide, NH: Ammonia, AL: Alkalinity, TR: Transparency. Values are given as mean ± SD of six treatments of each fish species. Values share a common superscript (a, b, c, d, e) and differ significantly at $p<0.05$ Duncan's Multiple Range Test (DMRT).

remained consistently in the alkaline range, fluctuating across the treatments and seasons. The stability of pH within the alkaline range indicates a relative resilience to external factors. It required maintaining a higher total alkalinity concentration in the water and assisting in mitigating. In fish ponds, EC is essential to have minimum salt content in water to assist fish in maintaining their osmotic balance. Additionally, EC can provide a rough estimate of the overall quantity of dissolved solids present in the water (Stone and Thomforde, 2004). EC and TDS displayed seasonal variations that may be attributed to precipitation, runoff, and land use changes (Zheng et al., 2022). Essential for aquatic life, Dissolved oxygen (DO) measures the oxygen in water, crucial for fish survival. Fish generally thrive when DO concentrations exceed 5 mg l^{-1} (Kramer, 1987). In this investigation, DO concentrations fluctuated seasonally across all treatments. Increased DO during the monsoon is likely influenced by enhanced aeration and oxygen saturation due to elevated water flow (Roberts, 1984). However, in some treatments, DO levels dropped <2 mg l^{-1}, potentially due to the occupied water surface by leaves, the turbidity caused by decomposed large leaves, and increased fish biomass. CO concentrations displayed seasonal variability across the treatments, indicating a complex interplay of biotic and abiotic factors affecting CO levels. NH is a common toxic substance from various sources, including waste, fertilizers, and natural processes. Fish primarily produce ammonia as a nitrogenous waste, which is also generated from the decomposition of organic matter (Randall, 2011). In present study, the measured ammonia levels from the experimental treatment were within an acceptable range. AL also indicates the bases in water, such as carbonate and bicarbonate, contributing to its buffering capacity. A decrease in AL below 20 mg l^{-1} can limit primary productivity in water. The recommended range for AL is 50-150 mg l^{-1} (Saraswathy et al., 2015). In the current investigation, the observed AL ranged between 50 and 125 ppm, within the acceptable range. NH and AL concentrations exhibited seasonal changes, potentially linked to nutrient cycling, overall inputs, and fish biomass. Transparency values fluctuated seasonally, which may be reflected by changes in nutrient dynamics and sedimentation processes. Treatment-specific trends indicate potential treatment effects on water quality, with some treatments consistently exhibiting higher or lower values for certain parameters.

The observed seasonal variations in water quality parameters suggest a dynamic interaction between treatments and environmental conditions. The ANOVA results confirm significant ($p<0.05$) differences across seasons for specific parameters, highlighting the need to consider seasonal variations in water quality assessments. The collective interpretation suggests a multidimensional relationship between periphyton counts, fish and makhana seed yield, and water quality parameters. The variations observed in each aspect highlight the importance of considering the holistic ecosystem response to different treatments. The higher periphyton counts in treatments coinciding with elevated fish and makhana yields might suggest a positive relationship between periphyton growth and overall ecosystem productivity. However, the complexity of these interactions necessitates further investigation. The identified variations in water quality parameters emphasize the sensitivity of the aquatic environment to experimental treatments, emphasizing the need for sustainable aquaculture practices. Understanding the interconnectedness of periphyton, yield, and water quality provides a foundation for optimizing treatment strategies to enhance productivity while maintaining a balanced and healthy aquatic ecosystem. It is important to acknowledge certain limitations in the study, such as the need for longer-term observations to capture more extended seasonal patterns and potential interactions. Future research could delve deeper into the mechanisms driving the observed relationships, exploring the role of individual treatment components and their impacts on ecosystem dynamics.

8 Conclusion

The objective of this research was to investigate the impact of periphyton on makhana cum fish culture, the compatibility of IMC, and assess water quality parameters. Among the different treatments, MF4 demonstrated a significantly higher fish yield compared to the others, while treatments MF3 and MF5 showcased the highest makhana yield. These findings emphasize the influence of treatment selection on both fish and makhana yields. The study of water quality parameters in several treatments, particularly those based on periphyton, demonstrated improvements in dissolved oxygen levels, total suspended solids, transparency, and other nutrient parameters. Incorporating periphyton substrate frames into the makhana cum fish culture system can enhance water quality and the availability of natural food, thereby contributing to increased fish yield without harm to makhana seed production. However, this study encountered several limitations that might have influenced the results. Makhana, being an annual hydrophyte, requires water availability throughout the year for its life cycle. Moreover, environmental variability, including seasonal changes affecting water quality parameters and pond-specific factors, likely impacted periphyton counts, nutrient availability, and overall productivity. For instance, the decomposition of makhana leaves influenced water transparency and led to the presence of suspended particles in water, impacting light penetration and potentially affecting periphyton growth. Regularly removing unwanted weeds was required to prevent competition with makhana and ensure sufficient space for fish rearing, including space for periphyton substrates. Vacant space management for fish rearing and adjustments to periphyton substrates introduced further variability, mainly as fish harvesting could only be possible after the makhana seed harvest. Additionally, the periodic replacement of periphyton substrates might have affected periphyton accumulation and availability as a natural food source. These limitations highlight that while the study provides valuable insights, future research should address these limitations and further enhance the understanding and implementation of periphyton-based systems in makhana cum fish culture.

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Conflicts of interest

All authors affirm that they do not have any identifiable competing financial interests or personal relationships that could have potentially influenced the findings and conclusions presented in this paper.

Data availability statement

The data supporting the findings of this study can be obtained from the corresponding author upon reasonable request.

References

Abwao JO, Boera PN, Munguti JM, Orina PS, Ogello EO. 2013. The potential of periphyton based aquaculture for nile tilapia (*Oreochromis niloticus* L.) production: a review. *Survival* 96: 91.

Altieri MA, Nicholls CI, Montalba R. 2017. Technological approaches to sustainable agriculture at a crossroads: an agroecological perspective. *Sustainability* 9: 349.

Anix A, Santhiya V, Athithan S, Ahilan B. 2020. Comparison of periphyton biomass on coconut coir and bamboo poles as natural substrates in earthen lined pond. *IJBSM* 11: 494–500.

Anix Vivek Santhiya A, Athithan S, Ahilan B, Stephen Sampath Kumar J, Srinivasan A. 2017. Evaluation of periphyton quantity on different natural substrates in Earthen lined pond. *J Appl Nat Sci* 9: 1630–1636.

APHA. 2017. Standard Methods of Water Analysis, 23rd edition. APHA

Apollos TG, Raji A, Modibbo U. 2016. Seasonal variation of water quality parameters of Zobe reservoir Dutsinma Katsina State, Nigeria. *Hydrol Curr Res* 7: 261.

Arthur JR, Lavilla-Pitogo CR, Subasinghe RP. 2000. *Use of chemicals in aquaculture in Asia, Southeast Asian Fisheries Development Center Aquaculture Department Tigbauan, Iloilo, Philippines*, pp 144.

Azim ME. 2009. Photosynthetic Periphyton and Surfaces. *Encyclopedia of Inland waters*, 184–191.

Azim ME, Wahab MA, van Dam AA, Beveridge MCM, Huisman EA, Verdegem MCJ. 2001. Optimization of stocking ratios of two Indian major carps, rohu (*Labeo rohita Ham.*) and catla (*Catla catla Ham.*) in a periphyton-based aquaculture system. *Aquaculture* 203: 33–49.

Azim ME, Wahab MA, Verdegem MCJ, van Dam AA, van Rooij JM, Malcolm ME, Beveridge CM. 2002. The effects of artificial substrates on freshwater pond productivity and water quality and the implications for periphyton-based aquaculture. *Aquat. Living Resour* 15: 231–241.

Baluyut AE. 1989. Aquaculture systems and practices: A selected review, united nations development programme Food and Agriculture Organization of the United Nations, Rome 4: 3–5. https://www.fao.org/3/t8598e/t8598e00.htm#Contents

Baluni P, Kumar K, Joshi HK. 2018. Ecology, Distribution pattern, density and diversity of periphyton in khankra spring fed stream of Garhwal Himalaya. India. *J Mountain Res* 12: 73–9.

Banerjea SM. 1967. Water quality and soil condition of fishponds in some states of India in relation to fish production. *Indian J Fish* 14: 115–144.

Beveridge MCM, Phillips MJ. 1993. Environmental impact of tropical inland aquaculture. *In* Environment and Aquaculture in Developing Countries (eds. R.S.V. Pullin, H. Rosenthal and J.L. Maclean). ICLARM Conf. Proc. 31. ICLARM, Manila. pp. 213–236.

Beveridge MCM, Wahab MA, Dewan S. 1994. Effects of daily harrowing on pond soil and water nutrient levels and on rohu fingerling production. *Prog Fish Cult* 56: 282–287.

Bhatnagar A, Singh G. 2010. Culture fisheries in village ponds: a multi-location study in Haryana, India. *ABJNA* 1: 961–968.

Bhujel RC. 2012. Small-scale aquaculture: global and national perspectives. In: Shrestha, M.K., Pant, J. (Eds.), Small-scale aquaculture for rural livelihoods: proceedings of the symposium on small-scale aquaculture for increasing resilience of rural livelihoods in Nepal. 5–6 Feb 2009. Kathmandu, Nepal. World Fish.

Boyd CE. 1992. Water Quality Management for Pond Fish Culture. Elsevier Science Publishers B.V., 1000 AH Amsterdam, The Netherlands. 316 pp.

Boyd CE, Lichtkoppler F. 1979. Water Quality Management in Fish Ponds. *Research and Development Series No. 22, International Centre for Aquaculture (J.C.A.A) Experimental Station Auburn University, Alabama*, 45–47.

Das M, Behera PR, Dash B. 2017. Periphyton communities in carp culture ponds treated with cow manure and biogas slurry. *Aquat Living Resour* 30: 25.

Das S, Der P, Raychaudhuri U, Maulik N, Das DK. 2006. The effect of *Euryale ferox* (Makhana), an herb of aquatic origin, on myocardial ischemic reperfusion injury. *Mol Cell Biochem* 289: 55–63.

David LH, Pinho SM, Romera DM, Campos DW, Franchini AC, Garcia F. 2022. Tilapia farming based on periphyton as a natural food source. *Aquaculture* 547: 737544.

Directorate of Horticulture. 2022. Bihar Makhana at a glance (*Euryale ferox* Salisb.) Bihar Horticulture development Society, Department of Agriculture, Bihar-01.

Dutta R, Dutta A, Bhagobaty N, Bhagabati SK. 2018. Periphyton community structure of Namsang stream, Arunachal Pradesh. *J Coldwater Fish* 1: 113–120.

Faruk-ul-Islam ATM. 1996. The use of bamboo substrates to promote periphyton growth as feed for Nile tilapia *Oreochromis niloticus* in small ponds. M. Sc thesis, BAU, Mymensingh, Bangladesh. 74 pp.

Hem S, Avit JLB. 1994. The results of “Acadja-Enclos” as an extensive aquaculture system (West Africa). *Bull Mar Sci* 55: 1038–1049.

Horne AJ, Goldman CR. 1994. Limnology (2nd ed.). McGraw-Hill. Chapter 4: Temperature.

Isbell F, Adler PR, Eisenhauer N, Fornara D, Kimmel K, Kremen C, Letourneau DK, Liebman M, Polley HW, Quijas S, Scherer-Lorenzen M. 2017. Benefits of increasing plant diversity in sustainable agroecosystems. *J Ecol* 105: 871–879.

James MR, Hawes I, Weatherhead M. 2000. Removal of settled sediments and periphyton from macrophytes by grazing invertebrates in the littoral zone of a large oligotrophic lake. *Freshw Biol* 44: 311–326.

Jha V, Barat GK. 2003. Nutritional and Medicinal properties of *Euryle ferox* Salisbury, In: Makhana, edited by Mishra RK, Jha V and Dehadrai PV (ICAR, New Delhi), 230–238.

Kapoor S, Kaur A, Kaur R, Kumar V, Choudhary M. 2022. Euryale ferox, a prominent superfood: Nutritional, pharmaceutical, and its economical importance. *J Food Biochem* 46: e14435.

Kaviyarasan K, Athithan S. 2019. Evaluation of periphyton quantity on different natural substrates in freshwater. *ASD* 39: 250–3.

Keshavanath P. 2014. Role of natural food in sustaining aquaculture. *J Aquat Biol Fish* 2: 6–13.

Kramer DL. 1987. Dissolved oxygen and fish behavior. *Environ biol fishes* 18: 81–92.

Kumar M, Raut SM, Bhatt BP, Kumar L. 2020. Scientific cultivation of Makhana for improving farmers' livelihood in eastern India. *BRT* 2: 670–672.

Kumar L, Gupta VK, Jha BK, Singh IS, Singh AK. 2011. Status of Makhana (*Euryale ferox* Salisb.). *Technical Bulletin No. R-32/ PAT-21, ICAR-RCER Patna-14*.

Kumari A, Jha V. 2016. Ethnic uses of Makhana (*Euryale ferox* Salisb.) in Mithila (north Bihar) and other parts of India. *JTFP* 4: 60–67.

LeGresley M, McDermott G. 2010. Counting chamber methods for quantitative phytoplankton analysis-haemocytometer, Palmer-Maloney cell and Sedgewick-Rafter cell. *UNESCO(IOC manuals and guides)*, pp. 25–30.

Letourneau DK, Armbrecht I, Rivera BS, Lerma JM, Carmona EJ, Daza MC, Escobar S, Galindo V, Gutiérrez C, López SD, Mejía JL. 2011. Does plant diversity benefit agroecosystems? A synthetic review. *Ecol Appl* 21: 9–21.

Levy A, Milstein A, Neori A, Harpaz S, Shpigel M, Guttman L. 2017. Marine periphyton biofilters in mariculture effluents: nutrient uptake and biomass development. *Aquaculture* 473: 513–520.

Liaquat M, Pasha I, Ahsin M, Salik A. 2022. Roasted fox nuts (*Euryale Ferox* L.) contain higher concentration of phenolics, flavonoids, minerals and antioxidants, and exhibit lower Glycemic Index (GI) in human subjects. *FPPN* 4: 1–2.

Likens GE. 1985. The aquatic ecosystem and air-land-water interactions. An Ecosystem Approach to Aquatic Ecology (pp. 430–435). Springer, New York.

Mandal RN, Saha GS, Sarangi N. 2010. Harvest and processing of Makhana (*Euryale ferox* Salisb.)-a unique assemblage of traditional knowledge. *IJTK* 9: 674–688.

Milstein A, Azim ME, Abdul Wahab M, Verdegem MCJ. 2003. The effects of periphyton, fish and fertilizer dose on biological processes affecting water quality in earthen fish ponds. *Environ Biol Fishes* 68: 247–260.

Mishra RK, Vidyanath J, Dehadrai PV. 2003. *Makhana*. *Indian Council of Agricultural Research, New Delhi*.

Nahiduzzaman M, Karim E, Nisheeth NN, Bhadra A, Mahmud Y. 2023. Temporal distribution of plankton and fish species at Mithamoin Haor: Abundance, composition, biomass and ecosystem based management approach. *Heliyon* 9: e22770.

Nath BK, Chakraborty AK. 1985. Studies on the Physico-chemical Properties of the Starch of Euryale ferox Salisbury. *J Food Sci Technol* 22: 293.

Negri M, Romera DM, Garcia F. 2023. Integrated multitrophic aquaculture in ponds using substrate for periphyton as natural source of food. *Boletim do Instituto de Pesca* 49: 12.

Nicholls C, Altieri MA, Vazquez L. 2016. Agroecology: principles for the conversion and redesign of farming systems. *J Ecosyst Ecogr* S5: 010.

Olah J, Szabo P, Esteky AA, Nezami SA. 1994. Nitrogen processing and retention in Hungarian carp farms. *J. Appl Ichthyol* 10: 215–390.

Paul BN, Adhikari S, Mandal RN. 2017. Training Manual on Application and Practices of Fish Feed in Aquaculture, ICAR-Central Institute of Freshwater Aquaculture, RRC, Rahara, Kolkata, West Bengal, India, pp: 1–130.

Raut S, Gupta N, Everard M, Singh IS. 2020. Commercially and medicinally significant aquatic macrophytes: potential for improving livelihood security of indigenous communities in northern Bihar, India. *J Threat Taxa* 12: 16819–16830.

Raut S, Kumar P, Singh J, Singh I, Ahirwal S, Nair S, Raman R, Jana B, Kumar M, Tarate S, Kumar, R. 2024. Enhancing productivity of the wetland ecosystem in North Bihar, India. *Indian Farming Digest* 3: 1–5.

Randall DJ. 2011. Nitrogenous-waste balance, *Excretion of Ammonia Encyclopedia of Fish Physiology*, 1437–1443.

Roberts PV. 1984. Dependence of oxygen transfer rate on energy dissipation during surface aeration and in stream flow. Gas transfer at water surfaces (pp. 347–355). Dordrecht: Springer Netherlands.

Saikia SK, Majumder S, Nandi S, Saha SK. 2013. Feeding ecology of the freshwater fish rohu *Labeo rohita* (Hamilton 1822): a case of intelligent feeding in the periphyton-based environment. *Zool Ecol* 23: 266–274.

Saraswathy R, Muralidhar M, Sundaray JK, Lalitha N, Kumararaja P. 2015. Water quality management in fish hatchery and grow-out systems. *Adv Mar Brackishwater Aquacul* 217–225.

Singh IS, Kumar M, Raut SM, Thakur AK, Singh SP. 2020. Integrated nutrient management package for field cultivation of makhana in North Bihar: INM for makhana cultivation. *J AgriSearch* 7: 138–141.

Singh IS, Thakur AK, Singh DK, Kumar PV, Raut SM. 2023. Assessment of nutrient accumulation capability of two economically important aquatic plants. *Pharma Innov J* 12: 2498–2504.

Stone NM, Thomforde HK. 2004. Understanding your fish pond water analysis report (pp. 1–4). Cooperative Extension Program, University of Arkansas at Pine Bluff, US Department of Agriculture and county governments cooperating.

Sukumaran K, Thirunavukkarasu AR, Kailasam M, Sundaray JK, Biswas G, Kumar P, Subhuraj R, Thiagrajan G, Venu S. 2017. Evaluation of Bamboo, Coconut Shell Substrates and Supplemental Feeding on the Growth of Pearlspot, *Etroplus suratensis* (Bloch) Fry in Low Volume Cages. *Fishery Technology* 54: 1–7.

USEPA/United States Environmental Protection Agency, *CADDIS*: 2. https://www.epa.gov/caddis-vol2/ph

Zheng J, Wang H, Liu B. 2022. Impact of the long-term precipitation and land use changes on runoff variations in a humid subtropical river basin of China. *J Hydrol Reg Stud* 42: 101136.

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