

Performance and sustainability of the makhana (*Euryale ferox* Salisbury) cum fish culture system in India

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Keywords:

Economic analysis, Fish integration, Makhana, Plankton, Proximate composition

Received : 11.01.2024

Accepted : 15.06.2025

Abstract

Makhana (*Euryale ferox* Salisbury) is a commercial aquatic crop cultivated in the small waterlogged areas of north Bihar, India. Given the importance of Makhana, the present study was designed to evaluate the performance and economic feasibility of semi-intensive integrated makhana cum fish farming. In the current investigation, two treatments were evaluated: sole makhana cultivation (T_1) and integrated makhana-fish culture (T_2). Both treatments were conducted in triplicate across four different locations in Bihar, India. In treatment T_2 , fingerlings of catla (*Labeo catla*), rohu (*Labeo rohita*), mrigal (*Cirrhinus mrigala*), bata (*Labeo bata*), Kawai (*Anabas testudineus*) and snakehead (*Channa striatus*) were stocked at a ratio of 3:2:2:1:1:1, respectively (6000 no. ha^{-1}). The results revealed that only makhana cultivation (T_1) yielded 1.78 ± 0.133 t ha^{-1} seed, whereas 1.55 ± 0.085 t ha^{-1} seed and 1.48 ± 0.15 t ha^{-1} fish were produced under the integrated makhana-fish culture system (T_2). Net benefits from T_1 and T_2 were ₹1,78,820 ± 18633.40 and ₹2,74,007.89 ± 83648, respectively. The estimated input cost, returns and revenue in the experiment significantly differed among the treatments, as assessed by the Wilcoxon-Mann-Whitney test ($p < 0.05$) (Wilcoxon, 1945). In T_2 , individual catla and rohu had an average weight of 574.82 ± 31.54 and 624.55 ± 11.5 g, respectively, which resulted in a total biomass of 0.74 ± 0.070 t ha^{-1} and 0.57 ± 0.087 t ha^{-1} , respectively. The biomass of catla and rohu was higher than the other stocked fish species, and they were compatible with makhana cultivation in an integrated aquaculture system. The biotic and abiotic parameters in each treatment were within the optimum range conducive for productivity. In addition, these semi-intensive technologies could help generate a surplus income of over 50% compared to traditional makhana cultivation methods and are more nutritionally sustainable than the monoculture method.

Introduction

Makhana (*Euryale ferox* Salisbury, Family: Nymphaeaceae) is a unique, prickly macrophyte typically found in the littoral zones of waterlogged areas. Natural growth of makhana plants occurs during the winter and summer months in the shallow regions of water bodies. In India, it is naturally available in the floodplain wetlands of Bihar, West Bengal and the north-eastern states (Kumar

et al., 2011). Makhana seeds, valued for their dual roles as a food source and in ayurvedic medicine, have been recognised for their diverse applications. These seeds are valued for their analgesic and aphrodisiac properties and they are traditionally consumed to help manage various conditions, including chronic diarrhea, vaginal discharge, kidney weakness, nocturnal emissions and impotence (Jha *et al.*, 2018; Mittal *et al.*, 2020). Furthermore, raw makhana seeds are rich in carbohydrates, proteins and essential minerals (Das *et al.*,

2006; Masram *et al.*, 2015; Raut *et al.*, 2020). The combination of their nutritional value and pharmaceutical significance has positioned them as a vital aquatic cash crop. North Bihar has many tributaries of the Ganga River, namely the Gandak, Kosi and Kamala-Balan, which are prone to frequent flooding during the monsoon season. Riverine catchments and nearby low-lying or depressed areas often experience water-logging or formation of stagnant pools. These areas locally known as Chaur along with oxbow lakes referred to as Mauns, formed by river meandering, constitute wet lands that are highly prone to flooding in the region. Flood-prone areas currently cover around 68 to 75 lakh ha; of which, over 8 lakh ha are affected by water-logging (Kumar *et al.*, 2013). The marginal areas of Chaur and Mauns, often contain small water ditches or ponds that are well suited for cultivating makhana and other aquatic crops like water chestnuts (Jana *et al.*, 2019). Makhana seeds have been traditionally collected from natural waterlogged areas, particularly in the districts of Darbhanga, Kishanganj, Katihar, Madhubani, Purnia, Samstipur, Saharsa and Supaul in North Bihar. With the increasing market demand for makhana seeds and their pops, commercial cultivation of makhana has been steadily increasing, especially in North Bihar (Kumar *et al.*, 2020). The demand for inland fish farming is on the rise, leading to an increase in its production. Many stakeholders and fishers are showing interest in makhana cum fish culture system. Although this cultivation system has primarily been practiced in ponds, only a small proportion of natural water bodies, such as Chaur and Mauns, have adopted this method (Singh *et al.*, 2017; Dana *et al.*, 2018). Traditionally, the fish cum makhana farming system mostly depends upon wild makhana species and fishes. The average fish production from the wetland systems in Bihar is currently around 0.3-0.4 t ha⁻¹ (Sarkar and Borah, 2018); while the estimated potential is around 2-2.5 t ha⁻¹ (Sugunan and Sinha, 2001; Bhattacharjya *et al.*, 2003). This may be due to improper management and production techniques, *i.e.*, using wild species of makhana, inadequate fish stocking, presence of predatory fishes and absence of manuring and feeding, resulting in low production of fish and makhana. Integrated aquaculture with makhana is not a new concept, as previous studies on makhana cum fish culture were conducted by Dehadrai (1972) and Verma *et al.* (1996) and they recorded around 1.2-3.6 t ha⁻¹ of catfish production. Catfishes are air-breathing fishes, well adapted to survive well in adverse and muddy environmental conditions due to the presence of accessory respiratory organs. Verma *et al.* (2008) recorded 2.1 t ha⁻¹ catfish with makhana. The average production of makhana cum fish culture system benefited >63% through the integrated nutrient management system (Pramanik *et al.*, 2013). Based on the production function, aquaculture is categorised into intensive, semi-intensive and extensive farming systems (Bayulut, 1989; Oddsson, 2020). In a semi-intensive culture system, feed and fertilisation are used for fish production purposes and most of the farmers in Bihar are adopting this method. Although some farmers are releasing fish into makhana ponds, there are currently no organised or systematic makhana-fish integrated farming activities. Moreover, there is a paucity of information related to selecting candidate species and their composition, feeding management practices, makhana plantation methodologies and creating a harmonic environment for makhana and fish in the same pond. Integrated farming of makhana and fish enables better utilisation of common natural resources and available space and thereby enhancing overall productivity per unit of aquatic resource. This, in turn contributes to improved income, livelihood and nutritional

security of the small and marginal farmers. Hence, the present study evaluated the productive performance of an integrated makhana-fish farming system, using makhana only cultivation as control.

Materials and methods

Study area

The experiment was conducted from January to October 2020 at four locations in northern Bihar. The locations included the ICAR-National Research Centre for Makhana (ICAR-NRCM) (26°11'15.93"N; 85°54'21.53"E) in Darbhanga, Benipur (26°02'47.05"N; 86°08'42.43"E) and Kapchhahi villages (26°05'55.95"N; 86°01'26.0"E) in Darbhanga as well as Raiyam Village (26°15'22.63"N; 86°12'43.8"E) in Madhubani District. In northern Bihar, Makhana plants are typically grown in natural water bodies. For this experiment, we used the high-yielding variety *Swarna Vaidehi*, which was developed by the ICAR-NRCM Darbhanga. The experiment consisted of two treatment sets: T₁ (control), where only Makhana was grown as a mono-crop and T₂, where Makhana and fish were integrated and reared together. Each treatment was replicated three times. The dimensions of each pond ranged from 800 to 1000 m² in area and had a depth of 1.2 to 1.5 m.

Makhana cultivation and transplanting

The cultivation of Makhana is carried out using two primary methods: the pond system method (PSM) and the field system method (FSM). The PSM begins by sowing makhana seeds in a nursery, which are then transplanted as saplings into main ponds with a water depth of approximately 1 m. As an aquatic crop, Makhana thrives in high water-retentive clayey soil rich in organic matter (Kumar *et al.*, 2017). In December, about 20 kg of healthy seeds were uniformly broadcasted in a 500 m² nursery pond for future transplantation into a one-hectare area. Throughout the growing period from December to April, the water depth in the nursery pond was maintained at about 0.3 m. These seeds were used for both T₁ and T₂ treatments. In March, healthy saplings were uprooted from the nursery and immediately transplanted into well-prepared ponds designated as T₁ and T₂. The saplings were spaced 1 m apart between rows and individual plants (Kumar *et al.*, 2020). There are generally two designs for integrating Makhana with fish during transplantation: the central vacant space design, which leaves 10-15% of the entire water area free in the centre and the peripheral vacant space design, which creates space around the edges of the pond (Mishra *et al.*, 2003). For this study, a peripheral vacant space design was adopted for T₂. This design allows solar radiation to penetrate the pond's bottom, providing sufficient natural fish food as well as facilitate management of sprawling makhana leaves in the peripheral region. After two months of transplantation, bright purple, solitary flowers begin to appear on makhana plants in May, though their blooming is not synchronised.

Stocking of fish fingerlings

Fingerlings of Indian major carps (IMCs) and other species were sourced from commercial vendors and quarantined using KMnO₄

at a concentration of 2 ppm in a separate tank for 15 days before being released into the treatment ponds (T_2) (Arthur et al., 2000). The species were stocked at a density of 6000 individuals per hectare, including catla (*Labeo catla*), rohu (*Labeo rohita*), mrigal (*Cirrhinus mrigala*), bata (*Labeo bata*), climbing perch (*Anabas testudineus*) and snakehead (*Channa striata*) in the specified ratio 3:2:2:1:1:1. It is important to avoid macro-phytophagous fish species in makhana cultivation ponds; and therefore in this experiment, sestonophagous and detritophagous fish species were selected (Pavlov and Kasumyan, 2002). The fish fingerlings were stocked in June, during the vegetative growth stage of makhana (Fig. 1). Supplementary feed was provided to the fish based on body weight, starting at 3-5% during the first two months and gradually reduced to 1-2% in the third month. A mixed feed comprising wheat or rice bran and mustard oil cake (MOC) in a 2:1 ratio was fed twice daily (Paul et al., 2017).

Economic analysis

Annual production was calculated based on the total biomass harvested per pond and expressed as $t\ ha^{-1}\ yr^{-1}$. Total input costs encompassed all types of expenditures, including labour, seed, feed, fertiliser, manure, energy and health management costs for pond management during one production year. Total revenue was defined as the sum of aquaculture revenue and makhana seed revenue, whereas aquaculture revenue represents the total sales value of aquaculture species and makhana seed revenue referred to the sales value of makhana seeds (Ahmed et al., 2023). The profit was calculated by considering the total input cost and the total sales value of both fish and makhana seed, as follows:

Net revenue (income) = Total revenue - Total input cost

Analysis of physicochemical parameters and plankton

Samples were collected every two weeks from each pond in the respective treatments. Soil samples were taken from the upper 20 cm layer of the pond bottom at three designated points within each pond, followed by thorough mixing. Approximately 1 kg of moist soil was collected from each treatment, labeled appropriately, securely packed in plastic bags and then transported to the laboratory. The collected samples underwent air-drying, crumbling and grinding processes before being stored in labeled plastic containers for subsequent analysis. Water samples were collected from each pond at the same locations as the soil samples, from a depth of 25 cm below the pond surface, from June to October. These samples were transferred into airtight bottles, sealed and preserved at $-20^{\circ}C$ until analysis (Kabir et al., 2020). *In situ* water quality parameters, including temperature ($^{\circ}C$), pH, electrical conductivity ($mS\ cm^{-1}$) and total dissolved solids (ppm), were measured using a standard portable instrument (Waco, model: WA-2015). Transparency (cm) was measured on-site using a Secchi disc. For the analysis of water samples, parameters such as ammonia-nitrogen were estimated colourimetrically using the indo-phenol blue method. Nitrite-nitrogen levels were determined using spectrophotometric method, total orthophosphate (also known as available phosphate) in the water was measured using the ascorbic acid method and dissolved oxygen (DO) levels were determined using Winkler method (APHA, 2005). The pH and electrical conductivity (EC) of the soil were measured by suspending it in distilled water at a ratio of 1:2.5, using the WA-2015 Waco Instrument pH/EC meter. Organic carbon content of the sediment sample was determined using Walkley and Black's wet chromic acid digestion method (Walkley and Black, 1934). Available phosphorus was measured using the Olsen method (Olsen et al., 1954), available nitrogen was

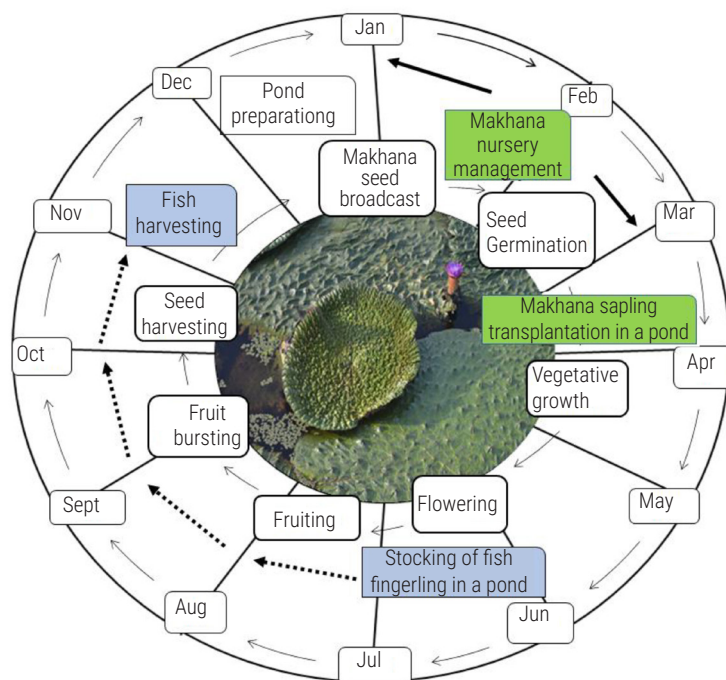


Fig. 1. Life cycle stages of makhana plant along with the appropriate timing for fish fingerling stocking and harvesting. $\longrightarrow$ indicates fish rearing period (June to October) and $\dashrightarrow$ indicates makhana nursery management period (January to March)

estimated by alkaline permanganate method and potassium levels were assessed using flame photometric method (Subbaiah and Asija, 1956). Plankton and zooplankton samples were randomly collected from each pond using a plankton net with a mesh size of 45 μm , between 10:00 and 12:00 hrs, from June to October. The collected samples were preserved in 4% formaldehyde solution. After preservation, plankton counts and species identification were conducted in the laboratory using Sedgewick Rafter counting chambers and an optical microscope (LYNX microscope, Lawrence and Mayo, India). Further calculations were performed using Shannon-Wiener species diversity index (H'), Evenness index (J) and Margalef richness index (D). Plankton were identified based on standard protocols (Reynolds, 2006; Kumar, 2015; Phan Doan Dang *et al.*, 2015; Chandra *et al.*, 2017; Fathibi *et al.*, 2017).

Proximate composition and mineral profile analysis

Makhana seeds were collected from the bottom of the experimental ponds at the end of October (Fig. 2a and b). The edible parts (kernel) were used to determine moisture (Proc. No. 925.10), ash (Proc. No. 923.03), fat (Proc. No. 920.85) and protein (Proc. No. 984.13) contents following AOAC (2005). Carbohydrate content was calculated by difference (100% minus the sum of water, ash, fat and protein percentages) and the energy value was expressed as Kcal 100 g^{-1} (Gopalan *et al.*, 1996). Mineral profile comprising phosphorus (P), potassium (K), sodium (Na), magnesium (Mg), calcium (Ca), iron (Fe), copper (Cu), manganese (Mn) and zinc (Zn), was estimated following AOAC method (985.35, Sec. 22.037) (AOAC, 2005).

Statistical analysis

Diversity indices (H' , J and D) were analysed using PRIMER 7 Version 7.0.23 (Plymouth Routines In Multivariate Ecological Research). Shapiro-Wilk test assessed data normality in IBM SPSS Version 20. Mann-Whitney U test ($p < 0.05$) was used for non-normally distributed data to analyse statistical parameters and comparisons.

Results

The water quality parameters are summarised in Table 1. In the T_1 treatment group, water temperature ranged from 25 to 32°C, while dissolved oxygen (DO) levels varied between 1.34 to 4.12 ppm and pH values ranged from 6.5 to 8.30. Total dissolved solids (TDS) ranged between 90 to 140 ppm, electrical conductivity (EC) from 166 to 271 $\mu\text{S cm}^{-1}$ and water transparency varied from 27 to 98 cm. Additionally, levels of phosphate (0.39 to 0.63 ppm), nitrite (0.03 to 0.09 ppm) and ammonia (0.024 to 0.04 ppm) were also recorded from June to October. In the T_2 treatment group, temperature, DO and pH values were similar, ranging from 25 to 32°C, 3.34 to 8.20 ppm and 6.50 to 8.30, respectively. The observed ranges for TDS (75 to 98 ppm), EC (151 to 214 $\mu\text{S cm}^{-1}$) and transparency (35 to 100 cm) were also significant. Levels of phosphate (0.30 to 0.71 ppm), nitrite (0.019 to 0.08 ppm) and ammonia (0.024 to 0.23 ppm) recorded in this group, are shown in Table 1. The estimated sediment parameters are also presented in Table 1. In the T_1 treatment group, pH ranged from 6.52 to 8.40, EC from 150 to 250 $\mu\text{S cm}^{-1}$ and organic carbon content varied from 0.6 to 7.8%. Nutrient parameters such as available nitrogen, phosphorus and potassium were in the range of 91.50 to 170 mg kg^{-1} , 6.60 to 13.14 mg kg^{-1} and 94.70 to 121 mg kg^{-1} , respectively. In treatment T_2 , pH, EC and organic carbon percentage ranged from 6.80 to 8.40, 160 to 260 $\mu\text{S cm}^{-1}$ and 0.70 to 0.88%, respectively. During the same period, the nutrient parameters viz., available nitrogen, phosphorus and potassium recorded in T_2 were 91.50 to 175 mg kg^{-1} , 6.60 to 15.40 mg kg^{-1} and 95.10 to 131 mg kg^{-1} , respectively. Mann-Whitney U test indicated that the values for dissolved oxygen, electrical conductivity and total dissolved solids in water as well as estimated soil parameters such as organic carbon, available nitrogen and available potassium showed significant differences between the treatments ($p < 0.05$). Comparison of physicochemical parameters between monoculture and integrated ponds provides valuable insights for decisions regarding pond management and environmental impact.

In this investigation, 24 species of phytoplankton were identified, predominantly from the groups Chlorophyceae (10 species), Cyanophyceae (6 species) and Bacillariophyceae (8 species) (Table 2). Diversity studies indicated that 24 species were recorded in location



(a)



(b)

Fig. 2. (a). Makhana fruit and (b) Dried makhana seed

Table 1. Physicochemical parameters of water and soil from T₁ and T₂ ponds (June-October 2021)

Water parameters	T ₁			T ₂			p-values
	Minimum	Maximum	Mean±SD	Minimum	Maximum	Mean±SD	
Temperature (°C)	25.00	32.00	29.35 ±2.36	25.00	32.00	29.35±2.40	1.00
DO (ppm)	1.34	4.12	3.18±0.88	3.34	8.20	5.36±1.42	0.00*
TDS (ppm)	90.00	140.00	111.65±17.92	75.00	98.00	88.10±7.92	0.00*
pH	6.50	8.30	7.35±0.51	6.50	8.30	7.29±0.49	0.59
EC (µS cm ⁻¹)	166.00	271.00	205.20±34.06	151.00	214.00	180.60±20.39	0.02*
Ammonia (ppm)	0.02	0.04	0.028±0.05	0.02	0.23	0.084±0.02	0.16
Nitrite (ppm)	0.03	0.09	0.066±0.02	0.019	0.08	0.089±0.02	0.23
Phosphate (ppm)	0.39	0.63	0.50±0.06	0.30	0.71	0.48±0.12	0.35
Transparency (cm)	27.00	98.00	75.70±25.07	35.00	100.00	78.70±21.51	0.56
Sediment parameter							
pH	6.52	8.40	7.17±0.35	6.80	8.40	7.17±0.38	0.06
EC (µS cm ⁻¹)	150	250	195±33.11	160	260.20	201.93±31.14	0.54
Organic carbon (%)	0.60	0.78	0.70±0.05	0.70	0.88	0.76±0.07	0.008*
Available nitrogen (mg kg ⁻¹)	91.50	170.00	114.95±20	91.50	175.00	129.55±25.61	0.04*
Available phosphorus (mg kg ⁻¹)	6.00	13.40	8.70±1.93	6.60	15.40	9.54±2.7	0.34
Available potassium (mg kg ⁻¹)	94.70	121	99±43	95.10	131.00	108±12.80	0.008*

Table 2. Overall phytoplankton and zooplankton diversity in the makhana pond system method

Phytoplankton		Zooplankton	
Chlorophyceae	<i>Ankistrodesmus</i> sp.	Rotifera	<i>Keratella</i> sp.
	<i>Oedogonium</i> sp.		<i>Cephalodella</i> sp.
	<i>Chlorella</i> sp.		<i>Trichocerca</i> sp.
	<i>Spirogyra</i> sp.		<i>Brachionus</i> sp.
	<i>Euglena</i> sp.		<i>Scardium</i> sp.
	<i>Mougeotia</i> sp.		<i>Sinantharina</i> sp.
	<i>Ulothrix</i> sp.		<i>Lepadella</i> sp.
	<i>Closterium</i> sp.		<i>Lecane</i> sp.
	<i>Cosmarium</i> sp.		<i>Asplanchna</i> sp.
	<i>Closterium</i> sp.	Cladocera	<i>Bosmina</i> sp.
Cyanophyceae	<i>Microcystis</i> sp.		<i>Moina</i> sp.
	<i>Anabaena</i> sp.		<i>Daphnia</i> sp.
	<i>Oscillatoria</i> sp.		<i>Alonella</i> sp.
	<i>Spirulina</i> sp.	Copepoda	Calonoid copepod
	<i>Nostoc</i> sp.		<i>Mesocyclops</i> sp.
	<i>Lyngbya</i> sp.		<i>Diaptomus</i> sp.
Bacillariophyceae	<i>Navicula</i> sp.	Ostracoda	<i>Cypris</i> sp.
	<i>Synedra</i> sp.		<i>Heterocypris</i> sp.
	<i>Diatoma</i> sp.		
	<i>Nitzschia</i> sp.		
	<i>Desmidium</i> sp.		
	<i>Cymbella</i> sp.		
	<i>Melosira</i> sp.		
	<i>Pleurosigma</i> sp.		

T₂, while 19 were noted in location T₁. The Shannon diversity index (*H'*) ranged from 0.93 to 2.42, with higher values observed in June and July at T₁ and from 0.94 to 2.58 at T₂, also peaking in June and July. The Pielou evenness index (*J*) was measured at a similar level in both locations: 0.85 to 0.94 in T₁ and 0.85 to 0.93 in T₂. Notably, the Simpson diversity index (*D*) was significantly higher in T₂ (ranging from 0.6 to 2.35) than in T₁ (ranging from 0.64 to 1.99)

(Fig. 3). Additionally, 18 species of zooplankton were identified, mainly from the groups Rotifera (9 species), Cladocera (4 species), Copepoda (3 species) and Ostracoda (2 species) (Table 2). The study of zooplankton diversity showed that *H'* index ranged from 0.93 to 2.42, with higher values recorded between June and July in T₁. In T₂, the *H'* index ranged from 1.84 to 2.51, again showing higher values in June and July. *J* index was fairly consistent between T₁ (from 0.89 to 0.97) and T₂ (from 0.90 to 0.97). In contrast, the *D* index was significantly higher in T₂ (ranging from 1.971 to 3.396) compared to T₁ (ranging from 1.864 to 2.524) throughout the experimental period (Fig. 3). Additionally, when examining phytoplankton diversity, certain species such as *Oedogonium* sp., *Chlorella* sp., *Euglena* sp. and *Spirulina* showed statistically significant differences among the treatments (*p*<0.05). Similarly, for zooplankton diversity, specific species, including *Sinantharina* sp., *Lepadella* sp. and *Heterocypris* sp., also exhibited significant differences among the treatments (*p*<0.05).

In this investigation, the average yield of makhana seeds was recorded at 1.78±0.13 t ha⁻¹ from treatment T₁ and 1.55±0.085 t ha⁻¹ from treatment T₂ (Table 3). In the makhana fish integrated pond (T₂), the dense canopy of makhana leaves and its vegetative structure over the water, made fish sampling difficult. As a result, only the initial mean weight (g) of fish fingerlings could be recorded at stocking in T₂. Final mean wet weight (g), fish survival rate (%) and fish yield (t ha⁻¹) were recorded at final harvest (Table 3). On termination of experiment T₂, the total fish yield was 1.48±0.15 t ha⁻¹ in October. Among the harvested fish, the average weights of catla and rohu were 574.82±31.54 and 624.55±11.49 g fish⁻¹, respectively (Table 3). Additionally, the total biomass of catla and rohu was recorded at 0.74±0.07 and 0.57±0.087 t ha⁻¹, respectively. Moreover, the average data on the growth performance of other individual species were pooled and analysed, as shown in Table 3. In addition, this study also examined the proximate composition and mineral profile of makhana seeds. The mean values for moisture, protein, carbohydrates, fat and ash content, along with the mineral profile components of phosphorus (P), potassium (K),

Table 3. Fish growth performance and makhana seed production analysis

Fish	Parameter	T ₁	T ₂ (Mean ± SD)
Catla	Initial mean weight (g fish ⁻¹)	--	10.30±2.4
	Final mean weight (g fish ⁻¹)	--	574.82±31.5
	Survival (%)	--	71.55±3.3
	Fish yield (t ha ⁻¹)	--	0.74±0.07
Rohu	Initial mean weight (g fish ⁻¹)	--	5.17±2.5
	Final mean weight (g fish ⁻¹)	--	624.55±11.5
	Survival (%)	--	68.50±4.2
	Fish yield (t ha ⁻¹)	--	0.57±0.09
Mrigal	Initial mean weight (g fish ⁻¹)	--	4.60±0.4
	Final mean weight (g fish ⁻¹)	--	170.09±5.8
	Survival (%)	--	41.75±3.0
	Fish yield (t ha ⁻¹)	--	0.085±0.005
Bata	Initial mean weight (g fish ⁻¹)	--	2.42±1.7
	Final mean weight (g fish ⁻¹)	--	94.10±4.6
	Survival (%)	--	69.73±0.5
	Fish yield (t ha ⁻¹)	--	0.0394±0.003
Anabas	Initial mean weight (g fish ⁻¹)	--	3.45±0.50
	Final mean weight (g fish ⁻¹)	--	45.82±1.1
	Survival (%)	--	79.50±2.0
	Fish yield (t ha ⁻¹)	--	0.0221±0.005
Channa	Initial mean weight (g fish ⁻¹)	--	15.92±1.0
	Final mean weight (g fish ⁻¹)	--	176.50±4.7
	Survival (%)	--	71.25±4.1
	Fish yield (t ha ⁻¹)	--	0.08±0.004
Makhana	Total fish yield (t ha ⁻¹)	--	1.48±0.15
	Makhana seed yield (t ha ⁻¹)	1.78±0.133	1.55±0.09

sodium (Na), magnesium (Mg), calcium (Ca), iron (Fe), copper (Cu), manganese (Mn) and zinc (Zn) in the kernel, are presented in Table 4. The economic analysis of input and output revealed that

Table 4. Proximate composition and mineral profile of raw makhana seed (kernel)

Proximate composition	Mean±SD	Mineral profile	Mean±SD
Moisture (%)	32.43±1.69	Phosphorus (mg 100 g ⁻¹)	66.1±0.26
Total ash (%)	0.3±0.05	Potassium (mg 100 g ⁻¹)	35.7±2.45
Fat (%)	0.3±0.08	Sodium (mg 100 g)	48.5±0.51
Protein (%)	7.2±0.25	Magnesium (mg 100 g ⁻¹)	11.3±0.11
Carbohydrate (%)	59.2±2.27	Calcium (mg 100 g ⁻¹)	9.5±0.51
Calorific value (Kcal 100 g ⁻¹)	360.3±1.52	Iron (mg 100 g ⁻¹)	0.8±0.02
		Copper (mg 100 g ⁻¹)	0.3±0.01
		Manganese (mg 100 g ⁻¹)	0.89±0.01
		Zinc (mg 100 g ⁻¹)	0.88±0.01

the input costs in the makhana cumfish ponds (T₂) were higher than those in the makhana-only pond (T₁). This was primarily due to additional labour engagement, expenses for supplementary feed and harvesting charges (Table 5). Both treatments incurred initial costs for seed sowing as they lacked an automatic seedling system. The total revenue for T₂ was ₹4,81,272±31584 ha⁻¹, while for T₁, it was ₹2,30,750±17638 ha⁻¹. The net benefits were ₹1,78,820±18633 for T₁ and ₹2,74,007±83648 for T₂ (Table 5). The estimated input costs, returns and revenue in this experiment showed significant differences among the treatments, as determined by the Wilcoxon-Mann-Whitney test ($p < 0.05$).

Discussion

Monitoring water and sediment quality is crucial for the growth and survival of aquatic plants and animals in aquaculture systems (Larsson, 1994). Table 1 illustrates the variations in different water and sediment quality parameters throughout the study period. Notably, water quality parameters tend to fluctuate during the

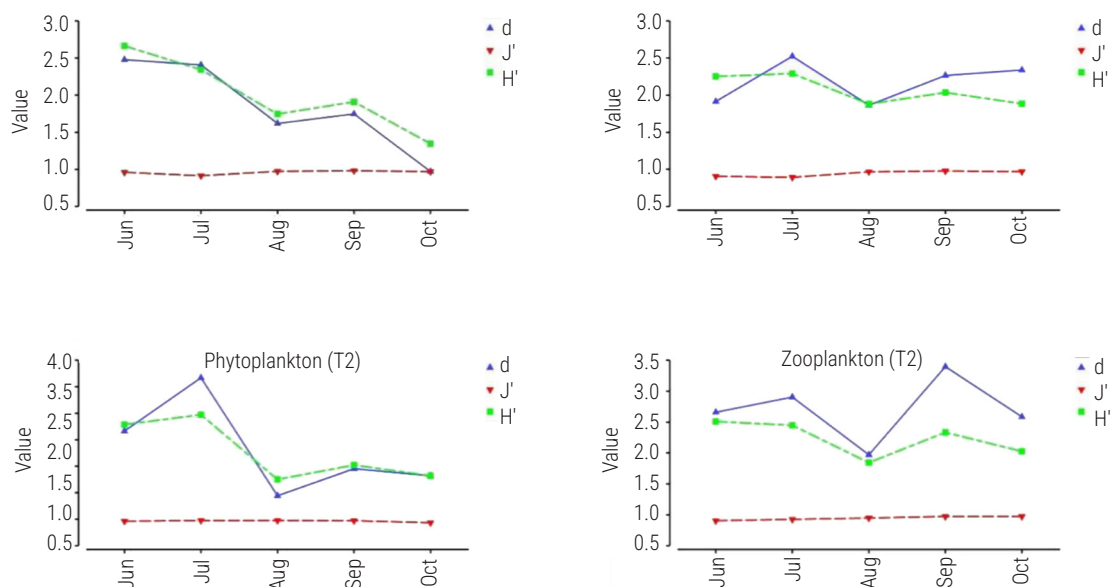


Fig. 3. Margalef richness index (D), evenness index (J') and Shannon-Wiener species diversity index (H') of both phytoplankton and zooplankton from T₁ and T₂

Table 5. Comparative economics analysis of Makhana (T_1) and Makhana cum fish integration (T_2) for 1 ha area

Particulars	T_1	T_2	Significance level
Total input cost (₹)	51,930.00±994.00	2,07,265.00±8380.29	
Return (₹)			
Makhana seed production (₹)	2,30,750.00±17638.00	2,01,250.00±11306.67	p<0.05
Fish production (₹)	–	2,80,022.00±27622.12	
Total revenue (₹)	2,30,750.00±17638.41	4,81,272.00±31584.03	
Net revenue (₹)	1,78,820±18633.40	2,74,007.89±83648	

growth stages of makhana. Total dissolved solids (TDS) reflect the overall ion concentration in water, which often rise in parallel with specific ions such as phosphate, nitrite and ammonia. The elevated values of TDS, electrical conductivity (EC) and transparency may be attributed to the emergence of fresh leaves, the decomposition of older leaves and the resulting plant residues (Zhao *et al.*, 2022).

Additionally, the dissolved oxygen (DO) level was found to be below the optimal range, likely due to shading from the makhana leaves. The shading effect from the macrophytes may result in reduced phytoplankton production, lower DO concentrations and increased levels of free carbon dioxide in the water, which can ultimately lead to fish mortality (Edwards, 1980). In the T_2 treatment ponds, water quality parameters varied throughout the growth stages of makhana and following the introduction of fish fingerlings. T_1 ponds exhibited significant levels of total dissolved solids (TDS) and electrical conductivity (EC), both of which were higher than those observed in T_2 . The elevated TDS levels may be attributed to the presence of dissolved organic salts such as phosphates, nitrites and ammonia. In general, the observed decline in temperature and dissolved oxygen (DO) levels during winter across both treatments, combined with the accumulation of decomposed plant residues, might have hindered nutrient absorption through photosynthesis and potentially impacted the metabolic rates of aquatic organisms (Bhateria and Jain, 2016; Day *et al.*, 2021). Consequently, concentrations of nutrients such as phosphate, nitrite and ammonia were found to rise in the water as October approached. Additionally, the accumulation of decaying makhana leaves in the ponds, which were not removed, might have contributed to further degradation of water quality in both T_1 and T_2 ponds. The better water quality observed in T_2 is likely attributed to its “peripheral vacant space” design, which facilitated sufficient sunlight penetration, thereby enhancing DO concentrations in this treatment.

Sediment pH is important in any aquatic ecosystem as it regulates many chemical processes necessary for maintaining productivity. According to Jhingran (1992), a pH level between 6.5 and 7.5 is ideal for achieving average to high productivity. In treatment T_1 , the maximum pH value of the bottom sediment was alkaline but remained within acceptable limits. The mean organic carbon content was approximately 0.70±0.05%, indicating a productive condition of the bottom sediment (Jhingran, 1992). The varying electrical conductivity (EC) values during the experimental period reflected the ions' solubility at the pond ecosystem's sediment-water interface. Nitrogen (N) and phosphorus (P) were identified as significant nutrient elements in the bottom sediment, playing a crucial role in determining the fertility of aquaculture ponds (Jhingran, 1992). Available nitrogen might have originated from organic nitrogen, exchangeable ammonium, ammonium present in pore water, dissolved nitrate and nitrogen gas (Francis *et al.*,

2004). In treatment T_2 , the recorded average EC, organic carbon, nitrogen, phosphorus and potassium values were higher than in T_1 . This increase may be attributed to the contribution of decomposed makhana biomass, fish excreta and external inputs such as supplementary feed and fertilisers (Horppila and Nurminen, 2003; Alvarez-Garcia *et al.*, 2019).

Phytoplankters are primary producers in aquatic ecosystems and form essential food source for carps (Billard, 1999). The density and community structure of phytoplankton reflect ecological and environmental quality of water bodies. With respect to plankton diversity, higher H' and D' indices were recorded in T_2 ponds compared to T_1 . This difference may be attributed to the ample surface water space available and the periodic application of fertiliser, which enhanced the diversity and growth of plankton. However, the dead makhana biomass produced suspended matter, resulting in a black colouration that decreased water transparency and could have potentially reduced phytoplankton abundance during the final month of the experiment. Similarly, the zooplankton community acts as an intermediary in the aquatic food web, transferring energy from primary producers (phytoplankton) to larger invertebrate predators and fish that feed on them. Zooplankton abundance can respond quickly to changes in the dynamics of phytoplankton in the ecosystem (Lou, 2013). Overall, T_2 exhibited higher zooplankton density and diversity, likely due to the sufficient availability of phytoplankton, regular loading of faecal matter, and soil scooping by fish during search for food.

Makhana cultivation is primarily practiced in waterlogged regions, particularly in the Chaur area, which showcases the untapped potential for cultivating profitable aquatic cash crops (Pramanik *et al.*, 2013). Generally, in makhana cultivation, seeds take 2 to 3 months to germinate in a nursery pond. Newly emerged leaves are red, turning green as they mature, featuring prickly spines. Plants with leaves over 10 cm in diameter are suitable for transplanting into pond systems. Once the roots of the saplings have developed adequately, the pond is filled to the appropriate depth for introducing fish fingerlings. Since the leaves typically reach over 1 m in diameter, it is necessary to maintain sufficient water surface space (10-15%) along the pond margins. It can be achieved by installing bamboo poles to prevent the leaves from spreading into open areas. In the present study, the average output of makhana seed from treatment T_1 was slightly higher than that from T_2 . In general, the field system method (FSM) of makhana cultivation has recorded seed production rates of around 2.8-3.0 t ha⁻¹ (Singh *et al.*, 2020). In contrast, the pond system method yielded lower quantity seeds. It may be attributed to the pond depth exceeding 1 m. At this depth, the plants expend more energy on upward growth and may remain stagnant, affecting their phenological characteristics, such as leaves and fruits. A study on the nutritional quality of raw edible makhana

seeds (i.e., kernels) revealed significant nutritional importance, with notable proximate composition and mineral profiles, including levels of phosphorus (P), potassium (K), sodium (Na), magnesium (Mg), calcium (Ca), iron (Fe), copper (Cu), manganese (Mn) and zinc (Zn). The highest estimated concentration of minerals was found for phosphorus (P), followed by sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), manganese (Mn), zinc (Zn), iron (Fe) and copper (Cu). The mean energy content (calorific value) was approximately 360.3 ± 1.52 Kcal 100 g^{-1} . Previous studies have also examined proximate composition and mineral profile of makhana seeds (Jana *et al.*, 2018; Kumar *et al.*, 2019). Similarly, research has been conducted on aquatic plants, such as lotus seeds (*Nelumbo nucifera* Gaertn) and *Trapa natans* (var. *bispinosa*) fruits, which have shown distinct nutrient profiles that include carbohydrates, protein, fat, vitamins, minerals and more (Adkar *et al.*, 2014; Bangar *et al.*, 2022).

Finally in T_2 , rohu and catla exhibited higher average weights than the other indigenous fish species. The organic matter produced by makhana plants along with that produced by various associated invertebrates benefits these fish. The remaining biomass from makhana plants (excluding the harvested seeds) decomposes into organic matter that enriches the substrate and contributes nutrients for subsequent crops and generates organic detritus, which is consumed by benthic fish such as *C. striata*. Additionally, it offers shelter for juvenile fish, helping to prevent cannibalism (Petr, 2000; Nazir *et al.*, 2023). The plants' prickly structures can deter other prey and bio-invaders. However, due to the insufficient availability of phytoplankton and zooplankton in the integrated system, adequate fertiliser and supplementary feeds were applied in T_2 according to the specific needs identified (Ayyappan *et al.*, 2011). Fish mortality in Indian major carps (IMCs) has typically been attributed to factors such as high plant density, the prickly characteristics of makhana plants, decomposed plant residues, decreased dissolved oxygen levels and suspended organic matter in the water column. Nonetheless, the average biomass of rohu and catla has been higher than that of other species, indicating that these two species are well-suited for co-cultivation with makhana. In addition, indigenous fish species such as bata, climbing perch and various murrel species can also be successfully raised alongside makhana. Therefore, integrated makhana-fish culture systems can function as integrated farmer managed systems, sustainably utilising all resources to boost productivity and increase farmers' incomes. The current study revealed that integrating makhana with fish farming yielded greater benefits than cultivating makhana as a mono-crop. Such semi-intensive farming techniques could lead to an additional income of over 50% compared to traditional makhana cultivation methods. Both makhana seeds and fish are aquatic resources that, when combined, have the potential to boost production. Furthermore, this integration can effectively utilise the underexploited natural resources found in the wetland regions of north Bihar.

The present study concluded that the makhana-fish system may provide mutual benefits regarding nutrients and organic matter, leading to synergistic effects. However, the research also identified potential issues, such as fish mortality and harvesting challenges, stemming from high plant density and the presence of prickly characteristics, as well as decomposed plant residues. These factors can lead to decreased dissolved oxygen levels in the water. Comparative economic analysis reveals that integrated aquaculture

systems outperform monocultures, potentially boosting surplus income by up to 50%. These findings highlight the effective use of pond ecosystems in integrated farming systems, making it a sustainable technology that can improve the livelihoods of rural fishers. However, further systematic studies are needed to standardise stocking densities, species combinations, feeding rates, and water quality parameters. Addressing these factors will help to optimise outcomes and enhance overall productivity from makhana-fish integration.

Acknowledgements

The authors wish to express gratitude to the Director, ICAR-RCER, Patna, for providing invaluable assistance and funding support for this project.

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