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Studies on factors influencing seed germination and seedling growth of Makhana (*Euryale ferox* Salisb.)

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Abstract

The aquatic food crop known as makhana (*Euryale ferox* Salisb.) or foxnut is typically cultivated in ponds or low depressions. This crop's significance is growing daily because of its high protein and carbohydrate content, low fat content, and religious contributions. Its production is constrained because of low seed germination and fungal infection of seeds. The present study aimed to evaluate the influence of temperature (15 °C, 20 °C, 25 °C, 30 °C, and 40 °C), seed treatment (fungicides and antibiotics), different concentrations of bleaching powder (0.25, 0.5, 1.0 g/litre), seed size (small, medium, and large), and light and dark conditions on germination and seedling growth of makhana seeds. Our results indicated that makhana seeds had significantly higher germination at 25 °C, an optimum temperature, than at the other temperatures. The most effective fungicide for controlling fungal infection in seeds and improving germination was Ridomil (a mixture of contact and systemic fungicides) @ 200 ppm for 24 hours. Bleaching powder also reduced makhana seed infection and improved seed germination at all tried concentrations, but at a low concentration (0.25 g/lit), seedling growth and root formation were faster. The percentage of makhana seeds that germinated was considerably higher for smaller seeds (44.6%), followed by medium seeds (28.6%) and large seeds (12.9%). Additionally, small seeds germinated sooner than medium and large seeds. Makhana seeds germinate equally under light and dark conditions, indicating that light is not involved in regulating makhana seed germination. However, seeds germinated under light had significantly higher leaf chlorophyll content and chlorophyll fluorescence than seeds grown in the dark. In addition, we observed that light is required for root development in makhana.

Keywords: *Euryale ferox*, makhana, seed germination, seedling growth

Introduction

Makhana (*Euryale ferox* Salisb.), sometimes referred to as gorgon nut or foxnut, is a popped seed derived from an aquatic crop that is a member of the Nymphaeaceae family (Raut *et al.*, 2025) ^[19]. It typically grows in bodies of stagnant water, such as lakes, ponds, and low depressions (Jha *et al.*, 1991; Raut *et al.*, 2020) ^[7, 17]. It is primarily grown in lowland ponds in Bihar, West Bengal, Orissa, and Assam. The districts of Darbhanga, Madhubani, Purnia, and Katihar in Bihar account for nearly 80% of all Makhana in India (Kumar *et al.*, 2011) ^[12]. Nearly 60,000 tonnes of makhana seeds are produced from the 35,000-ha area of north Bihar, which accounts for >85% of makhana seed production in India (Directorate of Horticulture, 2022) ^[2]. In a pond, about 10,000 plants/ha are grown, and the seed yield in the traditional system is around 1.8–2.0 t/ha (Khadatkar *et al.*, 2020) ^[10]. It provides a livelihood to thousands of fishing families in the region and is a potential earner of foreign exchange (Raut *et al.*, 2024; Singh *et al.*, 2023) ^[18, 20]. After being processed from a black hard nut to a white puff, they become a very nutritious food source. Makhana seeds provide outstanding dietary attributes since they are rich in vital nutrients and bioactive compounds such as carbs, protein, fiber, vitamins, minerals, and polyphenols (Kapoor *et al.*, 2022) ^[9]. Notably, they have favorable arginine-to-lysine/proline ratios and a high amino acid index (89% to 93%) (Nath and Chakraborty, 1985) ^[15]. Additionally, makhana seeds have shown promise in treating a number of illnesses, such as diabetes, digestive problems, heart problems, kidney problems, and reproductive abnormalities (Das *et al.*, 2006; Jha and Barat, 2003) ^[1, 8]. Additionally, roasted makhana pops have a low Glycemic Index (GI) of only 37%, making them

a nutrient-rich alternative that may be included in balanced diets and may help people with metabolic diseases, including diabetes and obesity (Liaquat *et al.*, 2022) ^[14].

Seed dormancy affects annual variation in population sizes and yield. Seed dormancy is an adaptive mechanism that ensures germination occurs in a suitable location and under suitable conditions (Fenner and Thompson, 2005) ^[13]. Seed dormancy and the germination characteristics of this species are poorly understood. Temperature and light are two environmental elements that encourage germination (Kumar *et al.*, 2017) ^[11]. For some species, seed size may be crucial to seed germination in addition to temperature and light (Leverett and Jolls, 2013) ^[13]. Fungal infection in makhana seeds also contributes to low germination. However, very few studies have been conducted to improve makhana seed germination. The aim of the present research was to quantitatively assess the germination of *E. ferox*, focusing on reducing fungal infection and studying the influence of temperature, light conditions, and seed size to improve germination for better production.

Materials and Methods

Makhana seeds were collected from two ponds in Darbhanga city, Bihar, and the experiments were conducted at the ICAR-Indian Institute of Water Management, Bhubaneswar, Odisha, during 2004-06. Seeds were stored in a plastic container for conducting different experiments. To study the effect of water temperature on makhana seed germination, 50 seeds in four replicates (total 200 seeds) were kept at 15 °C, 20 °C, 25 °C, 30 °C, and 40 °C. Fungal infection reduces makhana seed germination, so we treated 200 seeds with different fungicides and antibiotics @ 200 ppm for 24 hours to reduce infection, then kept them in distilled water until they germinated. In the control, seeds were kept in water, while in another treatment, we changed the water daily to reduce the infection load. We used 50 seeds per treatment in four replications. In another experiment to improve makhana seed germination, seeds were grown under various concentrations of bleaching powder, viz. 0.25, 0.5, 1.0 g/litre, and compared with 0 concentration (water), and germination and seedling growth were recorded. To study the effect of seed size on germination, we conducted another experiment, and we measured the maximum diameter of collected seeds with a digital caliper and divided them into three size groups: small (7-9 mm), medium (9-11 mm), and large (11-13 mm). We used about 581, 514, and 216 seeds for the germination test in each size group, respectively. Seeds in each size group were stored in separate plastic containers with four replicates, and the average fresh weight of the seeds in these three categories was recorded. We filled the containers with water for seed germination and replaced the water regularly. We kept fifty seeds in each of the four replicates under light and dark conditions to study germination, seedling growth, and physiological parameters. Leaf chlorophyll content of seedlings was measured by following the dimethyl sulfoxide (DMSO) method (Hiscox and Israelstam, 1979) ^[15], and chlorophyll fluorescence (Fv/Fm and ΦPSII) was measured by the Fluorescence Monitoring System (FMS-2, Hansatech), 10 days after germination of seeds. Leaves were dark-adapted for 30 minutes using leaf clips prior to measuring Fv/Fm. The experimental design was a completely randomized design (CRD), and statistical analyses were conducted using analysis of variance (ANOVA) in SAS 9.2 for Windows (Gomez and Gomez, 1984) ^[4]. At the 5% probability level, we evaluated differences between treatment means using Duncan's multiple range test (DMRT).

Results and Discussion

Effect of temperature on germination of makhana seeds

An experiment was conducted to study the effect of water temperature on makhana seed germination. Fifty seeds in four lots (total 200 seeds) were kept at 15 °C, 20 °C, 25 °C, 30 °C and 40 °C for 24 hours, and germination was recorded. Germination varied with water temperature, and the maximum number of seeds germinated at 25 °C (Table 1). Thus, the optimum temperature for makhana seed germination is 25 °C. Other researchers have reported similar findings (Imanishi and Imanishi, 2014; Kumar *et al.*, 2017) ^[6, 11]. At 30 °C, the germination frequency was significantly higher in light than in darkness due to the interaction between light condition and germination temperature; at 15°C, the reverse tendency was seen (Imanishi and Imanishi, 2014) ^[6].

Table 1: Effect of temperature on germination of makhana

Treatments	Total number of seeds tested	Germination (%)
15 °C	200	11.5 ^c
20 °C	200	36.0 ^b
25 °C	200	63.0 ^a
30 °C	200	31.5 ^b
40 °C	200	9.2 ^c

Four replications and 50 seeds were tested in each replication. Mean values followed by different letters in the column denote significant ($P \leq 0.05$) differences between the treatments by Duncan's multiple range test.

Effect of fungicides and antibiotics on germination of makhana seeds

White fungal growth in makhana seeds was observed to cause poor germination (Fig. 1). Microscopic studies showed that this was *Phycomyces* fungi. Makhana seeds were allowed to germinate in both disinfected soil and distilled water to determine how the pathogen spread. The infection was found under both conditions. Hence, this fungal attack was seed-borne.



Fig 1: Makhana seeds with fungal growth

Fungicides (Thiram, Bavistin, and Ridomil) and an antibiotic (Streptomycin) were used for seed treatment to control fungal infection. The seeds were treated with a solution of these fungicides and the antibiotic for 24 hours, then kept in distilled water until they germinated. In the control, seeds were kept in water, while in another treatment, we changed the water daily to reduce the infection load. Ridomil, a mixture of contact and systemic fungicides, was the most effective chemical, while the antibiotic streptomycin was the least effective (Table 2). Germination improved from 22 to 77.5%, a 3.5-fold increase. A significant improvement in makhana seed germination was also observed in the treatment where water was changed daily.

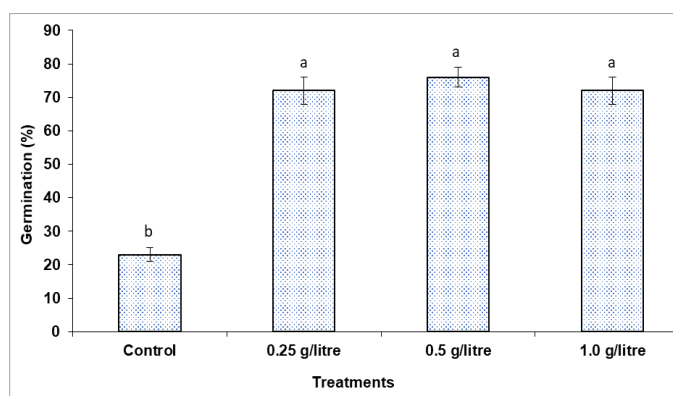
Table 2: Effect of fungicides and antibiotic seed treatment on germination of makhana

Seed Treatments	Total number of seeds tested	Germination (%)
Water (Control)	200	22.1 ^b
Thiram (Contact fungicide)	200	25.2 ^b
Bavistin (Systemic fungicide)	200	27.0 ^b
Ridomil (Mixture of both)	200	77.5 ^a
Streptomycin (Antibiotic)	200	19.3 ^b
Water (Daily change)	200	79.0 ^a

Four replications and 50 seeds were tested in each replication. Mean values followed by different letters in the column denote significant ($P \leq 0.05$) differences between the treatments by Duncan's multiple range test.

Effect of different concentrations of bleaching powder on germination and seedling growth of makhana seeds

To improve the germination of makhana seeds, they were grown under various concentrations of bleaching powder. The concentrations used were 0.25, 0.5, and 1.0 g/litre, compared with 0 concentration (water). It was found that the bleaching powder reduced the makhana seed infection and improved germination up to 76% at 0.5 g/lit concentration; however, no significant differences were observed in makhana seed germination at different concentrations of bleaching powder (Fig. 2). Significantly longer seedlings with more fresh weight were recorded in 0.25 and 0.5 g/lit bleaching powder solution. It was also found that, when germinated seeds were grown under these different solutions, roots did not form in the control and in the 0.5 and 1.0 g/lit bleaching powder treatments up to one month. However, seeds kept under 0.25 g/lit bleaching powder solution showed root formation after 7 days of germination (Table 3). These results indicated that a low concentration (0.25 g/lit) of bleaching powder not only improves seed germination but also triggers root formation in makhana seeds.

**Fig 2:** Effect of different concentrations of bleaching powder on seed germination of makhana**Table 3:** Effect of different concentrations of bleaching powder on growth and root development after 7 days of germination

Treatments	Seedling length (cm)	Leaves number	Fresh weight (g)	Root development
Control	12.0 ^b	1-3	0.80 ^b	No roots
0.25 g/litre	19.1 ^a	2-4	1.21 ^a	Roots
0.50 g/ litre	18.0 ^a	3-4	1.10 ^a	No roots
1.0 g/litre	10.8 ^b	3-4	0.78 ^b	No roots

Four replications and 50 seeds were tested in each replication. Mean values followed by different letters in the column denote

significant ($P \leq 0.05$) differences between the treatments by Duncan's multiple range test.

Effect of seed size on germination of makhana seeds

After seed collection, seeds were divided into three size groups: small (7-9 mm), medium (9-11 mm), and large (11-13 mm). 581, 514, and 216 seeds for the germination test in each size group, respectively. Average fresh weight of the small, medium, and large seeds was 0.45, 0.84, and 1.09 g, respectively (Table 4). Results indicated that small seeds exhibited a significantly higher germination percentage (44.6%), followed by medium (28.6%) and, least, large seeds (12.9%). Small seeds also germinated earlier than medium- and large-sized seeds. This may be because small seeds seem to complete dormancy in a shorter period. Imanishi and Imanishi (2014)^[6] and Kumar *et al.* (2017)^[11] also reported higher germination of smaller makhana seeds than larger seeds. Moreover, Imanishi and Imanishi (2014)^[6] found that the germination frequency of larger seeds peaked after 90 days of stratification at 4°C, but smaller seeds continued to have high germination frequencies for up to 180 days. Smaller and larger seeds may exhibit varied germination responses due to differences in the rate of dormancy induction in the annual dormancy cycle.

Table 4: Effect of seed size on germination of makhana

Categories	No. of seeds tested	Seed diameter (mm)	Seed fr. wt. (g)	Germination (%)
Small	581	7-9	0.45 ± 0.15 ^c	44.6 ^a
Medium	514	9-11	0.84 ± 0.12 ^b	28.6 ^b
Large	216	11-13	1.09 ± 0.07 ^a	12.9 ^c

Four replications and 50 seeds were tested in each replication. Mean values followed by different letters in the column denote significant ($P \leq 0.05$) differences between the treatments by Duncan's multiple range test.

Effect of light and dark conditions on germination, seedling growth and physiological parameters of makhana

Very few studies have examined the effect of light on makhana seed germination, but field observations showed that makhana seeds do not require light for germination (Okada, 1935; Wakita, 1959)^[16, 21]. In our study, we also observed no significant differences in makhana seed germination percentage under diffused light and complete darkness (Table 5). This indicates that light is not required for makhana seed germination. However, when germinated makhana seeds were kept under diffused light and complete darkness, we observed significant differences in seedling growth, development, and physiology. This experiment was planned to determine the response of germinated seeds that are either on the bottom of the pond bed or buried in the mud. Seeds at the bottom of the pond bed receive diffused light; however, seeds inside the mud remain in the dark. Results showed that seedlings grown under light had root development after 10 days of germination; however, seedlings grown in the dark had no root development (Fig. 3). Fresh weight of seedlings and number of leaves were significantly higher in seedlings grown under light than in the dark. However, seedling length was significantly greater under dark conditions (Table 5). Leaves of seedlings grown in the dark had protochlorophyll pigment, due to which they showed a lower quantum yield of Fv/Fm and ΦPS II and electron transport rate (ETR) as compared to the leaves of seedlings grown under light (Table 5). The chlorophyll content in the leaf was significantly higher in seedlings grown under light conditions than in the dark condition. According to Imanishi and

Imanishi (2014) ^[6], makhana seeds sprout in both light and darkness, indicating that light does not affect makhana seed germination. Kumar *et al.* (2017) ^[11] reported similar findings, but they did not study the effects of light and darkness on root development, seedling growth, and physiological performance.



Fig 3: Makhana seedlings grown under diffused light (left) and dark (right) after 10 days of germination

Table 5: Effect of light and dark on seed germination, seedling growth, and physiology of makhana after 10 days of germination

Parameters	Light	Dark
Germination (%)	38.0±4.1 ^a	42.6±3.8 ^a
Fresh weight of seedlings (g)	0.51 ± 0.03 ^a	0.28 ± 0.04 ^b
Leaf number	3.5 ^a	2.3 ^b
Seedling length (cm)	9.4 ^b	11.0 ^a
Root development	++	--
Chlorophyll a (mg/ g fr.wt.)	0.611 ± 0.036 ^a	0.015 ± 0.002 ^b
Chlorophyll b (mg/ g fr.wt.)	0.200 ± 0.01 ^a	0.024 ± 0.003 ^b
Total Chlorophyll (mg/ g fr.wt.)	0.811 ± 0.01 ^a	0.039 ± 0.004 ^b
Fv/Fm	0.816 ± 0.013 ^a	0.592 ± 0.017 ^b
ΦPSII	0.761 ± 0.01 ^a	0.407 ± 0.01 ^b
Electron transport rate (ETR)	4.480 ± 0.12 ^a	2.640 ± 0.06 ^b

Conclusion

The results of the present study are relevant to understanding makhana seed germination and provide insight into improving germination by controlling fungal growth, a factor causing low germination. It clearly demonstrated that small makhana seeds had higher germination than large seeds. An important finding for makhana growers is that they should use smaller seeds as seed material for the next crop. However, large seeds can be processed to produce bigger popped makhana to fetch a higher price in the market. Makhana cultivation involves several drudgery operations, viz., sowing, interculture operations, harvesting (seed collection), and processing, which are done manually. Therefore, the main challenge in makhana cultivation is mechanization for these operations. Other hurdles include the lack of quality seeds/varieties, limited skills in management practices, and the very small area under makhana cultivation. As demand increases day by day because of its benefits as a ready-to-eat food, these challenges make makhana costlier in the market. Makhana cultivation is a low-input-requiring crop; therefore, small and marginal pond owners can exploit its potential, and it can be a viable alternative aquatic crop for sustainable agriculture in rural India. Research is needed to integrate makhana with aquaculture co-production systems to increase income and profitability for

farmers. Government policy and support are needed to fund research and extend this important crop nationwide.

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