Effect of fertilizers on the growth performance of *Azolla pinnata*: A potential feed additive for fish culture in Bangladesh

SHAHIDA ARFINE SHIMUL, MASUMA KHANOM MUNTAHA, MOHAMMAD SHAKIL KHAN, SAIFUDDIN RANA, MD ARIFUL ISLAM MILON 1, INKIAD AHMED HIMEL, ANTAR SARKER AND SK. AHMAD AL NAHID*

Department of Fisheries Resource Management, Chattogram Veterinary and Animal Sciences University (CVASU), Khulshi-4225, Chattogram, Bangladesh

1Department of Marine Bioresource Science, CVASU, Khulshi-4225, Chattogram, Bangladesh

*Corresponding author’s Email: nahid83bau@gmail.com

Abstract. *Azolla* is among the most popular, low-cost, and high-nutrient-content fish feeds. *Azolla pinnata*'s growth performance was evaluated on the effects of fertilizers containing phosphorus and nitrogen. The study used a completely randomized design (CRD) over thirty-five days in 150 L water containers. Two sets of sub-experiments were established, each with three replications: one for the combined application of phosphorus and nitrogen and another for the sole application of phosphorus. The results showed that the combined application of phosphorus (46% P) and nitrogen (46% N) was more effective than the application of phosphorus alone. The highest cell number and protein content of combined P and N applications were (70.01±4.73 g and 33.71±2.3%) in the T4 treatment. On the other hand, the maximum cell number and protein content of the sole P application were 58.76±3.8 and 33.06±2.25%, respectively, in the T4 treatment. A significant variation was observed in terms of the mean cell number, weight, and protein content among the experimental treatments. This study’s primary focus was to demonstrate the effects of inorganic fertilizers on *Azolla* growth. The findings suggest that the combined application of phosphorus and nitrogen fertilizer can significantly enhance *Azolla* growth compared to the sole application of phosphorus.

**Keywords:** *Azolla pinnata*, Fish feed, Nitrogen, Phosphorous

Introduction

Bangladesh’s aquaculture is growing as a result, and as the industry shifts from low-density to high-density culture, the demand for feed and other inputs has greatly increased (Hossain *et al.* 2022). Aquaculture feed is expensive in many developing nations and must contain all necessary nutrients for intense fish production. Feed with low manufacturing costs and essential nutrients is required for financial viability because fish feed increases the cost of aquaculture production (Das *et al.* 2018).

The free-floating water fern *Azolla pinnata* is a member of the cyanobacteria-growing Azollaceae family. *Azolla* is a promising feed due to its high nutritional value and ease of production (Paryanto *et al.* 2023). It contains more essential amino acids than wheat bran, offal, and maize, making it a suitable source of protein (Mounes *et al.* 2020). It contains incredible proteins, vital amino acids, vitamin A, carotenoids, growth-promoting substances, and minerals. *Azolla* has a 25-35\% protein content, 10–15\% mineral content, and a mixture of 7–15\% biopolymers, bioactive compounds, and amino acids by dry weight (Kumar *et al.* 2020). This plant contains significant amounts of vitamin A,
precursor beta-carotene, and vitamin B and is rich in minerals such as iron, calcium, magnesium, potassium, phosphorus, and manganese (Anitha et al. 2016).

Ponds and ditches are home to *A. pinnata*, which is advantageous in fish farming. It grows rapidly in natural ponds. It can frequently be grown in captive media at a fair price if a large quantity is required (Paolacci et al. 2018). It is becoming increasingly appreciated as a feed ingredient and an essential source of protein for fish that consume plants. Fish productivity benefits from fresh *Azolla pinnata* and natural feeding levels (Refaey et al. 2023). *Azolla* is a supplementary food for fish, contributing to their growth performance and protein requirements (Da Silva et al. 2022). However, it can potentially be a low-cost feed for fish culture. Currently, it’s used as a feed additive in various fish culture systems, such as tilapia and carp (Magouz et al. 2020).

*Azolla* may grow rapidly in favourable environments, doubling in 2–5 days and producing extremely dense mats (Korsa et al. 2024). The survival of Azolla in indoor culture systems depends on the availability of fertilizer and water, but other water characteristics, such as temperature, pH, dissolved oxygen, and salinity, might occasionally affect their growth (Mramba and Kahindi 2023). Phosphorus is a micronutrient with a significant growth-restraining effect on Azolla. P is an important nutrient for these species’ successful and rapid growth (Temmink et al. 2018). The amount of nitrogen in the medium has a negligible effect on the speed of plant growth (Da Silva et al., 2022). Compared to this, 10 ppm phosphorus supplementation could promote Azolla development, and it is a growth-limiting factor (Hossain et al. 2021). Therefore, the study aimed to evaluate the growth performance of *Azolla pinnata* cultured with different nitrogen and phosphorus fertilizer concentrations. The study might provide a thorough grasp of the applications of nitrogen and phosphorus fertilizers and the best dosages. By determining the proper nutrient content for Azolla growth, the study may help increase the plant’s commercial culture for commercial use. The study will provide a higher understanding of the potentiality of Azolla-based systems in Bangladesh through the low-cost natural purification process.

**Materials and methods**

Before the experiment began, an Azolla (Azolla pinnata) sample was obtained from a natural pond in the Khulshi near Chattogram (22.36281416° E, 91.80432002° N). It was acclimated to the process for one month in a tank on the roof of the teacher’s quarter of the Chattogram Veterinary & Animal Sciences University. Urea and TSP (Triple superphosphate) were used as fertilizer sources. Urea comprised 46% nitrogen, and TSP provided 46% phosphorus. Both fertilizers were purchased from a local market.

**Experimental design**

Thirty-five days were spent growing the Azolla. The study used a completely randomized design. 150 L of water containers were used for the experiment. Twelve plastic containers
were placed in the lab to continue the experiment. Before culture, the sample’s original mean weight was recorded. Two parameters (nitrogen and phosphorous combined and phosphorous fertilizer) were used to evaluate Azolla’s growth performance. These two parameters were experimented with four different treatments. Each treatment had three replications. The detailed experiment design is given in Table I.

Table I. The detailed layout of the experimental treatment with their inclusion level (mg/L)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatment</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen and phosphorous Fertilizer (combined)</td>
<td>T1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>N=5 mg/L, P=2.5 mg/L</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>N=10 mg/L, P=4.0 mg/L</td>
</tr>
<tr>
<td></td>
<td>T4</td>
<td>N=15 mg/L, P=6.5 mg/L</td>
</tr>
<tr>
<td>Phosphorous fertilizer</td>
<td>T1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>2.5 mg/L</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>4 mg/L</td>
</tr>
<tr>
<td></td>
<td>T4</td>
<td>6.5 mg/L</td>
</tr>
</tbody>
</table>

Calculation of growth performance
To ascertain which treatment had the greatest results regarding Azolla cell number and weight, Azolla cells were collected by a scoop net and counted daily using a Petri dish, and their weight and number were measured by a weight machine. The mean cell count and final mean weight were calculated to assess growth efficiency. The Kjeldahl apparatus and Kjeldahl methods were used to determine the protein content (AOAC 2005).

Determination of water quality parameters
The ideal water quality conditions for the growth of Azolla were also identified using indicators of water quality such as dissolved oxygen (DO), temperature, and pH. Temperature and dissolved oxygen (DO) were monitored with an electronic probe (Model: JANEWAY-9500). Samples were gathered in gas-tight bottles to test the pH of the water. In a lab environment, pH was measured using the electrode-probe technique (Hanna HI-2211 model).

Statistical analysis
Microsoft Office Excel (Version 2007) and IBM SPSS Statistics (Version 25) were used for the statistical analysis. Means and standard deviations were used to present the statistical data. The significant variance among the treatments was found using one-way ANOVA (analysis of variance) and Tukey’s post hoc test.
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Results

Effects of Nitrogen and Phosphorous fertilizer on the growth performance
The present study evaluated Azolla’s growth performance regarding their mean cell number and weight. In the case of Phosphorus tanks, T1 had the lowest mean cell number (44.23±4.7) and mean weight (0.56±0.10 g), whereas T4 had the highest number of cells (58.76±3.8) and weight (0.78±0.11 g) (Table II). On the other hand, in Nitrogen and Phosphorous combined treatment, T1 also had the lowest mean cell number (44.38±4.80) and mean weight (0.57±0.13 g) in T1 and the highest cell number (70.01±4.73) and weight (1.03±0.27 g) in T4. From those results, the study indicates that the T4 treatment provides the highest cell number and weight for both treatments (P and N+P) (Table II).

Table II. Azolla pinata's growth performance when applying nitrogen and phosphorous fertilizers

<table>
<thead>
<tr>
<th>Factor</th>
<th>Treatment</th>
<th>Cell number (per tank)</th>
<th>Weight (g)</th>
<th>Protein content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N+P</td>
<td>T1</td>
<td>44.38 ± 4.80</td>
<td>0.57 ± 0.13</td>
<td>27.91 ± 2.28</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>45.23 ± 6.11</td>
<td>0.72 ± 0.09</td>
<td>29.74 ± 1.84</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>60.71 ± 5.09</td>
<td>0.98 ± 0.21</td>
<td>32.45 ± 2.11</td>
</tr>
<tr>
<td></td>
<td>T4</td>
<td>70.01 ± 4.73</td>
<td>1.03 ± 0.27</td>
<td>33.71 ± 2.3</td>
</tr>
<tr>
<td>P</td>
<td>T1</td>
<td>44.23 ± 4.70</td>
<td>0.56 ± 0.10</td>
<td>27.36 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>44.47 ± 3.13</td>
<td>0.53 ± 0.07</td>
<td>27.76 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>47.80 ± 1.80</td>
<td>0.59 ± 0.07</td>
<td>30.03 ± 2.6</td>
</tr>
<tr>
<td></td>
<td>T4</td>
<td>58.76 ± 3.80</td>
<td>0.78 ± 0.11</td>
<td>33.06 ± 2.25</td>
</tr>
</tbody>
</table>

Compared to the P application and combined N and P application, N and P showed better growth performance. The highest cell count, 58.76±3.8, was observed with only P, while the combined application of N and P resulted in a cell count of 70.01±4.73. Consequently, the mean weight for phosphorus was 0.78±0.11 g and for combined application was 1.03±0.27 g (Fig. 1). ANOVA analysis showed a significant variation between the cell number and weight in T4 treatment in both phosphorus and combined phosphorus and nitrogen application (p<0.05). The findings indicate that the combined application of phosphorus and nitrogen fertilizer was more effective and could boost Azolla's growth than the sole application of phosphorus.

Effects of Nitrogen and Phosphorous fertilizer on the protein content
The protein content was also analyzed for each treatment, and the increased dose of the fertilizer aids the protein of the Azolla. The highest protein content was found in T4 for both P and N+P-treated Azolla (Table II and Fig. 1). There was a significant variation among the experiment treatments observed in the protein content between N+P and P-treated Azolla (p<0.05).
Fig. 1. The comparison of the effect of nitrogen and phosphorous fertilizer application in the growth performance of *Azolla Pinnata*. In this graph, P is denoted as Phosphorous while Np is denoted as Nitrogen and phosphorous combined application.

Relationship between cell number and weight of Azolla’s based on fertilizer application

There was a significant correlation observed between cell number and weight among the experimental treatments ($p < 0.05$) (Fig. 2). In comparison with P and N+P, N+P cell number showed a stronger correlation ship than the P (NP: $r=0.956$, P: $r=0.890$). The relationship matrix showed the overall scenario of the relationship between cell number and weight of the experimental treatments. The relationship between cell number and weight in the N+P ($r=0.956$) treated fertilizer was stronger than in the P ($r=0.890$) treated fertilizer.
**Water quality parameter assessment**

The water quality metrics were recorded daily throughout the trial. The mean value of the water quality parameters is documented in Table III.

<table>
<thead>
<tr>
<th>Factor</th>
<th>N+P</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>22.62 ± 1.95</td>
<td>22.69 ± 2.03</td>
</tr>
<tr>
<td>Dissolved oxygen (mg/L)</td>
<td>6.36 ± 1.13</td>
<td>6.21 ± 1.12</td>
</tr>
<tr>
<td>pH</td>
<td>5.69 ± 0.57</td>
<td>5.58 ± 0.68</td>
</tr>
</tbody>
</table>
Discussion

The present study evaluated the effect of the N and P application on the growth performance of *Azolla pinnata* and found a positive response in the cell number increase and weight gain. T0 (without any fertilizer) showed the least cell number and weight in the case of both factors, meaning that P and N+P combined the applications. These findings showed a significant effect of N and P fertilizer application on the Azolla’s growth. The findings also agreed with Sadeghi *et al.* (2013) who stated that Azolla’s growth performance could be regulated by applying inorganic fertilizers such as N and P.

N fertilizer was found not to be a limiting factor in Azolla’s growth but can regulate their growth to some extent in indoor culture (Sadeghi *et al.* 2012). On the other hand, P acts as a limiting factor for their growth (Hossain *et al.* 2021). The present study findings also align with the above-mentioned studies as the P application showed better results but could be more effective with application N. Among P-treated tanks, 6.5 mg/L showed more effective results than the other treatment. The lowest growth among the P treated was found in the T1; this also agreed with the findings of Temmink *et al.* (2018) who also found similar trends in the results.

*Azolla pinnata* is considered a high protein source used extensively in agriculture (Paryanto *et al.* 2023). Its high protein content and other essential amino acids presence in its body tissue made it one of the most demandable feed additives in the aquaculture sector (Kumar *et al.* 2020). The present study found about 33% protein content in both N and N+P applications. Around 23% of the crude protein content was found in the cultured Azolla of a previous study (Cherryl *et al.* 2014), which is less than that in this experiment. There was no elaborate explanation in the previously published literature for the variations of the protein content of Azolla due to the application of inorganic fertilizer. The present study observed a trend of increasing protein content of Azolla due to rises in the application of N and P fertilizers.

Azolla growth performance could be regulated by different factors such as fertilizer, water availability, and cultured parameters (Azab *et al.* 2020). The study tried to find out the overall effects of applying inorganic fertilizers such as N and P and found that their growth performance was significantly influenced by those fertilizers. A few studies were conducted on *Azolla pinnata* growth performance, and this study’s findings could be helpful for the extension of the culture of Azolla worldwide. This study could boost the production of Azolla’s culture and thus benefit Bangladesh’s aquaculture feed sector.

Literature Cited

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