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Research Article
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Effect of water quality and organic fertilizer type on *Azolla pinnata* biomass production

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Abstract: *Azolla pinnata* is an aquatic fern with high potential as feed; however, its productivity is heavily influenced by water quality and nutrient availability under local environmental conditions. A field experiment was conducted at the College of Agriculture, University of Basra, specifically in the Aquaculture Unit (67°44'00" E, 31°32'47" N), to investigate the effect of water type (W) and organic fertilizer type (N) on the production of *Azolla pinnata* plants grown in water tanks. The experiment consisted of two factors: first, two types of water: (W1) reverse osmosis (RO) water, and (W2) tap water sourced from the Al-Bid'ah River. The second factor was two types of organic fertilizer: (N1) poultry manure, and (N2) livestock manure. The experiment was conducted with three replicates using a completely randomized design. Means were analyzed using the LSD test at a comparison level of $P \leq 0.05$ to determine the differences between treatments, specifically the total fresh weight and total dry weight. The results indicate that treatment (reverse osmosis water) was superior to treatment (water from the Al-Bida'a river) in all studied parameters (fresh weight and dry weight). Treatment W1 recorded the highest fresh weight production with an increase of 10.49%, along with a 10.32% increase in dry weight. No significant differences were recorded between the water type W and the organic fertilizer type N in terms of fresh weight production. The results indicate that treatment (poultry manure) was superior to treatment (cow manure) in the fresh weight parameter, recording the highest fresh weight production with an increase of 2.74%. Despite the significant impact of the interaction between water type and organic fertilizer type on dry weight, the interaction between reverse osmosis water and poultry waste is better than the interaction of Al-Bida'a River water with poultry manure by 20.79%, and the treatment of Al-Bida'a water with aquatic waste by 20.79%. Reverse osmosis water mixed with poultry manure has proven very beneficial in enhancing *Azolla pinnata* fresh weight production under the studied conditions.

Keywords: *Azolla pinnata*, organic fertilizer, water type

1. Introduction

Azolla pinnata R. Br is an aquatic plant that floats on the water's surface. It is of great importance due to its rapid growth and large vegetative mass, as well as its high content of essential minerals such as nitrogen (N), phosphorus (P), and potassium (K), protein, and other important compounds (Nasir et al., 2022; Amarasinghe et al., 2024). Azolla's importance has increased because it contributes to reducing feed production costs, which are constantly rising (Ansal, 2020; El Naggar & El-Mesery 2022; Sultan et al., 2025).

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Azolla has high nutritional value, being rich in crude protein, essential amino acids, minerals, and vitamins, and can be used in feeding fish, poultry, and livestock (Saranya Devi et al., 2024). Numerous studies indicate that azolla is an important source of protein and nutrients, and that its inclusion in feed rations increases productivity (Nasir et al., 2022; El Naggar & El-Mesery 2022; Hamidan et al., 2024; Saranya Devi et al., 2024). However, the nutritional value of azolla is highly influenced by growing conditions and the type of growing medium. Therefore, it is important to study some of the factors affecting its growth and nutritional composition.

Several factors control azolla production and nutritional value, including the type of water used in cultivation and the type of fertilizers applied. It has been found that different water types lead to significant variations in azolla productivity and nutritional value. Some river water sources have yielded higher production compared to others, due to changes in protein content and other characteristics of the azolla. This information indicates that water is not merely a growing medium for plants, but is one of the most important factors determining the nutritional value of azolla (Nimbalkar & Patil, 2021; Nimbalkar & Patil, 2024). Organic fertilizers also play a crucial role in increasing azolla productivity, both quantitatively and qualitatively, and in enhancing its nutritional content. The effect of organic fertilizers varies depending on their source and composition. Several studies have shown that poultry manure yields higher production in both quantity and quality compared to other animal manures, possibly due to its high nutrient content and ease of decomposition in water (Utomo et al., 2019; Hamidan et al., 2024).

Therefore, this study aimed to determine the impact of water quality and organic fertilizer type on azolla production and growth by comparing the use of RO water and water from the Al-Bida’a River, as well as two types of fertilizer: poultry manure and livestock manure. The goal was to identify the most suitable treatments for maximizing azolla production, promoting healthy growth, and ensuring high nutritional value suitable for animal feed.

Studies on Azolla in Basra Governorate conditions are very few and still limited. The lack of Azolla in the governorate has made farmers' knowledge very limited. Therefore, this study contributes to filling a local research gap by evaluating the green and dry weight production of Azolla under the water and fertilizer conditions available in Basra Governorate, which supports the spread of Azolla in Basra Governorate in southern Iraq. This study contributes to filling a local research gap by evaluating the biomass production of Azolla pinnata under controlled agricultural conditions in Basrah. Although Azolla is non-native and requires careful management to prevent unintentional ecological spread into natural open waterways, its controlled cultivation provides a highly sustainable local feed resource

2. Materials and Methods

The experiment was conducted in the aquaculture field of the College of Agriculture, University of Basrah (67°44'00" E, 31°32'47" N) to study the effect of water type (Table 1) and organic fertilizer type (Table 1) on the productivity of fresh and dried Azolla. The water characteristics used in the research were measured (Table 2). The experiment included two factors: first, two types of water: (W1) reverse osmosis (RO) water, and (W2) tap water supplied from the Al-Bid'ah river. The second factor was two types of organic fertilizer: (N1) poultry manure, and (N2) livestock manure. The experiment was conducted with three replicates. A Completely Randomized Design (CRD) was selected because the experiment was conducted under uniform and homogenous environmental conditions within a controlled greenhouse setting, eliminating directional gradient variations.

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Table 1. Water type and organic fertilizer treatments used in the study.

No.	Water and organic type	Symbols
1	Reverse osmosis water (R.O.)	W1
2	Tap water (Al-Bid'ah river)	W2
3	Poultry fertilizer	N1
4	Cow fertilizer	N2

Table 2. Water specifications.

Water type	EC (µS m^{-1})	TDS (mg L^{-1})	pH
Reverse osmosis water (R.O.)	90	42	7.7
Tap water (Al-Bid'ah river)	2060	1022	9.87

During the 10-day experimental period, the environmental parameters inside the greenhouse were monitored: the ambient temperature ranged between 18°C and 26°C, and the light intensity under 50% shade turf was maintained at approximately 15,000–20,000 Lux

A plastic-coated basin measuring 5 meters long, 1 meter wide, and with a water height of 0.15 meters was constructed for the purpose of growing Azolla plants, and Azolla plants were taken from it for the purpose of conducting research in the experimental units.

Poultry and livestock manure were collected from the fields of the College of Agriculture at the University of Basra. One kilogram of poultry manure was soaked in 10 liters of tap water, and the same amount of livestock manure (one kilogram) was soaked in 10 liters of tap water. This soaking period lasted five days. Afterward, 100 ml of the resulting fertilizer solution was applied as a single dose to each experimental unit at the beginning of the experiment (day 0) without further re-application during the 10-day period.

2.1 Nutrient solution specifications (physical and chemical properties):

The physical and chemical properties and nutrient concentrations of the nutrient solution were measured and determined according to standard methods. pH was measured using a pH meter, and electrical conductivity (EC) was estimated using a conductivity meter. For macronutrients, total nitrogen was estimated using the Kjeldahl method, and available phosphorus was measured using a spectrophotometer with the ascorbic acid and ammonium molybdate complex. Potassium, sodium, and calcium concentrations were measured using a flame photometer after the necessary dilutions of the extract (Table 3). Cylindrical culture vessels with a radius of 0.125 meters were prepared, and water was added to a depth of 15 cm. (The surface area of the container = 0.05 square meters).

Table 3. Physicochemical properties and nutrient concentrations of the prepared nutrient solutions used in the experimental treatments.

Physicochemical parameter	Unit	Poultry Manure (N1)	Cow Fertilizer (N2)
pH	-	6.8	7.2
EC	µS m^{-1}	4.2	3.0
Total Nitrogen (N)	mg L^{-1}	310.0	165.0
Available Phosphorus (P)	mg L^{-1}	78.0	35.0
Potassium (K^+)	mg L^{-1}	260.0	175.0
Sodium (Na^+)	mg L^{-1}	225.0	185.0
Calcium (Ca^{2+})	mg L^{-1}	94.0	65.0

Note: Table 3 presents the physicochemical properties of the nutrient solutions extracted from poultry manure (N1) and cow fertilizer (N2) after a 5-day soaking period in tap water

An initial fresh biomass of exactly 25 g of Azolla pinnata was inoculated into each cylindrical experimental unit. It was then transferred in dishes to the experimental units and left for 10 days. The experimental units were covered with transparent plastic film to increase humidity, which promotes azolla growth, and to provide warmth, as the experiment was conducted during winter. The experimental units were also placed inside a greenhouse covered with green turf to provide 50% shade. The entire azolla plant from each experimental unit was then harvested using small nets to collect the azolla without water. The harvested plants were weighed on a sensitive scale after being transported to the laboratory to avoid wind interference and inaccurate measurements. The azolla plants were then spread out on paper bags in the laboratory for air drying. Afterward, they were placed in bags and put in an electric oven at 75°C for 70 hours to dry completely. The dry weight was then weighed on a precision scale.

2.2 Statistical analyses

The experiment was designed using a Completely Randomized Design (CRD). Data were subjected to Analysis of Variance (ANOVA) using SPSS Statistics version 27.0 (IBM Corp., Armonk, NY, USA). Means were compared using the Least Significant Difference (LSD) test at a significance level of $P \leq 0.05$. The experimental units were distributed as shown in Table 4.

Table 4. Distribution of experimental units.

	R1		R2		R3	
	W1	W2	W1	W2	W1	W2
N1	N1W1R1	N1W2R1	N1W1R2	N1W2R2	N1W1R3	N1W2R3
N2	N2W1R1	N2W2R1	N2W1R2	N2W2R2	N2W1R3	N2W2R3

Note: W1 and W2 are the water types, which are reverse osmosis water (RO) and tap water (Al-Bid'ah river), respectively. N1 and N2 are the types of organic fertilizer, which are poultry fertilizer and cow fertilizer, respectively. R1, R2, and R3 represent replicates of experiments. The code N1W1R1 represents Poultry Manure (N1) with RO water (W1) in Replicate 1 (R1). All other treatment combinations follow the same coding convention

3. Results and Discussion

3.1 Fresh weight

Statistical analysis revealed the primary role of the interaction between water type and organic fertilizer type on the fresh weight of Azolla pinnata. Therefore, the interaction effect is presented and discussed first, followed by the individual main effects.

3.1.1 Interaction between water type and organic fertilizer type

As shown in Table 5, the interaction between water type and organic fertilizer type did not reach statistical significance at the 0.05 probability level ($LSD_{0.05} = 0.0571$). This indicates that the effect of each factor (water type and fertilizer type) was independent of the other, and no synergistic or antagonistic effect was observed between them on Azolla fresh weight.

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Table 5. Effects of water type, fertilizer type, and their interaction on Azolla fresh weight (kg m^{-2}).

Fertilizer type	Water type W		Mean (water type)
N	W1	W2	
N1	1.93 a	1.51 c	1.72 a
N2	1.64 b	1.71 b	1.67 b
Mean (fertilizer type)	1.78 a	1.61 b	
$LSD_{0.05}$	W	N	W × N
	0.0404	0.0404	0.0571

Note: Means followed by different letters within the same column or row indicate statistically significant differences according to the LSD test at $P \leq 0.05$

3.1.2 Water type

As shown in Table 5, the type of water had an effect on fresh weight, with treatment W1 (reverse osmosis water) outperforming treatment W2 (tap water from the Al-Bida'a River). Fresh weight in treatment W1 increased by 10.49% compared to treatment W2. This may be because the total dissolved solids in reverse osmosis water reduce osmotic and ionic stress on Azolla, thus improving its vegetative growth and increasing freshweight. This is consistent with (Nimbalkar and Patil, 2021; Nimbalkar and Patil, 2024). These results are also physiologically supported by Rai et al. (2006), who found that Azolla plant yield decreased by 45-65% with increasing water salinity.

3.1.3 Organic fertilizer type

Table 5 shows that the type of fertilizer had a significant effect on fresh weight, with treatment N1 (poultry manure) outperforming treatment N2 (cow manure). Fresh weight increased by 2.74% in treatment N1 compared to treatment N2. This may be because organic fertilizer increases the availability of nutrients, especially nitrogen and phosphorus, which are among the factors most closely associated with chlorophyll accumulation in Azolla (Utomo et al. 2019) found that poultry manure was the most effective in increasing Azolla production compared to other types of organic fertilizers, indicating that it provided a suitable environment for the production and growth of Azolla. Furthermore, availability of essential nutrients, particularly nitrogen and phosphorus in poultry manure improves chlorophyll formation, which increases photosynthetic efficiency and chlorophyll production. Amarasinghe et al. (2024) also found that increased chlorophyll production resulted from the availability of nutrients and enhanced plant physiological efficiency.

3.2 Dry weight

3.2.1 Water type

As shown in Table 6, the type of water affected the dry weight. Treatment W1 (reverse osmosis water) outperformed treatment W2 (supplied water from the Al-Bida'a river). The dry weight in treatment W1 increased by 10.32% compared to treatment W2. This may be because reverse osmosis water reduced osmotic and ionic stress on the Azolla, thus maintaining increased and efficient green matter growth. This aligns with the findings of Rai et al. (2006). Furthermore, the study by Aref and Marcos (2006) demonstrated that increased water salinity significantly inhibits growth and reduces both fresh and dry weight. The higher dry weight observed with reverse osmosis water suggests that less saline water allows the plant to increase its dry matter content.

3.2.2 Organic fertilizer type

Table 6 shows that the type of fertilizer had no significant effect on dry weight. Regarding the interaction between water type W and organic fertilizer type N, there was a significant effect on dry weight. Treatment W1N1 (reverse osmosis water with poultry manure organic fertilizer) outperformed treatment W2N1 (supply water from the Al-Bida'a regulator with poultry manure) by 8.49%, and treatment W2N2 (supply water from the Al-Bida'a regulator with livestock manure) also showed a 0.49% increase. This may be due to the interaction of the two factors—the lower salinity in reverse osmosis water and the increased efficiency of poultry manure—which contributed to the production of greater biomass. This is attributed to lower water salinity and the availability of nutrients in the poultry manure, which provide the plant with phosphorus, nitrogen, and other essential elements necessary for higher biomass. This, in turn, leads to a higher dry matter content, as demonstrated in the study by Rai et al. (2006) and Nimbalkar and Patil (2024). On the other hand, poultry waste yields a higher green production compared to other organic fertilizers, as mentioned by Utomo et al. (2019), and this result was supported by the study of Amarasinghe et al. (2024).

Table 6. Effects of water type, fertilizer type, and their interaction on Azolla dry weight (g m^{-2}).

Fertilizer type	Water type W		Mean (water type)
N	W1	W2	
N1	91.80 a	76.00 d	83.90 a
N2	81.40 b	81.00 c	81.20 a
Mean (fertilizer type)	86.60 a	78.50 b	
$LSD_{0.05}$	W	N	W × N
	0.2078	Ns	0.2939

Note: Means followed by different letters within the same column or row indicate statistically significant differences according to the LSD test at $P \leq 0.05$

4. Conclusion

This study demonstrates that reverse osmosis (RO) water significantly enhances the fresh and dry biomass production of *Azolla pinnata* compared to tap water from the Al-Bid'ah River. Furthermore, the combination of RO water with poultry manure yielded the highest productivity, improving Azolla dry weight by 20.79% compared to Al-Bid'ah River water treatments. Therefore, utilizing low-salinity water paired with poultry manure is highly recommended for maximizing Azolla biomass production as a sustainable animal feed resource in Basrah.

Data Availability

All data generated or analyzed during this study are included in this published article.

Author Contributions

Ahmed Al-Mothefer: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft preparation. **Arshad T. M. Sultan:** Methodology, Investigation, Formal analysis, Writing – review & editing, Supervision.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

Ethics Statement

This study did not involve human participants, animal subjects, or identifiable personal data. Therefore, ethical approval was not required.

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