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Growth Characteristics of *Azolla pinnata* under Light Limiting Conditions

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A B S T R A C T

Azolla (*Azolla pinnata*), a free-floating fern was cultured under tree canopy shading with an incident sunlight of 700 lux. The culture was treated with phosphorus, zinc and potassium for growth in treatment combinations. Though the specific growth rate and doubling time approximated to zero, biomass as yield was observed. The best biomass yield was observed with only phosphorus and zinc treatment. The work suggests that azolla grown under low illumination can be adapted for indoor cultures and can be used for composting and aesthetic purposes.

Introduction

Sustainable agriculture is a requirement that needs to be met by today's farming practices. This regenerative agricultural practice balances agricultural practices with environmental conservation with an aim to achieve targeted food production (Yang *et al.,* 2025). Amongst the available alternatives, Azolla (also called 'green gold'), a rapidly growing, free-floating fern in water bodies, helps not only to achieve agricultural sustainability but also climate resilience. This forms a symbiotic relationship with a cyanobacterium, *Anabaena azollae*, leading to fixation of atmospheric nitrogen. It acts not only as a biofertilizer, but also as a rich source of protein. Azolla is used also as a feed for livestock and fish. Additionally, it finds use in healthcare industry, water body purification, and carbon balancing (Matangi *et al.,* 2025; Marzouk *et al.,* 2023; Yang *et al.,* 2025; Korsa *et al.,* 2024).

Azolla also purifies water by absorbing heavy metals and pollutants, while serving as a high-protein livestock and aquaculture feedstock (25–33% crude protein), making it both a sustainable and economical supplement.

Beyond agriculture, this nutrient-dense resource has uses in industry and healthcare and has even been featured in space diets (Debnath, 2024; Yuan *et al.,* 2026; Prabakaran *et al.,* 2022; Yohana *et al.,* 2023; Matangi *et al.,* 2025). Thus, in low-income regions, azolla can enhance productivity, improve soil health, and help in sustainable farming.

For *Azolla* cultivation, a water temperature of 18^0 C to 28^0 C, a pH value of 5.5 to 6.5, and partial shade are essential. Optimal light intensity required is approximately 20,000 lux. Azolla prefers partial shade of 25 to 50 % for growth. Proper humidity (80-90%) and shallow water depth are also vital for rapid growth. A water depth of approximately 10 cm is essential for culturing, as well as phosphorus provides a faster growth. There are many elements required to culture azolla. However, of the nutrients, phosphorus is essential because it plays a role in symbiosis and impacts growth and is considered a vital nutrient for azolla culture (The Azolla Foundation). Deficiency of phosphorus leads to loss of anthocyanin pigments, and azolla changes color to red or purplish brown. Potassium is the other element that plays a role in osmo-regulation and impacts on azolla growth. Zinc as a micro-element and required in trace amount effects on impacting on growth and acts as a cofactor for several enzymes and helps to synthesize auxin (Gamachis *et al.,* 2024; The Azolla Foundation).

The present work was done to study Azolla growth under low sunlight, or light-limiting conditions. This study was undertaken to study the growth performance and biomass production of Azolla with different combinations of phosphorus, zinc, and potassium nutrient sources. This observational study is helpful to study Azolla growth under a tree canopy covered area, with low sunlight received at the ground soil.

Materials and Methods

Culture vessel, location and added inputs: *Azolla pinnata* was cultured in cement made semi-spherical shaped vessel with vessel area of 1 cubic square metre. Inputs added were: cowdung (100 gram per square metre area), soil (10 kg per square metre, azolla (100 gram per square metre area), Phosphorus as Single super phosphate (5 gram per square metre area). The experiment was carried out at the premises of School of Agriculture and Allied Sciences (latitude: 22.261679 and longitude: 88.19603), The Neotia University. Temperature inside green net measured using android application from Google play store was 38^0 C. The period chosen for the experiment was mid-July to mid October, 2024. Observations were recorded at every week interval, and a total of 13 weeks observation were recorded. The different nutrient treatment combinations used were: T_0/Experimental Control ($P_0K_0Zn_0$), T1 ($P_0K_0Zn_1$), T2 ($P_0K_0Zn_1$), T3 ($P_0K_1Zn_0$), T4 ($P_0K_1Zn_1$), T5 ($P_1K_1Zn_0$), T6 ($P_1K_1Zn_1$), T7 ($P_1K_0Zn_0$), T8 ($P_1K_0Zn_1$). Significance of '1' is presence/addition of a nutrient element, and '0 is absence/no addition of that element. The study being an observational study was not extended with replications apart from resource constraints.

Nutrient treatments: Nutrient combinations were applied as zinc sulphate for zinc, SSP for P, muriate of potash for potassium, each at a concentration of $5g/m^2$. Eight treatments were set up with an experimental control. The amount of zinc present in zinc sulphate is 21% (as zinc sulphate monohydrate); P as P_2O_5 in SSP is 16%; K as K_2O in MOP (muriate of potash) is 60%.

Light intensity and growth conditions: Light intensity was measured using a portable luxmeter of Sigma Instruments (Sigma Digital Luxmeter 101). Azolla was grown under tree shade with a light intensity of 700 lux (average reading across the study period) inside a green net chamber.

Measurement of specific/relative growth rate (RGR) and doubling time (DT)

i) RGR was measured using the equation: $RGR = ln(W_t - W_0)/t$, wherein

W_0 is initial weight, or biomass at start time t_0 or t_1,

W_t is biomass weight at the end of nth period of culture period, t_n

Culture period duration = $t_n - t_0 = t$ =total time taken for culture growth, and

Natural logarithm is *ln* (base *e* logarithm)

ii) Doubling Time (DT) was measured using the equation:

$$DT = ln(2)/RGR = 0.693/RGR$$

pH measurement: Culture vessel water pH was measured in the laboratory using a pH meter (Systonic Auto digital pH meter, Model: S-901).

Electrical Conductivity (EC): Culture vessel water EC was measured using Labtronics Deluxe conductivity meter, Model: LT-26.

Biomass yield: Fresh biomass yield was measured using portable electronic balance (Electronic compact scale balance SF-400C).

Root length measurement: Azolla root length was measured using a scale of 0-30 cm range. Root lengths were of an average 1 cm.

Statistical analysis: The statistical analysis was performed using Microsoft Excel, and no artificial intelligence tools were used.

Results and Discussion

Biomass measurement: Biomass measured has been shown in Table 1. The azolla culture showed a sigmoid growth curve (Figure 2). Average and Standard deviation values of the biomass data from Table 1 were considered to assess the mean values of actual/observed specific growth rate and doubling time (Table 2) of the azolla culture used in the experiment. Though specific growth rate and doubling time values approximated to zero, biomass yield was observed (Table 2 and Figure 3). However, the biomass yield was highest for growth with phosphorus and zinc, followed by growth with phosphorus only. The average biomass value recorded was 578 grams with a standard deviation of 168.83; the average specific growth rate (actual/observed specific growth rate was 0.004 with a standard deviation of 0.01, and the average doubling time observed was 0.288 with a standard deviation of 0.75.

pH measurement: Azolla was cultured at a water pH of 6.8. Azolla was cultured in vessels under tree shade with green net enclosure (Figure 1).

EC measurement: Water EC for culture was observed as 0.38.

Root length measurement: Azolla root lengths were an average of 1 centimetre long (Figure 4).

Table.1 Observation of biomass values week wise

T	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12	Week 13	Average
T1	266	229	259	247	275	424	429	484	523	481	435	422	401	375
T2	364	266	286	283	333	466	502	550	819	903	527	582	530	493.2
T3	350	256	281	286	368	464	424	494	543	521	444	457	430	409.1
T4	384	273	329	509	415	531	530	589	584	714	463	477	472	482.3
T5	389	278	337	328	480	839	826	849	947	940	855	601	429	622.9
T6	378	318	406	349	501	862	873	973	1114	891	496	429	752	641.7
T7	393	343	470	396	599	1036	933	1012	1009	1018	836	502	879	725.1
T8	380	356	503	568	610	1040	1426	1437	1680	1161	1039	592	529	870.8

Note: T=Treatments, Biomass values were recorded in grams every week.

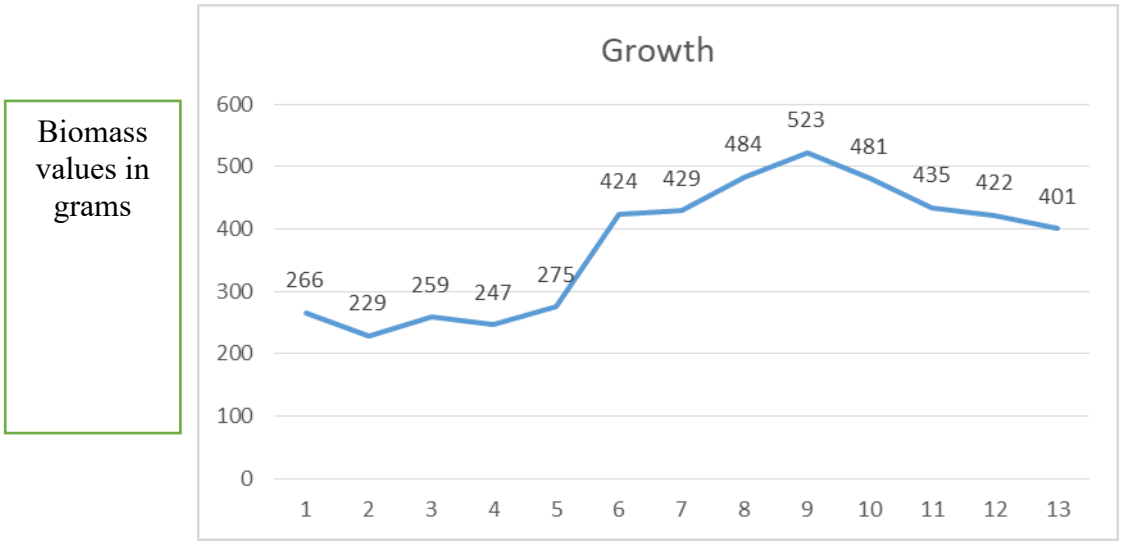
Table.2 Assessment of specific growth rate (RGR) and doubling time (DT)

	Treatments	Average biomass yield (grams)	Average RGR	Average DT
T1: P0K0Zn0	T1	375	0.008333	0.5775
T2:P0K0Zn1	T2	493.2	0	0
T3P0K1Zn0	T3	409.1	-0.00833	-0.5775
T4: P0K1Zn1	T4	482.3	-0.00833	-0.5775
T5: P1K1Zn0	T5	622.9	0	0
T6: P1K1Zn1	T6	641.7	0.008333	0.5775
T7: P1K0Zn0	T7	725.1	0.025	1.7325
T8: P1K0Zn1	T8	870.8	0.008333	0.5775
AVERAGE		578	0.004167	0.28875
Standard Deviation		168.8312	0.010911	0.756125

Figure.1 Culture of Azolla in vessels under green net area

Azolla was cultured at the premises of School of Agriculture and Allied Sciences, The Neotia University.

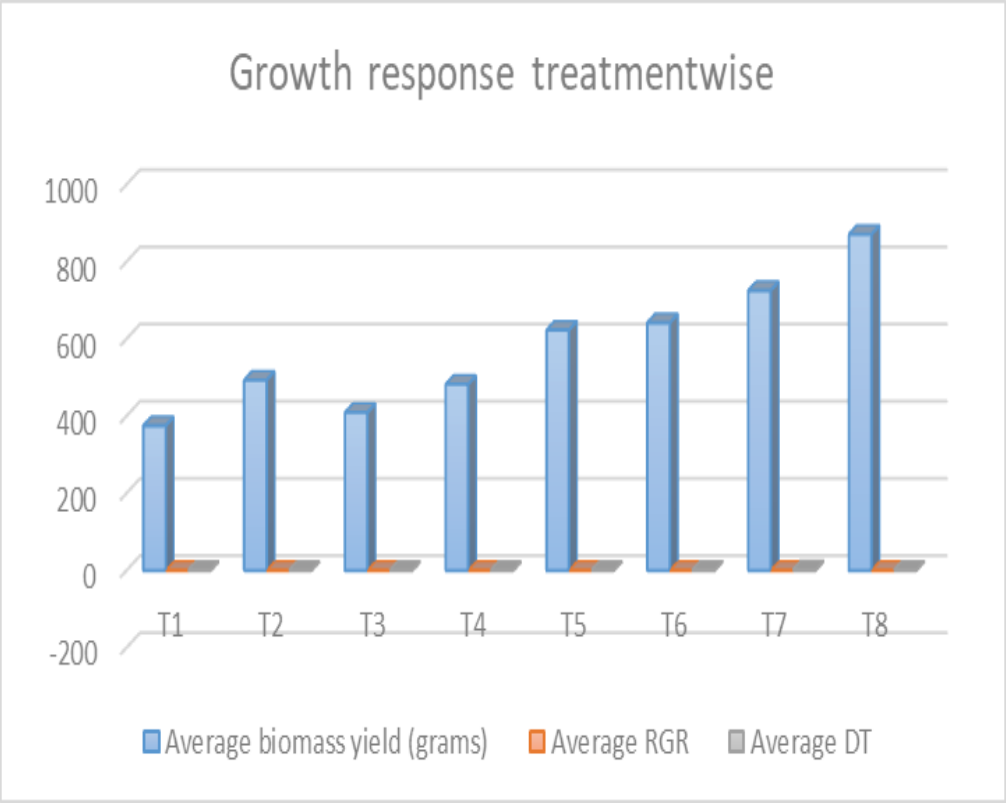
Figure.2 Growth curve of *Azolla pinnata*. Y-axis recorded biomass values in grams, X-axis: week-wise distribution of the biomass values



Week wise distribution of the biomass values

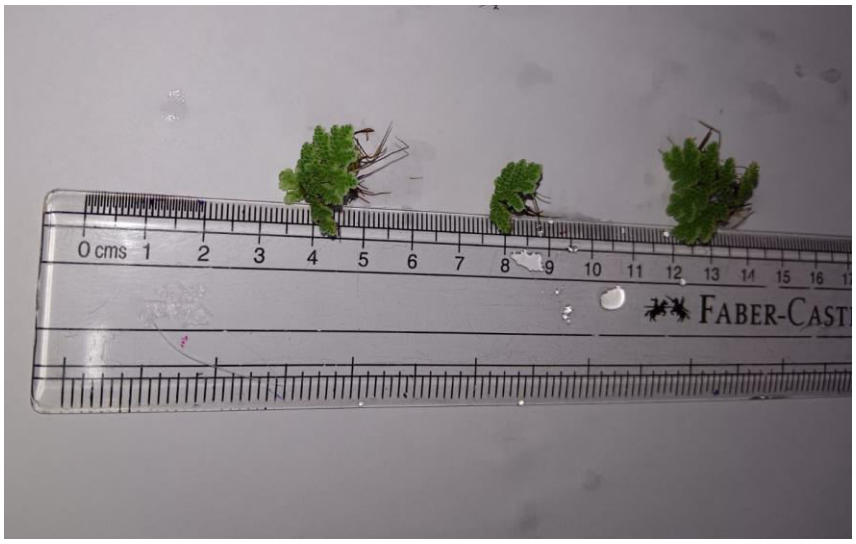
The curve depicts biomass (by weight) changes with the study period, and shows a lag, log, stationary and decline phases of growth.

Figure.3 Growth response treatment-wise.



The graph observed treatment wise growth responses

Figure.4 Measurement of root length



Average root length of Azolla pinnata in the study was observed.

The study being an observational was with the limitations of adequate and required daylight to grow azolla (15,000 to 20,000 lux), variations of daylight intensity, replications of the treatment, and observing pH and EC changes throughout the growth period.

The study was also done under tree canopy shaded area. However, pH and EC used to grow the culture matched the standard requirements for azolla cultivation (Tran, 2015). Duration taken for cultures to grow for a long time is due to non-provision of required sunlight to grow azolla. Under optimal conditions, *Azolla pinnata* achieves a specific growth rate (relative growth rate) of about 0.321 per day, resulting in a doubling time of approximately 2.16 days (da Silva *et al.,* 2022).

Additionally, with a drop in light intensity less than 1,500 lux, growth of this fern significantly reduces. Such light-limiting conditions induces stress which triggers profound metabolic reprogramming to survive, and with continued such situation thresholds can be crossed resulting in metabolic dys-functioning (da Silva *et al.,* 2022; Thepsilvisut *et al.,* 2024).

The present limitations have compelled the culture to observe actual specific growth rate equalling to the maximum specific growth rate leading to a zero kinetics of growth. This has led to the achievement of the biological threshold, and reduced accumulation of photosynthates. However, achievement of biomass yield concludes that with regard to the amount of daylight provided to grow the culture, adaptation can be done for indoor and within human habitat room, or office cultivation of azolla, as well as growth amidst tree canopy covers apart from composting and aesthetic purposes.

Authors Contribution

The author confirms contribution to the paper as follows: study conception and design: SB; data collection: SB; analysis and interpretation of results: SB; draft manuscript: SB; reviewing the results and approval of the final version of the manuscript: SB.

Availability of Data and Material

All data was generated independently by the author without any external commercial assistance. The dataset supporting this study is available from the corresponding author only upon justified request.

Conflict of Interest

The author declares no conflict of interest, financial or otherwise.

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AI tool usage: No AI tool has been used in the preparation, analysis, or review of the manuscript.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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