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BIOMASS GROWTH AND COMPOSITION OF AZOLLA (*Azolla pinnata* R. BR.) SUPPLEMENTED WITH INORGANIC PHOSPHORUS IN OUTDOOR CULTURE

M.A. Hossain[1*], S.A. Shimu[1], M.S.A. Sarker[3]
M.E. Ahsan[2] and M.R. Banu[2]

[1] Department of Aquaculture, [2]Department of Fisheries Management
Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh
[3]School of Agriculture and Rural Development, Bangladesh Open University
Gazipur, Bangladesh.

ABSTRACT

An experiment was conducted to know the effect of supplemental phosphorus on biomass growth and composition of a floating aquatic fern, *Azolla pinnata,* cultured in a pit system for 21 days with 4 levels of phosphorus (0, 5, 10, and 15 ppm) supplementation. Water quality parameters of the pits were within a suitable range for *A. pinnata* culture. It was observed that fresh and dry weights of *A. pinnata* increased with phosphorus supplementation up to 10 ppm. Doubling time was the fastest when the culture medium was supplemented with 10 ppm of phosphorus. Phosphorus content of *A. pinnata* was proportional to the phosphorus supplementation in the culture medium. Supplementation of phosphorus also improved the protein and lipid contents of *A. pinnata*. It was concluded that supplementation of 10 ppm phosphorus to water used for culturing *A. pinnata* is optimum under outdoor conditions.

Keywords*:* Azolla, Nutrient, Growth, Phosphorus

INTRODUCTION

Azolla is a free-floating aquatic fern, which is a member of the family Salviniaceae. It is a dichotomously branched plant and naturally available on moist soils, ditches, and marshy ponds. This fern grows extensively in association with nitrogen-fixing bacteria *(Anabaena azollae),* which allows it to thrive on waters low in nitrogen but containing phosphorus. *Azolla* grows on floating water surfaces in the temperate and subtropical regions (Katole et al., 2017). It can reproduce sexually, by forming spores. However, it mainly reproduces vegetatively by breaking off side branches at a rapid rate. Under ideal conditions, it grows exponentially, doubling its biomass every 2 to 5 days (De et al., 2015; Kathirvelan et al., 2015).

[*] Corresponding author: amzad@bsmrau.edu.bd

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Azolla is a good source of protein. It contains almost all essential amino acids, minerals such as iron, calcium, magnesium, potassium, phosphorus, manganese, etc., apart from appreciable quantities of vitamin A's precursor, beta-carotene, and vitamin B_{12}. It is also found to contain probiotics and biopolymers (Bhaskaran and Kanappan, 2015). Thus, *Azolla* appears to be a potential source of nutrients and has a considerably high feeding value (Anitha et al., 2016).

Azolla is used as a feed or feed supplement for a variety of animals, including broiler chicken (Balaji et al., 2009), laying hens (Alalade et al., 2006), black tiger Shrimp (Sudaryono, 2006), tilapia (Das et al., 2018; Hundare et al., 2018) and buffalo calves (Indira et al., 2009). *Azolla* is also used in diets for sows and for partial replacement of protein for growing or fattening pigs (Leterme et al., 2010). Due to easy cultivation and high biomass yield, *Azolla* can be an ideal feed substitute for animals. Apart from animal feed, *Azolla* is also widely used as a bio-fertilizer for paddy cultivation. It is a mosquito repellent and bio-scavenger as it takes away all heavy metals from water (Bhuvaneshwari, 2012; Bhuvaneshwari and Singh, 2015).

Phosphorus (in the form of phosphate) is the primary limiting nutrients for *Azolla* growth and yield. The importance of phosphorus on *Azolla* growth has been confirmed in the Anzali wetland (Sadeghi et al., 2013). In laboratory experiments, Janes (1998) found that increasing phosphorus supply led to increased sporulation in Azolla. There have been few reports about the requirement of phosphorus for sustained *Azolla* spp. growth (Herzalla et al., 2003; Cheng et al., 2010). Most of those studies have been conducted with pure nutrients in laboratory conditions. However, scientific information on phosphorus's effect on *Azolla* culture in the outdoor conditions is limited. Therefore, the present study was undertaken to know the impact of supplemental phosphorus on biomass growth and composition of *A. pinnata*.

MATERIALS AND METHODS

The experiment was conducted during September and October, 2018 in the Faculty of Fisheries, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh. Twelve outdoor pits, each having a dimension of 1.5 m ×1.5 m × 0.3 m, were made for *A. pinnata* culture. Silpaulin sheets of 2.0 m × 2.0 m were spread out over each of the pits. 15 kg of sieved fertile soil (pH 6.2, organic matter 2.85%, available phosphate 0.11 P_2O_5/100 g dry soil) was uniformly spread over the sheet. Two kg of cow dung mixed with 10 liters of water was poured over it. Finally, the water depth of the pits was raised to 20 cm with underground water.

The experiment was laid out in a complete randomized design (CRD) with 3 replications. The P was added through triple superphosphate (TSP) at 4 levels, 0, 5, 10, and 15 ppm and designated as P0, P5, P10, and P15, respectively. The required amount of TSP was measured, dissolved in an aliquot of water and mixed well with the respective pit's water. *A. pinnata* inoculum, used in this experiment, was collected from the rice fields of Bangladesh Rice Research Institute (BRRI), Gazipur,

Bangladesh. The inoculum was starved by keeping it in demineralized water for 7 days before inoculating into the culture pit. Water was periodically added to maintain 20 cm water depth. *A. pinnata* was harvested after 21 days of inoculation.

The water temperature (°C) was recorded using a thermometer (9337U20, Thomas) and pH by a digital pH meter (HQ11D, HACH) at the spot. Ammonia (mg/l) determination of water sampled in leveled 250 ml black plastic bottles were done in the laboratory with a spectrometer (DR 6000, Hach Co., Colorado, USA).

In order to monitor growth of *A. pinnata*, 100 plants were collected on every third day, blotted and weighed, and the average weight was calculated. At the end of 21 days experimental period, fully grown *A. pinnata* was harvested from the pits, washed, blot-dried gently and weighed for calculating fresh weight, relative growth and doubling time. The harvested *A. pinnata* sample from each pit was dried in an oven at 105° C for about 24 hours until constant weight, and the dry value was calculated. The dried sample was put into a polythene bag and kept into the refrigerator until further analysis. Dry matter, crude protein, crude lipid, and crude ash contents were determined, following the methods described by AOAC (2007). For P determination, the oven-dried sample was digested with a nitric acid-perchloric acid mixture, and P in the digested sample was determined following the method followed by Hossain and Furuichi (2000).

Growth was measured in terms of total fresh weight (kg), dry weight (kg), relative growth rate (RGR), and doubling time (DT). In order to determine the RGR and DT, the formulae $RGR= (\log W_t - \log W_0) /t$ and $DT = t \times \log 2 [\log (Wt\ Wo^{-1})]^{-1}$ were used, respectively, where DT is the doubling time (days), RGR is the relative growth rate expressed as g/g per day, t the experiment duration (days), Wt the final weight, and Wo the initial weight.

Data were analyzed with one-way analysis of variance (ANOVA) to determine whether there was any significant difference among treatments mean, while LSD test was used to compare the treatment means (Hofmann, 2008).

RESULTS AND DISCUSSION

There was no variation in water quality parameters during the culture of *A. pinnata* with different levels of P. Temperature, pH, and ammonia varied from 24.3-25.7 °C, 6.85-7.46, and 0.02-0.32 mg/l, respectively in all the P levels and did not differ significantly as a result of P supplementation. These three parameters were within suitable range for growth of *A. pinnata*. It grows relatively well in a temperature range of 20-30°C (Cheng et al., 2010; De et al., 2015) and pH 5-8 (Sadeghi et al., 2013). Maejima et al. (2001) reported that the lower levels of total ammonia in water are better for *Azolla*.

The dry weight of *A. pinnata* (mg/plant) is shown in Table 1. The weight did not vary much with P supplementation up to the 6^{th} day as compared to the control but was

significantly more later. The maximum weight (4.65 mg/plant) was observed on 21st day in P10. Sadeghi et al. (2013) observed that deficiency of P affects the growth of *A pinnata*.

Table 1. Growth of *Azolla pinnata* cultured under different P supplementation (dry weight mg/plant)

Days	Dry weight mg/plant*			
	P0	P5	P10	P15
0	3.51 ± 0.15	3.51 ± 0.28	3.51 ± 0.28	3.51 ± 0.28
3	3.74 ± 0.25	3.69 ± 0.28	3.64 ± 0.33	3.64 ± 0.29
6	3.68 ± 0.42	3.69 ± 0.46	3.72 ± 0.44	3.65 ± 0.41
9	3.81 ± 0.34^{b}	3.95 ± 0.39^{a}	3.97 ± 0.33^{a}	3.93 ± 0.39^{a}
12	3.85 ± 0.43^{b}	4.11 ± 0.35^{a}	4.15 ± 0.22^{a}	4.14 ± 0.37^{a}
15	3.76 ± 0.21^{b}	4.21 ± 0.26^{a}	4.20 ± 0.35^{a}	4.12 ± 0.12^{a}
18	3.62 ± 0.22^{b}	4.37 ± 0.31^{a}	4.55 ± 0.42^{a}	4.14 ± 0.13^{a}
21	3.42 ± 0.21^{c}	4.25 ± 0.292^{b}	4.65 ± 0.32^{a}	4.44 ± 0.29^{c}

*Mean ± SD. Data in the same row bearing different letters are significantly different ($p<0.05$).

The doubling time was calculated, which was the minimum (3.71 days) when the culture medium was supplied with 10 ppm P (Table 2). However, further increase in P supplementation level could not decrease the DT. DT was the highest (4.08 days) without P supplementation to the water. Fresh weight, RGR and dry weight of *A. pinnata* were enhanced by the supplementation of P and were maximum (5.01 kg/m^2, 0.82 g/g per day and 0.25 kg/m^2, respectively) with 10 ppm P. De et al. (2015) observed that under P depletion, *A. pinnata* had recorded a significant restriction of growth by dry matter loss. Temmink et al. (2018) in a laboratory experiment observed significant increase in RGR in *A. filiculoides*, when culture medium was supplied with 0.3 ppm P and further increase in P up to 10 ppm could not improve the RGR. Gerek (2001) reported that 1122 g/m^2 of fresh *Azolla mexicana* can be harvested after15 days with the initial fresh weight of 300 g/m^2 of Azolla. The final fresh weight at harvest was more in the present study due to the difference in *Azolla* species and relatively longer culture period (21 days).

Table 2. Effect of P supplementation on different growth parameters of *A. pinnata*

P in medium (mg/L)	Doubling time	RGR (g/g)	Fresh weight (kg/m^2)	Dry weight (kg/m^2)
P0	8.39 ± 0.12^{a}	0.36 ± 0.02^{c}	3.90 ± 0.21^{c}	0.27 ± 0.05^{b}
P5	5.47 ± 0.21^{b}	0.42 ± 0.06^{b}	4.51 ± 0.18^{b}	0.32 ± 0.03^{b}
P10	3.74 ± 0.31^{c}	0.54 ± 0.05^{a}	5.92 ± 0.17^{a}	0.41 ± 0.07^{a}
P15	3.83 ± 0.18^{c}	0.43 ± 0.04^{b}	4.60 ± 0.15^{a}	0.39 ± 0.05^{a}

*Mean ± SD. Data in the same row bearing different letters are significantly different ($P<0.05$).

There have been variations in the levels of phosphorus reported by researchers for sustained growth of *Azolla*. In laboratory experiments, a concentration of around 0.06 ppm was reported to be adequate to sustain *Azolla* growth. However, a range between 0.3 and 10 ppm was suggested from field surveys (Cheng et al., 2010). The response of *Azolla* to different concentrations of phosphorus for optimum growth varies with the species (Herzalla et al., 2003). Field surveys of *Azolla* in the Philippines showed that P concentrations of *A. microphylla* were higher than those of *A. pinnata* var. *imbricata*, and the available P contents of the soils where this species was growing were higher (Sadeghi et al., 2013). In a pot experiment, Cheng et al. (2010) observed that maximum biomass occurred when *A. pinnata* received 1 ppm of N and 5 ppm of P, whereas *A. filiculoides* required 10 ppm N and up to 20 ppm P.

Supplementation of P influenced the dry matter, protein and lipid contents of *A. pinnata* (Table 3).

Dry matter (4.65%), protein (28.52%) and lipid (4.15%) were the highest with 10 ppm P at the end of 21 days experimental period. Dry matter was the lowest in control group. De et al. (2015) reported that the limitation of P resulted in a significantly lower dry matter and protein contents of *A. pinnata*.

Table 3. Proximate composition of *A. pinnata* cultured in water with different levels of supplemental P.

Parameters	Supplemental P level			
	P0	P5	P10	P15
Dry matter (%)	4.05 ± 0.48^{a}	4.90 ± 0.29^{b}	4.65 ± 0.69^{b}	4.85 ± 0.28^{ab}
Protein (% dm)	26.84 ± 0.70^{ab}	26.02 ± 0.63^{b}	28.52 ± 0.51^{a}	27.23 ± 0.19^{b}
Lipid (% dm)	5.55 ± 0.70^{b}	$4.15 \pm 0.58b^{c}$	7.77 ± 0.71^{a}	2.65 ± 0.13^{c}
Ash (% dm)	18.37 ± 0.10^{b}	17.37 ± 0.82^{b}	21.36 ± 0.99^{a}	14.65 ± 0.21^{c}

*dm= dry matter basis. *Mean ± SD. Data in the same row bearing different letters are significantly different ($P<0.05$).

Oyange et al. (2019) attributed variations in the nutrient composition of *Azolla* to differences in the response of *Azolla* strains to environmental conditions such as temperature, light intensity and soil nutrient which consequently affect their growth, morphology and composition. Cheng et al. (2010) concluded that the N accumulation potential of *A. filiculoides* under future climate warming depends primarily on the temperature change and P availability. The average crude protein content of *A. pinnata* estimated in the present study matched with the previous findings. The P supplementation also affected tissue P content of *A. pinnata* (Figure 1) and it was maximum in P15 treatment. Tissue P content in *Azolla* was positively correlated with the concentration of P in water used for culturing (Oyange et al., 2019).

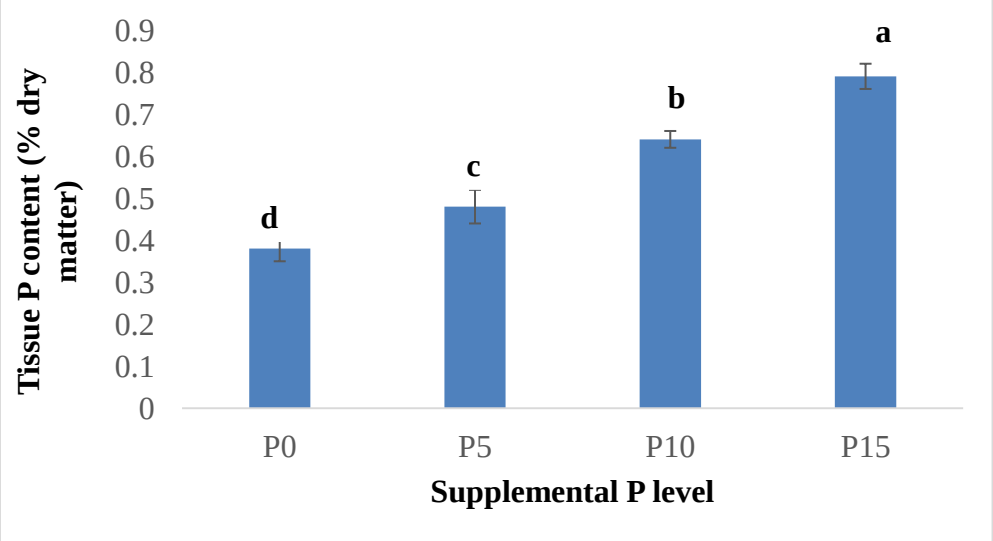

Figure 1. Effect of P supplementation in culture water on the tissue P content of *A. pinnata* (Different letters indicate significant difference). The different alphabets on each bar show level of significance ($P<0.05$).

CONCLUSION

Among the many inorganic nutrients required by plants, P is one of the most important elements that significantly affect plant growth and metabolism. In the present study, P supplementation @10 ppm to the water enhanced the growth, decreased the doubling time and increased nutrient content of *A. pinnata* suggesting that it is the optimum dose for the outdoor cultivation of *A. pinnata* in the ponds.

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