Effect of different growing nutrient solutions on *Azolla pinnata* productivity under Egyptian conditions

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Abstract

A field experiment was conducted from 24 June to 31 December, 2023 in a private farm (30° 22' 01.0" N and 31° 36' 26.1" E), Egypt, to test the effects of two nutrient solutions compared with farmer practice on *Azolla pinnata* fresh biomass, tissue-chemical constituents, tissue-NPK concentrations and the amount of water applied and its water productivity. Results showed that average fresh yields were 54.9, 44.1, and 40.9 t/ha/month respectively for nutrient solution A, solution B and farmer practice. Average *Azolla pinnata* fresh yield during summer season was higher than that recorded during autumn season. In Azolla's tissues, average Total Carbohydrates (TC) values were 32.3, 31.4, and 32.7%, average Total Fiber (TF) contents were 15.9, 15.7, and 15.8%, average AA values were 14.0, 12.6, and 13.1%, and average Crude Protein (CP) values were 14.0, 12.6, and 13.1% for solution A, solution B and farmer practice treatments, respectively. Average tissue-N values were 2.41, 2.21, and 2.14%, tissue-P values were 0.54, 0.40, and 0.40%, and average tissue-K values were 1.22, 1.21, and 1.25% for the same respective treatments. Total amounts of applied water during the growing period was 4071 m^3/ha and average water productivity values were 96.4, 80.4, and 73.6 kg fresh yield/m^3 for the three respective treatments. It could be concluded that, the multiple contents of growing nutrient solution significantly increase *Azolla pinnata* biomass. It also increased the chemical constituents of the plant, tissue-NPK, and water productivity. Ponded water contains macro- and micro-nutrients and can be used to irrigate other crops in the farm.

Keywords: *Azolla pinnata*, biomass, chemical constituents, tissue-NPK, Applied water, water productivity

INTRODUCTION

Azolla is a genus of aquatic ferns and small-leafed floating plants, native to the tropics, subtropics and warm temperate regions of Africa, Asia and the Americas. It is a common free-floating fern up to 10 to 30 millimeters in diameter with roots hanging down to about 40 millimeters below the water surface. Azolla ferns float on the surface of the water individually or as large mats (Mosha, 2018). Azolla species are used as animal feed, human food and medicine, biofertilizer, water purifier, green manure, hydrogen fuel, biogas producer, weed and insect controller, and reduces ammonia volatilization after chemical nitrogen application. It improves the water quality by removing excess quantity of nitrates and phosphorus. Also, Azolla has many advantages and is known as a source of many essential bioactive compounds and nutrients (Ray *et al.*, 1979; Wagner, 1997; Pabby *et al.*, 2003; Chris *et al.*, 2011; Sadeghi *et al.*, 2013; Selvaraj *et al.*, 2014; Rashad, 2021).

Few research reported the water consumption by Azolla plants through the growing season. The experiments by Amro (2022) showed that the monthly water consumption rate by the Azolla plant is approximately equal to 120 m^3/dunum (1200 m^3/ha/month).

Azolla plants support nitrogen fixing bacterium, which allows it to use nitrogen from the water and air for its own growth. It can fix atmospheric nitrogen due to the presence of blue-green algae (*Anabaena azollae*) located in cavities of the ferns' lobes (Adzman *et al.*, 2022). It is a good source of protein and contains almost all essential amino acids and minerals like iron, calcium, magnesium, potassium, phosphorus and manganese (Brouwer *et al.*, 2018; Patil and Patil, 2020).

Under optimum growth conditions, including water depth, nutrient concentration, pH, relative humidity, air and water temperatures, and sunlight exposure, Azolla spreads across water surface until it covers the whole surface of the water in a dense cover. Azolla can double its leaf area in seven days if conditions of high nutrient levels and water temperatures persist (Adzman *et al.*, 2022).Results by Chatterjee *et al.* (2013) showed that, nutrient composition of Azolla species varied depending on the environmental conditions, including temperature, light intensity, and soil nutrients. These factors would therefore have an impact on growth morphology and its nutrient composition.

Watanabe *et al.* (1977) conducted laboratory studies which showed that Azolla, grown in a nitrogen free solution, can double its mass in 3-5 days and can accumulate 30-40 kg N ha^{-1} in two weeks. Results by Kannaiyan *et al.* (1981) and Kannaiyan (1982) indicated that P, K, Ca, Mg, Fe, Mo, Co, and Zn have been shown to be essential for Azolla growth and N-fixation. The main macronutrients and other essential nutrients that are necessary for optimizing Azolla growth and N fixation are P, K, Ca, Mg, Fe, Mo, Co, and Zn (Carithers *et al.*, 1979; O'Hara, 2001; Kannaiyan, 1982). Previous studies showed that, Ca and P deficiencies had a considerable effect on Azolla growth and N fixation compared to K

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and Mg deficiencies (Watanabe *et al*., 1977; Subudhi and Singh, 1978; Kannaiyan *et al*., 1981). As the P level drops in the growth medium, it will affect growth rate and N fixation.

Also, Azolla growth was reduced in low concentrations of Fe, Ca, or P. Nordiah *et al*. (2012) stated that, Azolla expands its population depending on the availabilities and contents of nutrients in the growing media, while water without phosphate showed low Azolla growth. Hossain *et al*. (2021) concluded that, phosphorus content of *Azolla pinnata* was proportional to the phosphorus supplementation in the culture medium. The supplementation of 10 ppm phosphorus to water used for culturing *Azolla pinnata* is optimum. It also improved the protein and lipid contents of *Azolla pinnata* under outdoor conditions.

Results by Adzman *et al*. (2022) indicated that Azolla growth was the best in water depth of 20 cm, the nutrient concentration of 812.5 ppm, pH of 7 and under 100% sunlight exposure. It can survive within a water pH range of 3.5 to 10, but optimum growth occurs in the pH range of 4.5 to 7 and temperature range of 18°C to 26°C.

The tested hypothesis here is that, the combined solutions of essential nutrients improve Azolla's yield and increase nutrient concentrations in the Azolla plant tissue. Therefore, the overall objectives of the implemented field experiment were to:

- Test the effects of different nutrient solutions on the productivity of *Azolla pinnata* under field conditions;
- Test the effects of nutrient solutions in the Azolla growing medium on total carbohydrates, total fiber, total amino acids, and crude protein as well as on NPK nutrient concentrations in the Azolla plant tissue.
- Determine the amount of water required to grow *Azolla pinnata* since very few data were reported on water used by Azolla plants and on its water productivity.

MATERIALS AND METHODS

Experimental site

A field experiment on the response of *Azolla pinnata* to different nutrients fertilization was carried out for 190 days (24 Jun. – 31 Dec., 2023) in a private farm (30° 22' 01.0" N and 31° 36' 26.1" E), Sharqia governorate, Egypt (Figure 1). Based on Köppen–Geiger classification, the climate of the site is arid desert-hot (BWh) of the Mediterranean type, with most of the rainfall occurring in the winter season (Beck *et al*., 2018). The main daily weather data (https://power.larc.nasa.gov/data-access-viewer/), including maximum and minimum air temperature (°C), relative humidity (%), wind speed (m/s) and rainfall (mm), characterizing the experimental site is illustrated in Figure 2. Annual rainfall at the experimental farm from January to December 2023 was 96.1 mm, while it was 25.7 mm during the growing period (24 June to 31 December, 2023). Average maximum temperature ranged from 41.6 °C (Jul.) to 24.1 °C (Dec.), while average minimum temperature varied from 23.4 °C (Aug.) to 13.4 °C (Dec.). The mean relative humidity values varied from 43.2% (Jul.) and 67.2% (Dec.). The obtained weather data were used to calculate reference evapotranspiration (ETo) values by applying the FAO-56 Penman-Monteith equation (Allen *et al*., 1998) in the FAO-CROPWAT 8 model.

Effective rainfall

The effective rainfall during the growing period was calculated on daily basis according the following relation (Dastane, 1974):

$$\begin{aligned} Re &= 0 && when \quad P < 6.25\ mm \\ Re &= P && when \quad 6.25 < P \leq 75\ mm \\ Re &= P - (P - 75) && when \quad P > 75\ mm \end{aligned}$$

Where:

Re: effective rainfall (mm), P: depth of rainfall (mm)

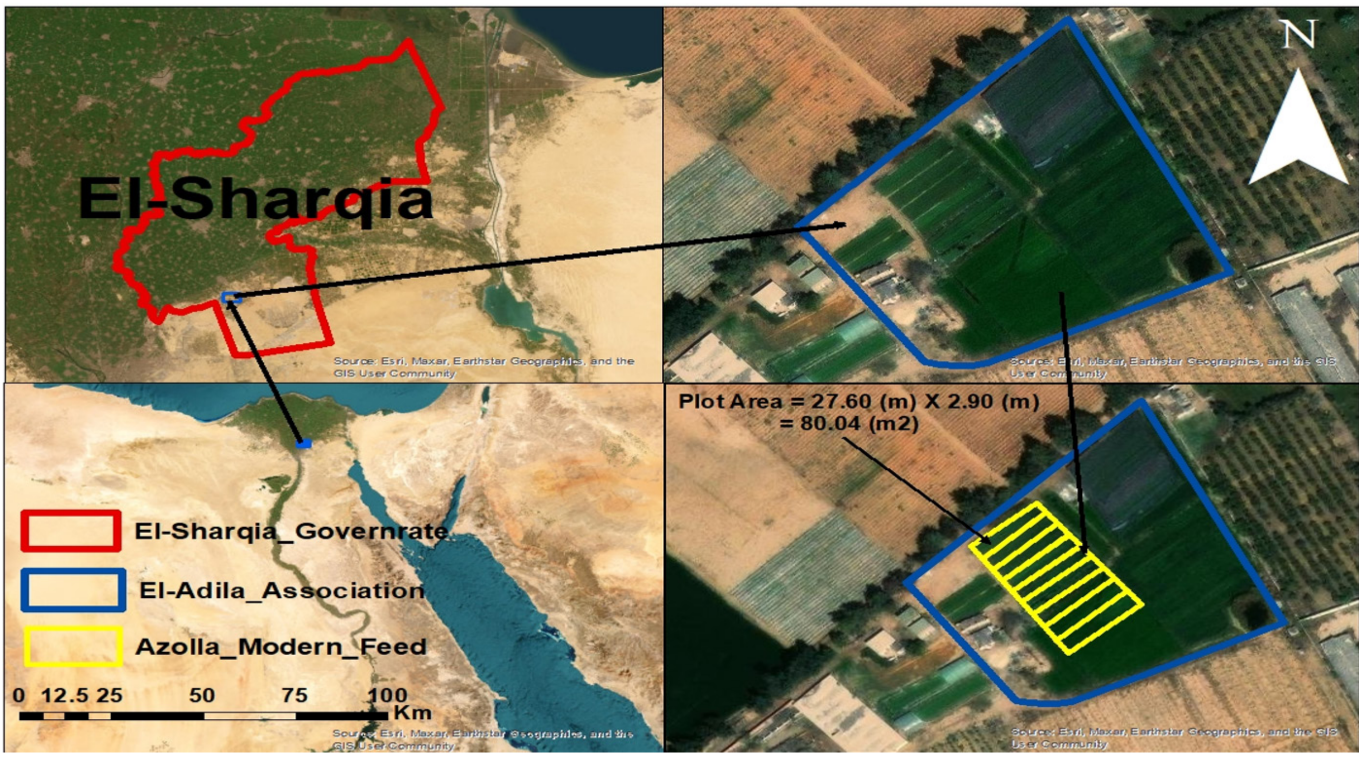

Figure 1: Location of the experimental site

Experimental design

A randomized complete blocks design (RCBD) with three replicates was used to conduct the field experiment. Three treatments, including two different combined nutrient solutions and farmer practice, were tested in this experiment.

Tested variables

The constituents of the two nutrient solutions and farmer fertilization practice tested in the experiment are:

- **Solution A:** Calcium Chloride ($CaCl_2.2H_2O$), Calcium Nitrate ($Ca(NO_3)_2.6H_2O$), Potassium Phosphate Monobasic (KH_2PO_4), Magnesium Sulfate Heptahydrate ($MgSO_4.7H_2O$), Phosphoric acid (80%), Boric acid (H_3BO_3, Boron 13%), Fe (EDTA, 13%), Zn (EDTA, 13%), and Mn (EDTA, 13%).
- **Solution B:** Calcium Nitrate ($Ca (NO_3)_2.6H_2O$), Phosphoric acid (80%, 50% of A), Boric acid (H_3BO_3, Boron 13%), Fe (EDTA, 13%), Mn (EDTA, 13%), $MnSO_4.7H_2O$, $CuSO_4$, Na_2MoO_4. $2H_2O$, and Manure.
- **Farmer practice:** Calcium Super Phosphate (15.5%), Manure, and Foliar spray of some macro- and micro-nutrients.

Cultural practices for growing *Azolla pinnata* (var. pinnata R. Brown)

Nine rectangular open-top aquaculture earth ponds (27.6 m x 2.9 m x 0.35 m) were prepared at the private farm to grow *Azolla pinnata*. The earth ponds were covered with plastic sheets (high density black PE, 50 µm) to prevent water seepage. The plastic sheets were covered

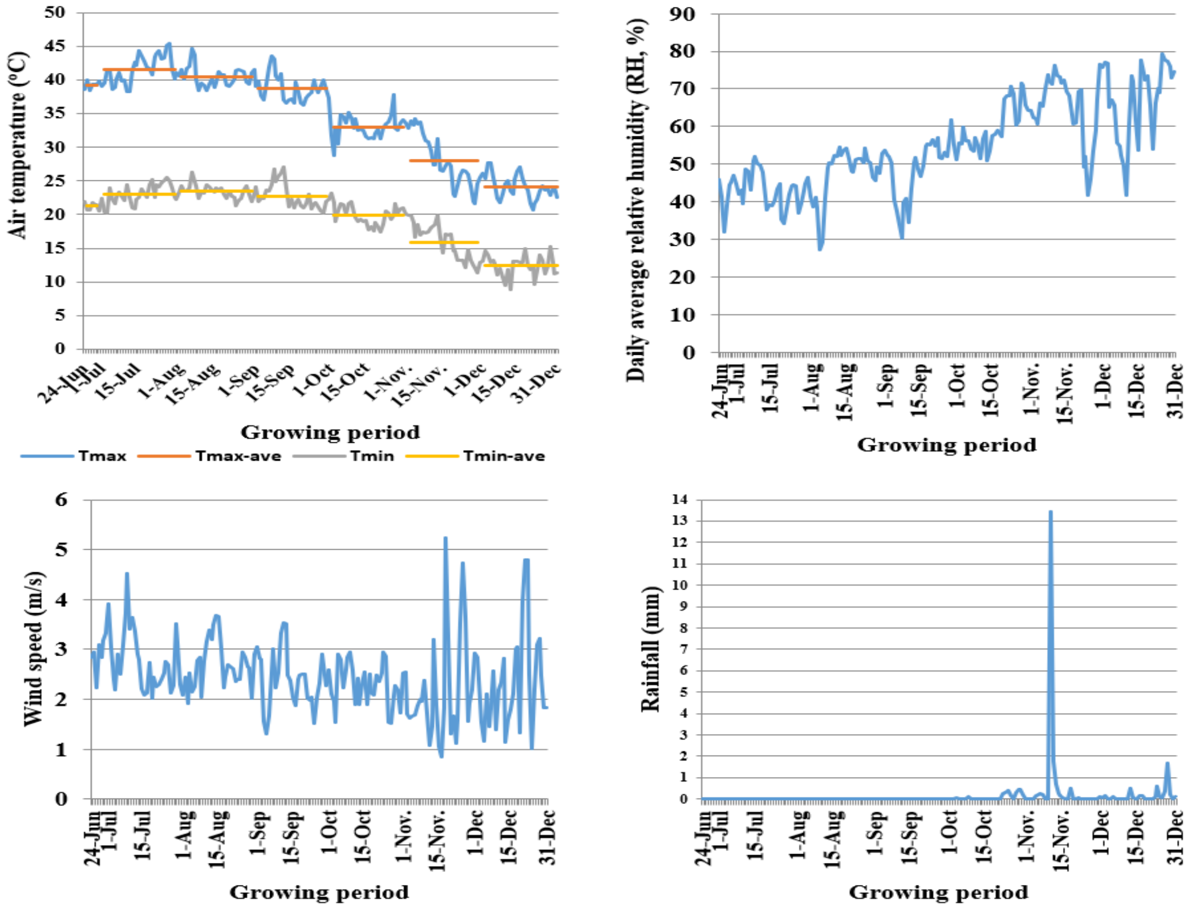

Figure 2: Daily weather data at the experimental site during Azolla growing period

Table 1: Chemical analysis and macro- and micro-nutrient contents of the soil added to the ponds

Chemical characters		Macro- and micro-nutrients (mg/kg)	
pH (1:2.5)	7.4	N	90
EC (dS/m)	11.1	K	411
Anions (meq/L)		P	7.12
CO_3^{2-}	-	Cu	0.02
HCO_3^-	5.5	Fe	7.12
Cl^-	61.5	Mn	0.62
SO_4^{2-}	43.9	Zn	0.27
Cations (meq/L)		**Others**	
Ca^{2+}	35.5	SP	69
Mg^{2+}	26.4		
Na^+	47.3		
K^+	1.65		

with a thin (10 – 15 cm) soil layer. The analysis of the soil used to fix the plastic sheet was done according to Tan (1996) and the obtained values are given in Table 1. All sides of the ponds were secured properly by placing bricks over the side walls.

The field experiment started on June 24, 2023 and each pond was filled with 18 m^3 of fresh water for *Azolla pinnata* propagation. The analysis of water (Tan, 1996) used to fill the ponds is given in Table 2. The amounts of water applied were measured by flow meters. Water was applied to the ponds every two weeks and maintained at water levels between 20 and 22 cm for proper propagation.

Table 2: Chemical analysis of the fresh water used to fill the ponds

Element	Value
EC (dS/m)	0.35
pH	7.27
Anions (meq/L)	
CO_3^{2-}	-
HCO_3^-	1.52
Cl^-	1.78
SO_4^{2-}	0.20
Cations (meq/L)	
Ca^{2+}	1.44
Mg^{2+}	0.77
Na^+	1.10
K^+	0.19
Macro- and micro-nutrients (mg/L) & SAR	
$N\text{-}NH_4$	6.30
$N\text{-}NO_3$	8.00
B	0.02
Cu	0.01
Fe	2
Mn	< 0.50
P	0.05
Zn	< 0.03
SAR	1.05

The ponds were inoculated with *Azolla pinnata* (pinnata variety) at the rate of 40 kg fresh ferns/pond (0.5 kg/m^2). There was a subsequent harvesting of Azolla at weekly interval during the period from July to October and every two weeks during November and December due to the unsuitable weather conditions for Azolla propagation (high wind speed and low temperatures).

Fertilization

The main fertilizers used in conducting the field experiments, element concentrations, doses, and dates of applications are presented in Table 3. For nutrient solutions A and B, macro- and micro-nutrients were added by the venturi fertilizer injector while filling the ponds with water. The farmer applied fertilizers by broadcasting and foliar methods. In solution B and farmer practice ponds, 0.5 m^3/pond of manure was added. Manure analysis is given in Table 4.

Table 4: Manure analysis

Element	%
Total N	1.24
P	0.8
K	1.5
S	15.8

Pest control

Two insecticides were used to control the pests (mainly larva) affecting Azolla. The two insecticides were Lambda cyhalothrin (5%) and Clorzane (48%). These insecticides were used once a week at the rate 10 cm^3/20 liters of water. In case of infection and changing Azolla plants from green to brown color, the insecticides were used 3 times/week.

Data collection and measured parameters

The parameters measured in this study were the amounts of water applied during the growing period using water flow meter and the fresh biomass. Plant samples were

Table 3: Fertilizer types, element concentrations, and dates and doses of applied fertilizers per pond during the growing period

Fertilizer Type & element concentration	Solution A			Solution B			Farmer Practice
	24 Jun.	21 Sep.	13 Nov.	24 Jun.	21 Sep.	13 Nov.	
$CaCl_2.2H_2O$ – [Ca = 40 ppm]	3 kg	1.5 kg	1.5 kg	x	x	x	10 g/20 L/week (foliar spry)
$Ca(NO_3)_2.6H_2O$ – [Ca = 0.2 ppm]	50 g	x	x	50 g	x	x	x
KH_2PO_4 – [K = 40 ppm, P = 32 ppm]	3.5 L	1.75 L	1.75 L	X	x	x	10 g/20 L/week (foliar spry)
$MgSO_4.7H_2O$ – [Mg = 40 ppm]	8.0 L	4.0 L	4.0 L	20.5 g	10.25 g	10.25 g	10 g/20 L/week (foliar spry)
Phosphoric acid (80%) – [P = 32 ppm]	2 L	1 L	1 L	1 L	0.5 L	0.5 L	10 cm^3/20 L/week (foliar spry)
H_3BO_3 (Boron 13%) – [B = 0.2 ppm]	50 g	25 g	25 g	50 g	25 g	25 g	5 g/20 L/week (foliar spry)
Fe (EDTA, 13%) – [Fe = 2.0 ppm]	775 g	388 g	388 g	775 g	388 g	388 g	5 g/20 L/week (foliar spry)
Zn (EDTA, 13%) – [Zn = 0.01 ppm]	100 g	50 g	50 g	x	x	x	5 g/20 L/week (foliar spry)
Mn (EDTA, 13%) – [Mn = 0.5 ppm]	100 g	50 g	50 g	x	x	x	5 g/20 L/week (foliar spry)
$ZnSO_4.7H_2O$ – [Zn = 0.01 ppm]	x	x	x	2.25 g	1.125 g	1.125 g	x
$MnSO_4.7H_2O$ – [Mn = 0.5 ppm]	x	x	x	125 g	62.5 g	62.5 g	x
$CuSO_4$ – [0.01 ppm]	x	x	x	2 g	1 g	1 g	x
$Na_2MoO_4.2H_2O$ – [Mo = 0.017 ppm]	x	x	x	2 g	1 g	1 g	x
Calcium Super Phosphate (15.5%)	x	x	x	x	x	x	5 kg/month (broadcasting)
Manure	x	x	x	0.5 m^3/pond			0.5 m^3/pond

collected and analyzed for total carbohydrate (%), total fiber (%), total amino acids (%), protein content (%) and NPK macro-nutrients. The chemical composition of Azolla was analyzed according to AOAC (2016). Also, water samples from the bonds were collected for EC, pH, cations, anions, $N\text{-}NH_4$, $N\text{-}NO_3$, and phosphorus and B, Cu, Fe, Zn, and Mn micro-nutrient analysis.

Statistical analysis

All obtained data were statistically analyzed using the MSTAT-C computer software package. For determining the effect of growing months and fertilizer treatments on the fresh yield, a two-way analysis of variance (ANOVA) was performed. The least significant difference (LSD) method was used to test the differences between treatment means at the 5% level of probability as described by Snedecor and Cochran (1981).

RESULTS AND DISCUSSION

Effect of tested treatments on *Azolla pinnata* fresh biomass

There were significant effects of the nutrient solutions used as well as the growing period on the fresh yields of *Azolla pinnata* (Table 5). The fresh yields, although fluctuated during the growing period, were consistently higher in A-solution treatment compared to those obtained from the other two treatments. Applying nutrient solution A recorded the highest average fresh yield (54.9 t/ha, 1.71 t/ha/day), followed by nutrient solution B (44.1 t/ha, 1.39 t/ha/day), while the lowest average yield (40.8 t/ha, 1.28 t/ha/day) was obtained from farmer practice (Table 5). The obtained results were close to those reported by Abdull Aziz (2012) who indicated that the fresh yield of *Azolla pinnata* varied from 820 to 1220 kg/

Figure 3: Photographs of the Azolla pinnata as affected by weather conditions. Images show a comparison between normal weather conditions during July (a), and high wind speed during November (b)

Table 5: Effect of tested treatments on average *Azolla pinnata* fresh yields (t/ha, t/ha/day, and kg/m²)

Fresh Yield										
Treatment / Month	Solution A			Solution B			Farmer Practice			Average (t/ha)
	t/ha	t/ha/day	kg/m²	t/ha	t/ha/day	kg/m²	t/ha	t/ha/day	kg/m²	
24 Jun. – 31 Jul.	80.7 a	2.18	8.07	54.7 c	1.48	5.74	52.0 cd	1.40	5.20	62.5 a
Aug.	66.4 b	2.14	6.64	54.2 c	1.75	5.42	50.1 cde	1.61	5.01	56.9 b
Sep.	54.3 c	1.81	5.43	46.3 de	1.54	4.63	42.7 ef	1.42	4.27	47.8 c
Oct.	62.5 b	2.01	6.25	53.8 c	1.74	5.38	51.8 cd	1.67	5.18	56.0 b
Nov.	27.2 hi	0.91	2.72	21.9 ij	0.73	2.19	19.7 j	0.66	1.97	22.9 e
Dec.	38.2 gh	1.23	3.82	33.5 gh	1.08	3.35	28.5 hi	0.92	2.85	33.4 d
Average	54.9 a	1.71	5.49	44.1 b	1.39	4.41	40.8 c	1.28	4.08	

Means that do not share the same letter are significantly different.

ha/day. The results were also similar to those of Hossain *et al.* (2021), who showed that the fresh biomass of *Azolla pinnata* varied from 3.90 to 5.92 kg/m^2 and close to those reported by Amro (2022), who indicated that the fresh yield of Azolla plant was equivalent to 6-7 kg/m^2. Results indicated also that, nutrient solution (A) that included multiple nutrients (i.e. Ca, K, P, Mg, B, Fe, Zn and Mn) significantly increased *Azolla pinnata* fresh yield by 24.5 and 34.5% as compared with the fresh yields of solution B and farmer practice, respectively. The obtained results were similar to those reported by Kannaiyan (1982) and Sadeghi *et al.* (2013), who showed that Azolla requires all macro and micro nutrients (i.e. P, K, Ca, Mg, Fe, Mo, Co, and Zn) which are essential for Azolla growth and N-fixation. Results agreed also with those of Nordiah *et al.* (2012), who indicated that a combination of more than one nutrient or multiple nutrient contents explained the increase in biomass of *Azolla pinnata*.

Results showed significant decrease in the fresh yields obtained during September as compared with those recorded in August and October. Yield reduction was due to severe shortage of the fresh water diverted to the experimental farm with direct effect on water depths in the ponds which decreased by 2 to 3 cm. The obtained results could be supported by those of Biswas *et al.* (2005) and Sadeghi *et al.* (2012a,b), who concluded that the optimal growth and biomass production of Azolla could have a close relation to water depth since low water depths might slow down the growth and reduce its biomass production. The obtained results could be explained by what was reported by Adzman *et al.* (2022) who stated that, higher volume of water could hold more dissolved oxygen (DO) providing the roots of Azolla with sufficient DO than lower volume of water. Also, water at deeper level is colder compared to at the surface which is exposed to direct sunlight making it warmer than water at deeper level. The cold water hold more DO because water molecules are closely packed together making it difficult to release into the atmosphere and the solubility of oxygen is decreased with warm water making it holding lesser DO.

Results showed also that, average *Azolla pinnata* fresh yield of 59.7 t/ha obtained during summer season (Jul. and Aug.) was higher than the average yield of 42.2 t/ha recorded during autumn season (Sep., Oct. and Nov.). The highest average fresh yield of 62.5 t/ha occurred during July, while the lowest average yield (22.9 t/ha) was recorded during November. The optimum weather conditions during summer, including air temperature, relative humidity, and wind speed, resulted in higher yield compared to the yield during autumn and winter seasons (Figure 3). The lowest yields during November were due to high wind speed. The obtained results were in line with those reported by Amro (2022), who found that growth rates of Azolla were higher during the summer. Abdul Aziz (2012) reported low productivity during winter and spring was attributed to low humidity, light intensity, day length and temperature. Also, Sadeghi *et al.* (2013) stated that wind and turbulent water can fragment and kill Azolla.

From the obtained results it could be concluded that, applying nutrient solution (A) that include multiple nutrients (i.e. Ca, K, P, Mg, B, Fe, Zn and Mn) by injecting it to the ponded water using vensuri during summer season gives the highest yield of *Azolla pinnata*.

Effect of tested treatments on total carbohydrates (TC), total fiber (TF), total amino acids (AA), crude protein (CP) in the *Azolla pinnata* tissues

Results illustrated in figure 4 showed no effect of the tested nutrient solutions on total carbohydrates (TC) values measured in Azolla's tissues. Average TC values were 32.9, 31.7, and 32.9% for solution A, solution B and farmer practice treatments, respectively. The obtained results were close to carbohydrate contents reported by Mohamed *et al.* (2018)with 30.5% on dry matter basis.

Meanwhile, average total fiber (TF) contents in Azolla's tissues were 16.0, 15.6, and 15.6% for solution A, solution B and farmer practice treatments, respectively. The results of crude fiber content obtained in *Azolla pinnata* tissues were slightly higher than the values reported by Chatterjee *et al.* (2013) with 13.4%, Kumar *et al.* (2018) with 11.2%, Wagh *et al.* (2021) with 14.7%, and Yee *et al.* (2022) with 12.2%. The slight difference between the result obtained and previous studies in the crude fiber values may be due to a change in dry matter content and maturity level of the Azolla that was collected at different intervals (Bhatt *et al.*, 2020).

Results in figure 4 showed also that, average values of total amino acids (AA) were 14.0, 12.6, and 13.1% for the three respective treatments. For A nutrient solution treatment, the AA contents in Azolla's tissues were 11.5 and 6.9% higher than the AA contents in B nutrient solution and farmer practice treatment, respectively. The obtained results were higher than the values reported by Mohan *et al.* (2020), who showed that Azolla has 7-10% amino acids.

Results showed also that, average crude protein (CP) values were 15.7, 13.5, and 13.4% for the three respective treatments. In Azolla's tissues, the crude protein values obtained from A nutrient solution treatment were 16.5 and 17.6% higher than the values obtained from B nutrient solution and farmer practice treatments, respectively. The obtained results were less than the reported crude protein values from other studies showing the CP% values of 17.6% (Van Hove and Lopez, 1987), 21.2 % (Sujatha *et al.*, 2013), 21.4% (Alalade and Lyayi 2006), 21.7 %, (Kavya 2014), 22.5 % (Ashraf and Sharma 2015), (Brouwer *et al.*, 2018),26.5% (Bhatt *et al.*, 2020), 28.5% (Hossain *et al.*, 2021), 24.1% (Yee *et al.*, 2022), 27.1% (Adzman *et al.*, 2022), and 25-30% (El-Naggar and El-Mesery, 2022).The low protein values could be related to the high maximum temperatures that exceeded 35 °C for several months at the experimental site with direct effect on N-fixation. The obtained results were explained by Bhatt *et al.* (2020), who related the variation in crude protein percentage to several conditions including air and water temperatures, nutritional content of the water, and pest growth that may affect *Azolla pinnata* growth and composition.

Effect of tested treatments tissue-NPK of *Azolla pinnata*

The effect of tested treatments on nitrogen (N, %), phosphorus (P, %), and potassium (K, %) in *Azolla pinnata*'s tissues is illustrated in Figure 5. Results indicated that tissue-N contents (%) in *Azolla pinnata* varied between 3.1 –2.1%, 2.6 – 1.9%, and 2.8 – 1.9% for nutrient solution A, nutrient solution B, and farmer practice treatments, respectively. Average tissue-N values were 2.51, 2.16, and 2.14% for the three respective treatments. The obtained values were less than reported for *Azolla pinnata* by Kushari and Watanabe (1991) with N (%) and by Shome (2024) with 4% tissue-N content. The low tissue-N content values obtained under the current experiment could be due to the high air temperatures that exceeded 35 °C for several months with direct effect on N-fixation by Azolla plants. The results agreed with those reported by Reddy (1987) who sated that maximum nitrogenase activity was observed between 20 – 30 °C and the rate of N_2 fixation was found to be highest at 30 °C.

As for tissue-P contents (%) in *Azolla pinnata*, results showed that average P values were 0.54, 0.40, and 0.40% for nutrient solution A, nutrient solution B, and farmer practice treatments, respectively. The high tissue-P values of 0.89, 0.62, and 0.61% recorded during December were due to the application of the 3rd dose of fertilizer by the end of November. The obtained results were close to those reported by Kushari and Watanabe (1991), who reported that P (%) in *Azolla pinnata* tissues varied from 0.40 to 1.04% as P-concentration in the growing media varied from 4.64 to 13.9 μg/cm^2/day. Results were also close to those of Bhatt *et al.* (2020) who reported that mineral profiling of *Azolla pinnata* phosphorus was 0.31%. The obtained results were less than what was reported by Shome (2024), who conducted research on *Azolla pinnata* under open area conditions and showed that the plants had the capacity to accumulate a good quantity of tissue-P of 1.45% at higher concentration of media-P.

Regarding tissue-K contents (%), results indicated that tissue-K contents (%) in *Azolla pinnata* varied between 1.34 - 1.05%, 1.33 – 1.13%, and 1.4 – 1.14% for nutrient solution A, nutrient solution B, and farmer practice treatments, respectively. Average tissue-K values were 1.22, 1.21, and 1.25% for the three respective treatments. The obtained results were less than those reported for *Azolla pinnata* by Kushari and Watanabe (1991) with K (%) between 4.4 – 5.0%, Bhatt *et al.* (2020) with K-mineral profiling of 2.68%, and Shome (2024) with tissue-K of 4.30% in open area.

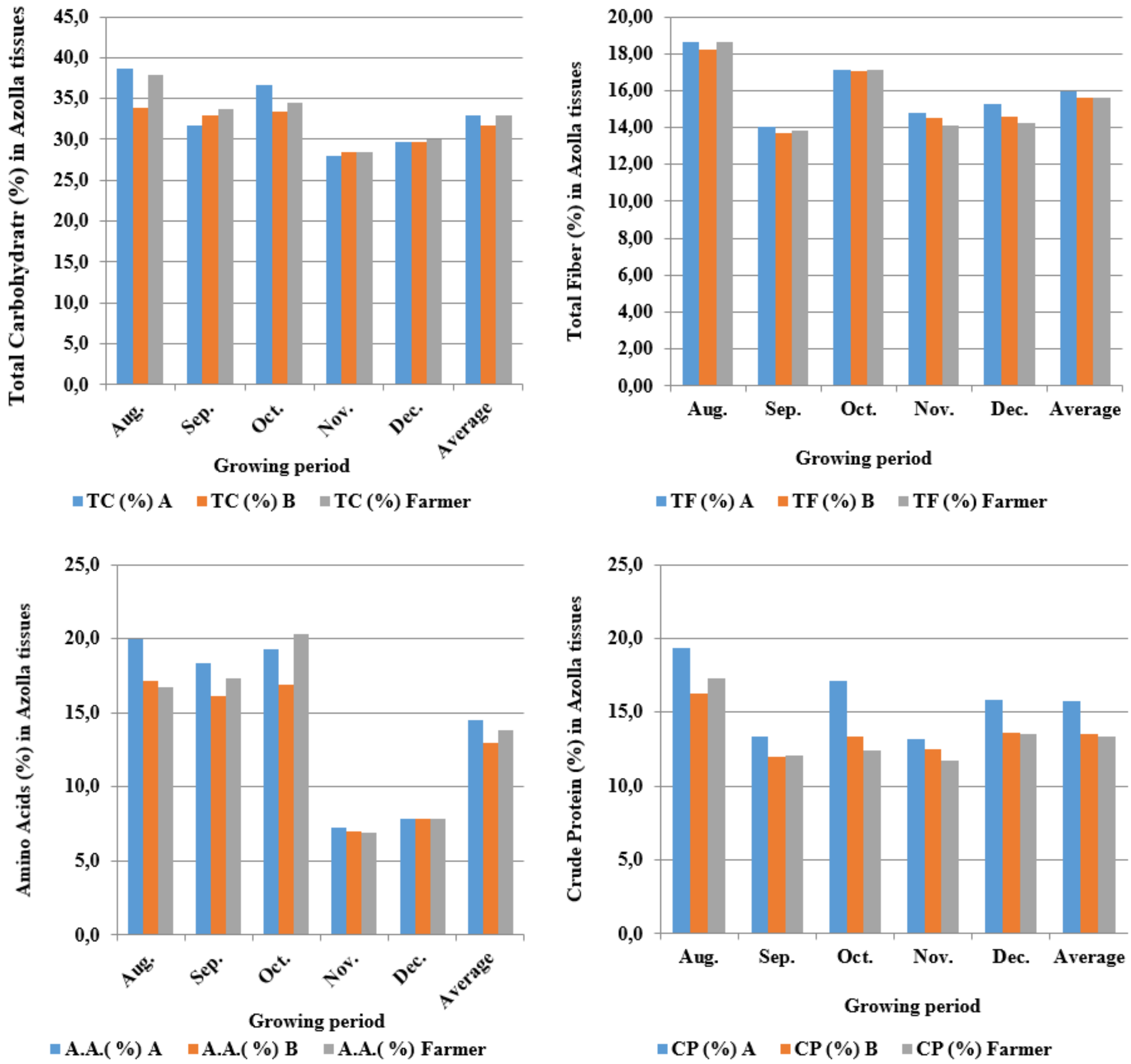

Figure 4: Total carbohydrate (TC, %), total fiber (TF, %), total amino acids (AA, %), and crude protein (CP, %) contents in Azolla pinnata's tissues as affected by tested treatments

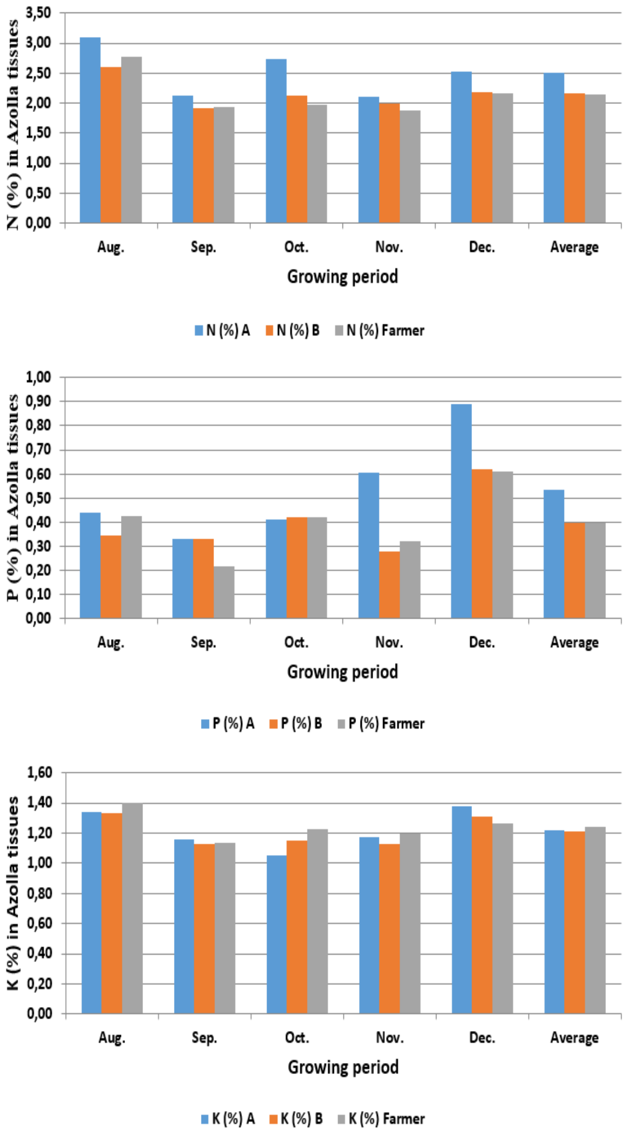

Figure 5: Nitrogen, phosphorus, and potassium (K) percentages in Azolla pinnata's tissues as affected by tested treatments

Water applied (WA) and its productivity (WP)

This experiment was the first in Egypt to measure and report the amounts of water needed to grow Azolla plants. Results in Table 6 showed that the total amount of applied water, including the effective rainfall during November, through the growing period (24 June – 31 December 2023) was 4071 m^3/ha. The highest amount (1406 m^3/ha) was added during July, while the lowest amount (281 m^3/ha) was added during December. This trend is logical due to the fluctuation in the obtained Azolla yields and the prevailing weather condition at the experimental site during the growing period. The obtained results were different than those reported by Amro (2022) who reported that the monthly water consumption rate by Azolla plant was approximately equal to 120 m^3/dunum (1200 m^3/ha/month). The differences in the results could be due to the cultivated Azolla variety, fertilizers used and the prevailing weather conditions at the two sites.

Results indicated also that, monthly and total depths of water applied were much less than the corresponding ETo values (Table 6). The AW/ETo ratios varied from 0.23 during September to 0.44 during July with an average growing period value of 0.37. The results showed that, floating Azolla plays a significant role in decreasing evapotranspiration from the growing surface. The obtained results agreed well with those reported by Kimani *et al.* (2020) who reported that Azolla cover significantly decreased evapotranspiration (ET) losses compared with open water surfaces and green polyester covered mats. They concluded that the obtained results may be attributed to a greater total reflectance of the incoming solar radiation and enhanced modification of the surrounding microclimate by the dense mat of the floating Azolla. The obtained results were also close to those of Diara and Van Hove (1984) and Liu and Zheng (1992), who reported relative reduction in ET due to floating Azolla cover.

Table 6: Monthly and total depths/amounts of water applied (WA), the calculated reference evapotranspiration (ETo), and AW/ETo ratios during the growing period

Month	Applied water (AW)		ETo (mm/mon.)	AW/ETo
	Depths (mm/mon.)	Amounts (m^3/ha/mon.)		
24 June – 31 July	140.6	1406.0	321.3	0.44
August	104.5	1045.0	243.9	0.43
September	46.2	462.0	201.7	0.23
October	44.2	442.0	140.5	0.31
November	30.0	300.0	-	-
Effective rainfall (mm, m^3) in November	13.46	134.6	-	-
Total applied + effective rainfall in November	43.46	434.6	103.4	0.42
December	28.1	281.0	78.7	0.36
Total (mm, m^3/ha)	407.1	4071.0	1089.5	
Initial amount of water applied to fill the ponds (m^3/ha)	-	2249.0	-	
Total water applied (m^3/ha)	-	6320.0	-	

Results showed that, the highest average water productivity value (96.4 kg fresh yield/m^3 water applied) was recorded for solution A treatment, while the lowest value (73.6 kg fresh yield/m^3 water applied) was recorded for famer practice treatment (Table 7). Also, the highest average WP value of 126.8 kg fresh yield/m^3 water applied was obtained during October and the lowest value of 44.4 kg fresh yield/m^3 water applied was obtained during July. The interaction effect of the tested nutrient solutions and the growing period showed that, the highest WP value of 141.3 kg fresh yield/m^3 water applied was recorded for Solution A treatment during October, while the lowest value of 37 kg fresh yield/m^3 water applied was recorded for farmer practice during July.

Table 7: Water productivity (kg fresh yield/m^3 water applied) of *Azolla pinnata* plants

Month	Solution A	Solution B	Farmer Practice	Average
July	57.4	38.9	37.0	44.4
August	63.6	51.9	47.9	54.5
September	117.5	100.2	92.5	103.4
October	141.3	121.8	117.3	126.8
November	62.7	50.3	45.4	52.8
December	136.1	119.2	101.4	118.9
Average	96.4	80.4	73.6	83.5

The initial amount of water added to fill the ponds (2249 m^3/ha) was not included as water consumed because it can be reused easily for irrigating other crops in the farm. The chemical analysis of the ponded water by the end of the experiment is shown in Table 8. The chemical analysis indicated that the ponded water can provide essential crop nutrients.

CONCLUSION

Applying nutrient solution A that include multiple nutrients (i.e. Ca, K, P, Mg, B, Fe, Zn and Mn) significantly increased *Azolla pinnata* biomass and produced, on average, 54,9 t/ha/month.

Injecting the nutrients by vensuri to the ponded water was more effective than adding the nutrients by broadcasting or foliar spry.

Highest biomass production was obtained during summer.

Applied amount of water during the growing period, 24 June – 31 December 2023, was 4071 m^3/ha.

An average water productivity value of 96.4 kg fresh biomass/m^3 applied water was recorded for nutrient solution A.

The ponded water of 2249 m^3/ha can be used to irrigate different crops in the farm.

Table 8: Chemical analysis of the ponded water by the end of the experiment

Element	Treatment					
	Solution A		Solution B		Farmer practice	
EC (dS/m)	1.19		0.62		0.64	
pH	6.1		6.24		5.9	
Anions						
Unit	**meq/L**	**g/m^3**	**meq/L**	**g/m^3**	**meq/L**	**g/m^3**
CO_3^{2-}	-	-	-	-	-	-
HCO_3^-	4.1	250.1	4.0	244.0	4.2	256.2
Cl^-	3.17	112.5	1.56	55.4	1.69	60.0
SO_4^{2-}	4.63	222.2	0.66	31.7	0.53	25.4
Cations						
Unit	**meq/L**	**g/m^3**	**meq/L**	**g/m^3**	**meq/L**	**g/m^3**
Ca^{2+}	5.28	105.6	2.85	57.0	3.44	68.8
Mg^{2+}	4.2	50.4	1.95	23.4	1.3	15.6
Na^+	1.95	44.85	1.33	30.6	1.52	35.0
K^+	0.65	25.4	0.09	3.5	0.06	2.3
Macro- and micro-nutrients						
Unit	**mg/L**	**g/m^3**	**mg/L**	**g/m^3**	**mg/L**	**g/m^3**
$N\text{-}NH_4$	4.9	4.9	4.1	4.1	4.2	4.2
$N\text{-}NO_3$	0.7	0.7	1.0	1.0	1.0	1.0
Total N	5.6	5.6	5.1	5.1	5.2	5.2
P	0.04	0.04	0.02	0.02	0.09	0.09
B	0.276	0.276	0.21	0.21	0.03	0.03
Cu	0.01	0.01	0.01	0.01	0.01	0.01
Fe	0.07	0.07	0.17	0.17	0.05	0.05
Mn	0.17	0.17	0.02	0.02	0.09	0.09
Zn	0.01	0.01	0.01	0.01	0.01	0.01

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