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Assessment of Suitable Growth Condition of *Salvinia* spp. for Sustainability of Floating Agriculture to Mitigate Climate Change Challenges on Crop Cultivation

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Abstract

Floating agriculture is an indigenous cultivation practice in the southern waterlogged areas of Bangladesh. Water hyacinth and other macrophytes were utilized in this agricultural method to prepare the floating bed. Among the numerous macrophytes used as mulch in floating beds, Salvinia spp. is a common species. To meet the local farmer's demand, it is necessary to improve the growth rates of these macrophytes so that they can be available year-round for preparing floating beds. For this purpose, an experiment was conducted to assess the growth conditions of Salvinia spp. under various nutritional media. *Salvinia molesta* and *Salvinia cucullata* were cultivated in four healthy media to carry out the experiment. The experimental design used in the present investigation was a split-plot arrangement in a Randomized Block Design (RBD), where four nutrients (NO_3^-; PO_4^{3-}; NH_4^+ and Urea) were assigned to the main plots, while five different concentrations of nutrient (0, 1, 5, 10 and 15 mM) were given to the sub-plots. *Salvinia* spp. growth was examined in terms of morphological and physiological responses and the distribution of nutrients. The highest relative growth rate (RGR) and biomass production (g DW) were found in the medium with one mM and five mM $PO4_3^-$ in the case of *S. molesta* and one mM NH_4^+ in the case of *S. cucullata*. It was also observed that the plant growth rate became suppressed when this macrophyte grew above the 5mM of nutrient concentration, and this suppressed condition turned into a toxic state when the organic nutrient concentration reached a concentration of 15 mM or above. In the case of Urea, this harmful condition starts with a concentration of 5 mM or more for *S. molesta* and any concentration for *S. cucullat*a.Nonetheless, very few variations were found in how various plant tissues and species absorbed mineral concentrations. The overall findings from this experiment indicate that both PO_4^{3-} and NH_4^+ concentrations of 1 mM and 5 mM are comparatively more suitable than NO_3^- and Urea for the growth and multiplication of *Salvinia* spp. However, more research is required in addition to varying the quantities of phosphate and ammonium to make a definitive choice.

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Keywords: Floating agriculture, Sustainability, *Salvinia*, Nutrient, Growth, and Development

Introduction

Bangladesh is among the countries on Earth where climate change is seen as a severe challenge due to its location. Aside from this situation, practically every year, two-thirds of Bangladesh's land wetland stays underwater for eight months (Islam *et al.*, 2011), greatly endangering the country's

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agriculture industry. The available research suggests that climate change will severely hinder crop output, putting the nation's food security at risk (Karim *et al.*, 1999; Palop *et al*., 2010; Warrick and Ahmad 2012). One creative, indigenous knowledge and technique-based, climate-smart agricultural method that can help with these issues is floating agriculture. This method of cultivating vegetables creates new chances for the local environment (Chowdhury 2004). Farmers in Bangladesh's southern floodplains, especially in the districts of Barishal, Gopalganj, and Pirojpur, have been engaged in this agricultural technique for over a century (Asaduzzaman 2004; Islam and Atkins 2007; Haq and Nawaz 2009; Irfanullah 2009; IUCN Bangladesh 2005).

Local farmers in Bangladesh's south prepare floating beds using a variety of macrophytes, including water hyacinth (*Eichhornia crassipes*), Asian water moss (*Salvinia molesta*), Kariba weed (*Salvinia cucullata*), Topapana (*Pistia stratiotes*), Khudipana (*Lemna minor*), Kutipana (*Azolla pinnata*), and Sonapana (*Spirodelapolyrhiza*). Local farmers frequently use Salvinia spp. as a mulching material for their cultivation procedure out of all these floating macrophytes. Local farmers assert that macrophytes (*Salvinia* spp.) are placed on the floating bed several times during the growing season. Because of this, a significant number of *Salvinia* plant samples are required to preserve the quality of the floating bed, including improved crop growth and nutritional status. *Salvinia cucullata* and *S. molesta* are two of the many species that live longest on floating beds and give crops more nutrients and support. Farmers mostly use these species in floating beds for crop cultivation.

Salvinia is a visually appealing, rapidly increasing, and free-floating aquatic fern that grows naturally in various settings, particularly in water rich in nutrients. Salvinia must be improved upon to sustain a floating bed and be readily available throughout the year. Farmers add urea to water bodies to promote Salvinia's vegetative growth, although the dosage quantity can occasionally have adverse effects. Additionally, it is still being determined how successful urea is at promoting Salvinia growth. In addition, plants may experience mineral deficiencies and NH_4^+ toxicity when there is a significant nitrogen (N) input to water bodies. These factors highly influence the amount of sunshine, salvinia growth, and water quality. Earlier research had conducted several studies to determine *Salvinia*'s ideal growing conditions and doubling period. Jampeetong *et al.* (2012) examined the growth and morphological responses, nitrogen uptake, and resource allocation in four aquatic macrophytes using three distinct inorganic nitrogen treatments, i.e. NH_4^+, NO_3^-, or/both $NH_4^+$and NO_3^-. An experiment was carried out in Bangladesh to learn more about these species' nitrogen intake and mineral allocation. This requires measuring the ideal nutrient combination for *Salvinia* spp.'s rapid growth and understanding how nutrients affect the species' growth rate, morphological responses, and nutrient allocation.

In this background the objective of the present investigation was to estimate the growth rate and doubling time, to determine the morphological responses and vegetative growth patterns under different nutrient conditions and to measure the allocation of nutrients taken up by different parts of *Salvinia* spp. from the water bodies.

Materials and methods

Experimental site

The Department of Botany at the University of Barishal, which is situated at 22° 39′ 46.0″ N latitude and 90° 21′ 53.5″ E longitude, is where the current experiment was carried out in a net house.

Plant materials

Salvinia molesta and *S. cucullata*, two free-floating species of water fern were used at the present investigation which were taken from ponds at Regional Agriculture Research Station (RARS), Rahamatpur, Barishal.

Treatments and design of the experiment

The current study employed a split-plot arrangement within a Randomized Block Design (RBD). Five nutrient concentrations [T_0 = 0 mM, T_1 = 1 mM, T_2 = 5 mM, T_3 = 10 mM, T_4 = 15 mM] were assigned to the sub-plots and replicated three times, while four nutrients [KNO_3; KH_2PO_4; $(NH4)_2SO_4$; Urea were used as a source of Nitrate (NO_3^-); Phosphate (PO_4^{3-}); Ammonium (NH_4^+); urea, respectively] were assigned to the main plots.

Cultural Practices and Growth Study

The collected plants were washed thrice with distilled water. The homogeneously sized fronds were placed in a hydroponic system and were thought to be inoculum. Half-strength Hoagland solution (HS) was used in the 1.5 L plastic containers for the experiments, and the pH was adjusted to 7. For every treatment, three replications were conducted, using six inoculums in each dose, while the control (0 mM) was kept in a similar state. To make up for the water volume lost in the pots, distilled water was added. After 28 days, the plants were cleaned and harvested to collect morphological data. The equation RGRw = ($\ln W_2 - \ln W_1)/(t_2-t_1)$ was used to calculate the relative growth rate (d^{-1}). W_1 and W_2 stand for the plant's beginning and final dry weight, and t_1 and t_2 stand for the starting and end time. Moretti et al. (1988) provided the following formula for doubling time (in days): t $\log_2$[log (wtw0−1)]1.

Amount of accumulated ions

Sodium (Na^+) and potassium (K^+) ions content were determined from plant tissue according to Begum (1993) using a flame photometer (Labtronics LT-671, India). A spectrophotometer (T60 U Spectrophotometer, India) was used to quantify the ion content of phosphate (PO_4^{3-}) and nitrate (NO_3^-) at wavelengths of 440 nm and 410 nm, respectively (Cataldo et al. 1975; Jackson 1967). A modified salicylate method (Quikchem method no. 10-107-06-3-B; Lachat Instruments, Milwaukee, WI, USA) was used to estimate the ammonium (NH_4^+) in the plant tissue extracts, and a UV-Vis spectrophotometer (T60 U Spectrophotometer, India) was used to assess the samples' absorbance at 690 nm.

Contents of chlorophyll

A spectrophotometer (Model: T60 UV Spectrophotometer) was used to measure the total chlorophyll content, chlor-a, and chlor-b at 645 and 663 nm (Yoshida et al., 1976).

Statistical analysis

Analysis of variance (ANOVA) and significance of the difference among the means was evaluated according to the least significant difference test (LSD) at the 5% level of probability (Gomez and Gomez 1984) using STATISTIX 10.

Results and Discussion

Morphological parameters

Number of leaves and leaf area

Leaf number and total leaf area are the primary factors that affect a plant's photosynthesis and, subsequently, it′s biomass production (Reddy and Matcha, 2010). Our findings align with this conclusion as we also observed a direct correlation between plant leaf number and total leaf area with biomass production. They affected the total number of leaves and leaf area (Table 1). In the case of both *Salvinia* spp., the most significant number of leaves and leaf area were seen at 1 mM of ammonium, followed by 1 mM and 5 mM of phosphate.

Root number and root length

Different combinations and concentrations of nutrients highly affected the root number/plant and length of the root (Table 2). The highest number of roots were seen at 1 mM ammonium and 5 mM phosphate, respectively, suggesting fresh shoot development occurs more quickly under these nutritional conditions. However, the longest roots of *S. molesta* were found at 1mM of phosphate treatment, and root length was reduced with increasing concentration above 5mM for all nutrient-fed plants.

Table 1. Combine effect of different concentrations of nutrients on leaves number and leaf areaof *Salvinia* spp.

Nutrients	Conc.	Leaf number		Leaf area	
		S. molesta	*S. cucullata*	*S. molesta*	*S. cucullata*
Nitrate	T_0	136.50±19.6^{fgh}	99.00±9.8^{cd}	50.36±15.6^{def}	22.41±3.9^{bc}
	T_1	214.50±38.2^{de}	33.00±2.94^{d}	57.25±1.2^{cde}	12.02±1.7^{c}
	T_2	157.88±94.3^{efg}	25.37±4.2^{d}	38.27±28.3^{efg}	9.26±3.2^{c}
	T_3	175.25±21.1^{efg}	24.75±6.3^{d}	60.55±24.8^{cde}	9.88±3.3^{c}
	T_4	100.75±26.9^{gh}	28.25±2.4^{d}	27.79 ±1.6^{fg}	10.06±1.8^{c}
Phosphate	T_0	136.50±19.6^{fgh}	99.00±9.8^{cd}	50.36±15.6^{def}	22.41±3.9^{bc}
	T_1	355.50±97^{ab}	240.25±70^{abc}	95.14±41.8^{a}	52.13±15.3^{a}
	T_2	367.50±10.8^{ab}	360.50±5.8^{ab}	88.79±17.7^{ab}	68.82±6.7^{a}
	T_3	193.25±17.2^{ef}	242.75±85.7^{abc}	50.01±8.53^{defg}	44.44±12.2^{ab}
	T_4	181.50±20.6^{ef}	289.50±10.7^{ab}	49.08±2.3^{defg}	51.89±11.9^{a}
Ammonium	T_0	136.50±19.6^{fgh}	99.00±9.8^{cd}	50.36±15.6^{def}	22.41±3.9^{bc}
	T_1	418.38±32.6^{a}	401.30±55.3^{a}	95.38±9.1^{a}	67.87±13.6^{a}
	T_2	335.50±22.5^{bc}	301.80±67.8^{ab}	66.97±23.4^{bcd}	60.26±15.7^{a}
	T_3	272.75±46.5^{cd}	223.00±14.7^{bc}	80.56±9.8^{abc}	45.40±4.6^{ab}
	T_4	155.75±51.4^{efg}	121.75±50.4^{cd}	51.88±13.9^{def}	22.50±5.2^{bc}
Urea	T_0	136.50±19.6^{fgh}	99.00±9.8^{cd}	50.36±15.6^{def}	22.41±3.9^{bc}
	T_1	360.00±96^{ab}	—	96.22±37.1^{a}	—
	T_2	69.50±18.6^{hi}	—	24.53±2.06^{gh}	—
	T_3	—	—	—	—
	T_4	—	—	—	—
Significance		**	NS	**	*

* ($P \leq 0.05$), ** ($P \leq 0.01$), NS (non-significant). Means (± SD) were calculated from three replicates for each treatment. Values with different letters are significantly different at $P \leq 0.05$ applying the LSD test. Blank entries indicate plants were not survived)

Table 2. Combine effect of different concentrations of nutrients on root number and root length of *Salvinia* spp.

Nutrients	Conc.	Root number		Root length	
		S. molesta	*S. cucullata*	*S. molesta*	*S. cucullata*
Nitrate	T_0	62.00±7.8^{fgh}	45.00±9.80^{de}	1.43±0.14^{cdef}	1.90±0.13^{cd}
	T_1	101.00±19.6^{de}	12.50±2.94^{e}	1.67±0.13^{ab}	2.75±0.42^{b}
	T_2	73.00±15.68^{efg}	9.00±1.96^{e}	1.33±0.13^{defg}	3.22±0.10^{a}
	T_3	81.50±10.78^{efg}	8.50±2.94^{e}	1.23±0.06^{g}	3.20±0.39^{a}
	T_4	44.50±12.74^{gh}	9.50±2.94^{e}	1.2±0.13^{g}	2.03±0.18^{c}
Phosphate	T_0	62.00±7.8^{fgh}	45.00±9.80^{de}	1.43±0.14^{cdef}	1.90±0.13^{cd}
	T_1	171.50±20.58^{ab}	116.00±15.68^{abc}	1.77±0.32^{a}	1.75±0.42^{cde}
	T_2	177.50±14.7^{ab}	161.00±17.64^{ab}	1.53±0.06^{bc}	1.72±0.16^{cde}
	T_3	90.50±8.82^{ef}	116.00±17.64^{abc}	1.48±0.10^{cd}	1.55±0.03^{e}
	T_4	85.00±9.8^{ef}	140.00±3.92^{ab}	1.25±0.09^{g}	1.55±0.10^{e}
Ammonium	T_0	62.50±7.8^{fgh}	45.00±9.80^{de}	1.43±0.14^{cdef}	1.90±0.13^{cd}
	T_1	202.50±14.7^{a}	175.00±14.70^{a}	1.57±0.06^{bc}	3.27±0.19^{a}
	T_2	162.00±11.76^{bc}	147.00±13.72^{ab}	1.47±0.13^{cde}	1.72±0.23^{cde}
	T_3	130.00±19.6^{cd}	107.00±11.76^{bcd}	1.28±0.03^{fg}	1.62±0.03^{de}
	T_4	71.50±9.8^{efg}	55.50±12.74^{cde}	1.32±0.03^{efg}	1.53±0.13^{e}
Urea	T_0	62.00±7.8^{fgh}	45.00±9.80^{de}	1.43±0.14^{cdef}	1.90±0.13^{cd}
	T_1	174.00±9.8^{ab}	—	1.55±0.03^{bc}	—
	T_2	29.00±9.8^{hi}	—	1.27±0.06^{g}	—
	T_3	—	—	—	—
	T_4	—	—	—	—
Significance		**	*	**	**

*($P \leq 0.05$) and ** ($P \leq 0.01$). Means (± SD) were calculated from three replicates for each treatment. Values with different letters are significantly different at $P \leq 0.05$ applying the LSD test. Blank entries indicate plants were not survived)

Total biomass

The findings showed that the nutrients and their concentrations significantly impacted the fresh and dry biomass of *Salvinia* spp. (Fig. 1). Plants grown with ammonium and phosphate produced the highest fresh and dry weight. For all nutrients, *Salvinia* spp. Exhibited the highest fresh and dry weight when exposed to 1 mM to 5 mM concentrations. In *S. molesta*, urea-fed plants showed the maximum biomass at 1 mM, then decreased and could not survive above 5 mM, whereas *S. cucullata* could not survive above 1 mM. On the other hand, Nitrate-fed plants showed lower biomass than ammonium and phosphate. In a study of *Salvinia* species, the highest dry matter yields were obtained from plants that received NH4-N l-1 to compare with the same level of NO3-N or urea-N (Cary and Weerts 1983a).

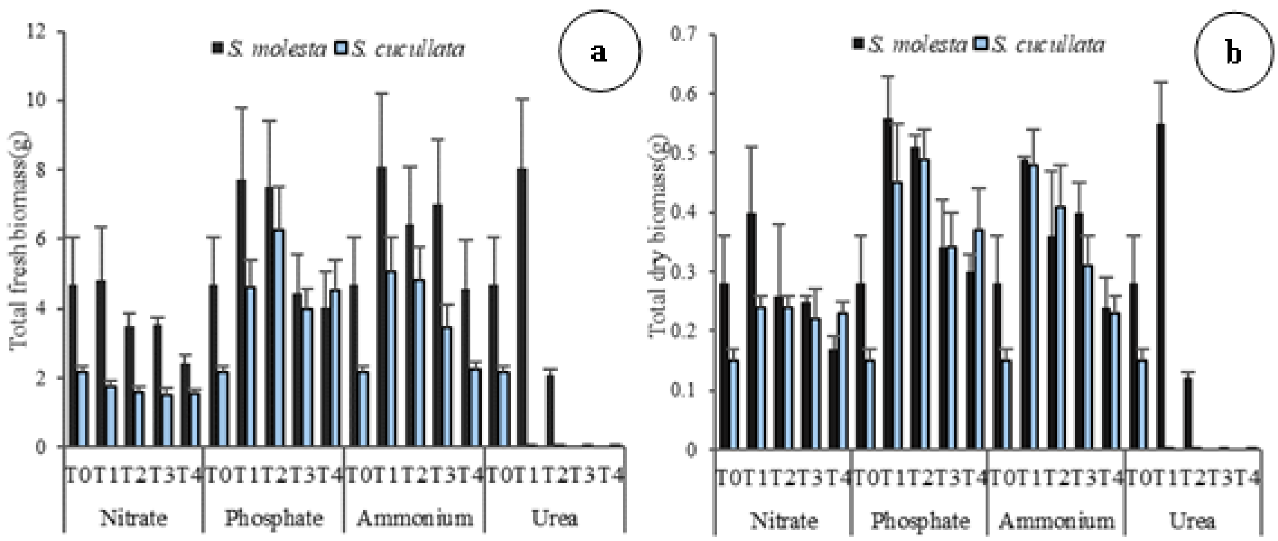

Figure 1. Combine effect of different concentrations of nutrients on (a) total fresh biomass production, (b) total dry biomass production (mean ± SD) of *Salvinia* spp.

Relative growth rate and doubling time

The nutrients and their concentrations impacted the growth of *Salvinia* spp. (Fig. 2). The plants' relative growth rates (RGRs) regarding nutrients were higher in phosphate and ammonium than in nitrate and urea. Similar results were found in *S. molesta* (Cary and Weerts 1984) and *Glyceria maxima*, where plants grown in NH_4^+ had a higher growth rate than NO_3^- (Tylova-Munzarova *et al*., 2005). The maximum relative growth rate (0.085 mg g^{-1} day^{-1}) was observed in *S. molesta*, which grew at 5 mM nutrient concentration of phosphate. In *S. cucullata*, the highest (0.067 mg g^{-1} day^{-1}) RGRs were observed at 1 mM of NH_4^+, but at higher concentrations of NH_4^+, the RGRs of the plants were significantly lower. Ammonium is more favorable over nitrate as an N-source for many species of aquatic macrophytes, possibly because of the lower energy needed for its uptake and assimilation (Miller and Cramer 2005; Fang *et al*., 2007; Jampeetong and Brix 2009a).

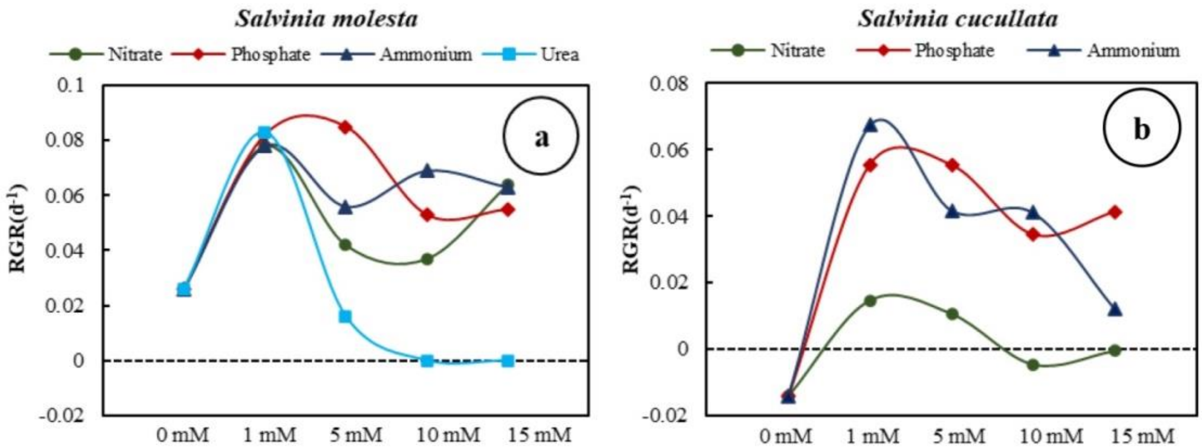

Figure 2. Relative growth rates (RGR) on a dry weight basis for (a) *S. molesta* and (b) *S. cucullata* grown at different concentrations (0 mM, 1 mM, 5 mM, 10 mM and 15 of nutrients (Nitrate, phosphate, ammonium and urea)

The minimum doubling time (DT) of the frond number was recorded (4.17 days) in 1 mM of NH_4^+, and the maximum DT of the frond number was recorded (6.84 days) in 1 mM of urea for *S. molesta*. In the case of *S. cucullata*, *the* minimum DT of the frond number was recorded (5.41 days) in 5 mM of PO_4^{3-} and the maximum DT of the frond number was recorded (22.10 days) in 10 mM of NO_3^- (Fig. 3). It was apparent that the sources of NH_4^+ and PO_4^{3-} were superior in resulting in higher numbers of leaves, slightly higher relative growth rates, and shorter doubling times than plants fed with the same level of NO_3^-N or urea-N. A similar pattern of results was also observed by Cary and Weerts (1983a), Finlayson (1984), and Mitchell and Tur (1975) for *Salvinia*.

The accumulated ion content in plant tissues

The accumulated ion contents (NO_3^-, Na^+, K^+, PO_4^{3}, $NH_4^{+)}$ in both leaves and roots differed between *S. molesta* and *S. cucullata*. NO_3^- and NH_4^+ content in leaves and roots were significantly affected in *S. molesta*. On the other hand, K^+, PO_4^{3-}, and NH_4^+ contents were affected considerably in the leaves and roots of *S. cucullata.* However, Na^+ and K^+ contents were reversely involved in the leaves and roots of both plants (Table 3, Fig. 4). Accumulation of ion contents in tissues varied with species and types of tissues, but differences between treatments were negligible. Konnerup and Brix (2010) observed that the NO_3^- uptake by *Cannabis indica* L. was not affected by the type of nitrogen sources. In this study, NH_4^+ content in plant tissue was higher in ammonium-fed plants than in other nutrient-fed plants because of the availability and less energy to uptake. Different studies have indicated that NH_4^+fed aquatic macrophytes grow well because less energy is needed for NH_4^+uptake than other nitrogen sources (Room and Thomas 1986; Fang *et al*., 2007; Jampeetong and Brix 2009a; Konnerup and Brix 2010).

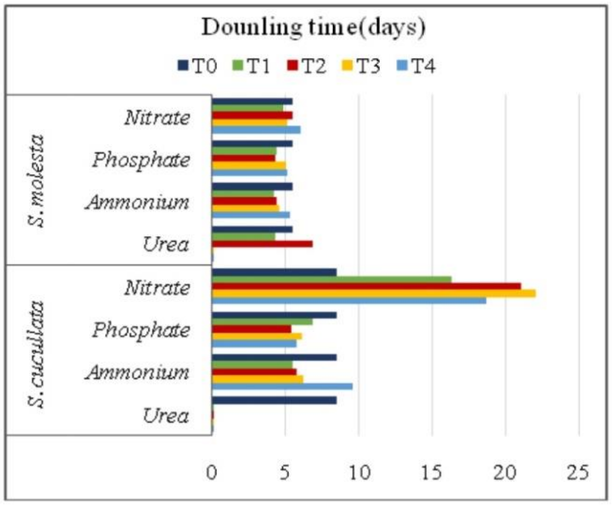

Figure 3. The doubling times (DT in days) of *S. molesta*and *S. cucullata*plants in different concentrations of nutrients.

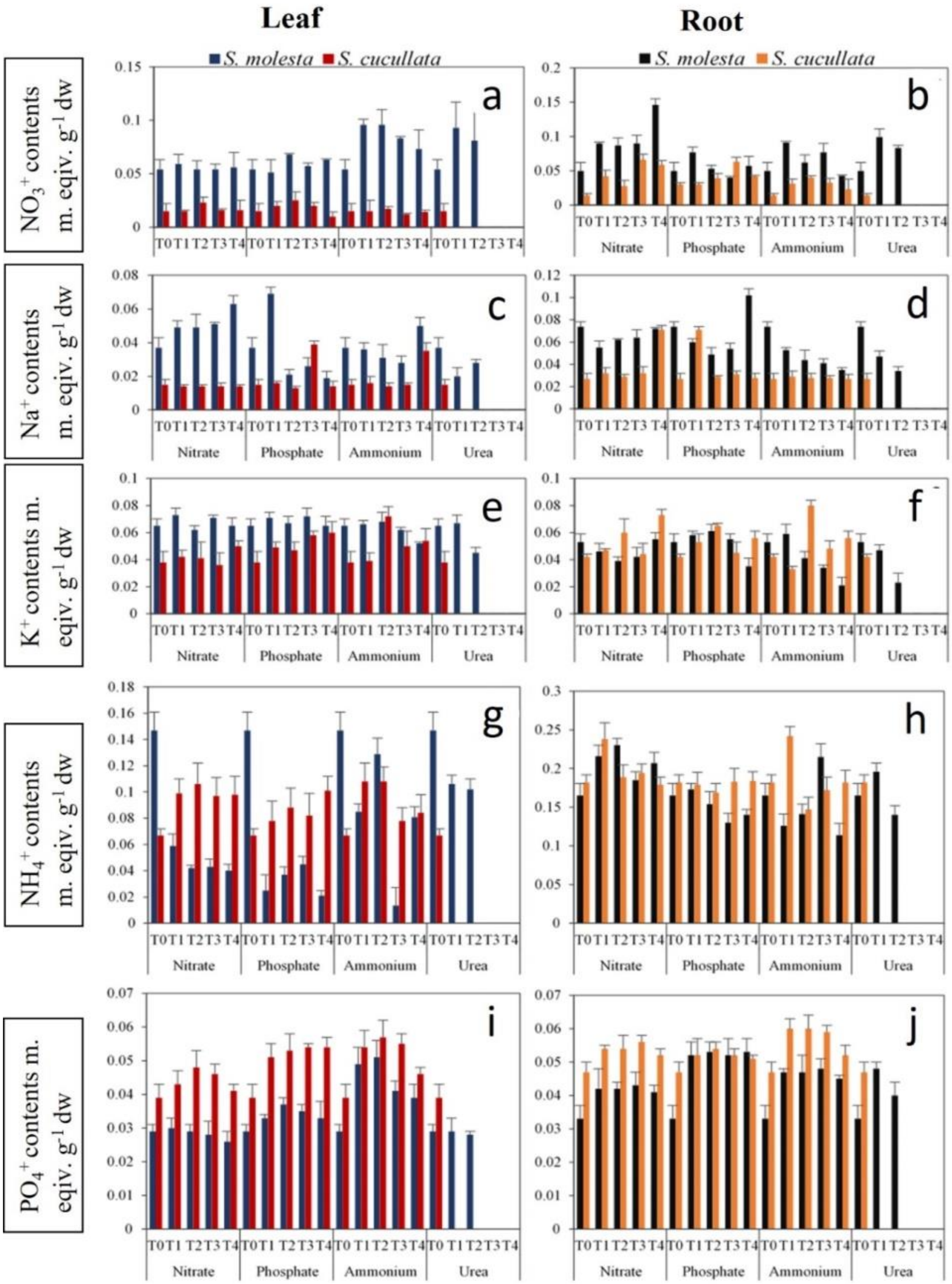

Figure 4. The accumulated ion contents (NO_3^-, Na^+, K^+, PO_4^{3-}, NH_4^+) in both leaves and roots of *S. molesta* and *S. cucullata*grown in different concentrations of nutrients.

Table 3. Results of two-way ANOVA (F-value) of Chlorophyll contents and accumulated ion contents in *S. molesta* and *S. cucullata* grown in different concentrations of nutrient.

	S. molesta			*S. cucullata*		
	Main effect		Interaction	Main effect		Interaction
	Nutrients	Conc.	Nutrients × Conc.	Nutrients	Conc.	Nutrients × Conc.
Chl-a (mg g^{-1}FW)	8.80**	10.98**	3.35**	7.97**	2.21^{NS}	2.31*
Chl-b (mg g^{-1}FW)	7.08**	6.40**	2.66*	35.89**	2.22^{NS}	6.19**
Total Chla+b (mg g^{-1}FW)	10.89**	11.23**	3.88**	38.89**	2.22^{NS}	6.19**
NO_3^- contents (m. eqiv.g^{-1} DW)						
Leaves	4.44*	6.24**	4.43**	3.15*	3.42*	0.70^{NS}
Roots	22.81**	14.13**	13.87**	2.61^{NS}	16.93**	7.18**
Na^+ contents (m.eqiv.g^{-1} DW)						
Leaves	5.84*	1.07^{NS}	1.18^{NS}	0.63^{NS}	0.45^{NS}	2.02^{NS}
Roots	8.97**	4.66*	4.15**	3.10*	1.12^{NS}	1.52^{NS}
K^+ contents (m.eqiv.g^{-1} DW)						
Leaves	13.07**	6.64**	3.38**	15.45**	2.61^{NS}	4.72**
Roots	4.55*	3.91^{NS}	1.09^{NS}	3.17*	4.43*	2.88*
PO_4^{3-} contents (m.eqiv.g^{-1} DW)						
Leaves	7.99**	13.81**	1.69^{NS}	42.22**	14.00**	17.23**
Roots	2.34^{NS}	1.76^{NS}	1.33^{NS}	16.86**	18.57**	15.98**
NH_4^+ contents (m.eqiv.g^{-1} DW)						
Leaves	19.91**	4.63**	2.82*	609.55**	3.74**	39.63**
Roots	748.80**	219.94**	213.69**	2055.70**	33.49**	130.74**

* ($P \leq 0.05$); ** ($P \leq 0.01$); NS (non-significant)

Chlorophyll contents

The concentrations of nutrients impacted the amounts of chlorophyll (Fig. 5). At 1 mM and 5 mM concentrations of NH_4^+, respectively, the maximum amount of chlorophyll was found in *S. molesta* and *S. cucullata*. Still, the chlorophyll contents declined as the NH_4^+concentration in the media increased. According to Jampeetong *et al*. (2012a), *Salvinia* spp.'s chlorophyll contents decreased as NH_4^+ supply increased, mainly when NH_4^+ levels were above 10 mM. This reduction in chlorophyll content may impact the plants' rate of photosynthetic activity. Plants fed ammonium had higher levels of chlorophyll than those provided phosphate, nitrate, or urea.

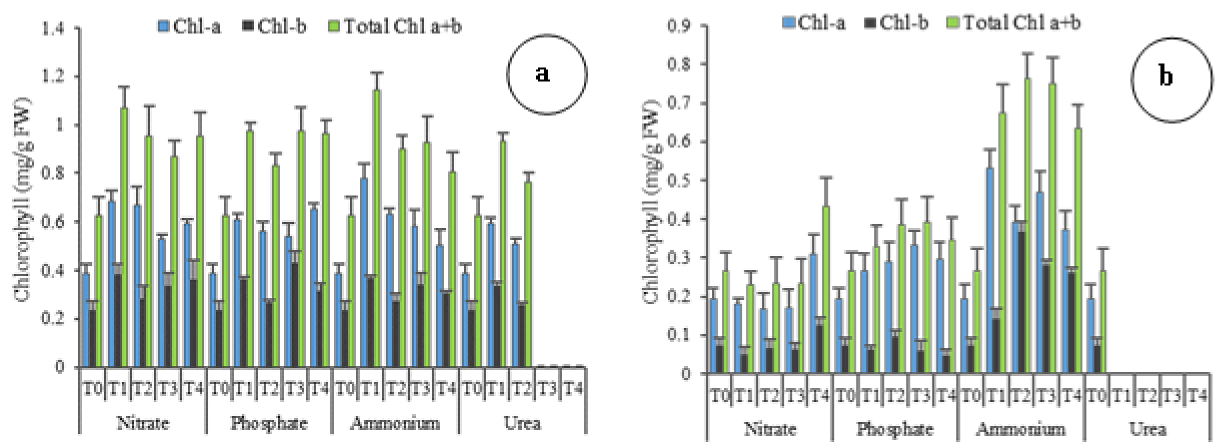

Figure 5. Amount of chlorophyll (mean ± SD) in the fresh leaf tissue of (a) *S. molesta* and (b) *S. cucullata*under different concentration of nutrients.

Conclusions

The results indicated that ammonium and phosphate-fed plants had better RGR, leaf number, total biomass, leaf area per plant, chlorophyll content, and less doubling time than nitrate and urea. In terms of concentration, nutrient concentrations between 1 mM and 5 mM produced better results. The results of the study suggest that plants responded reasonably well to individual doses of 1 mM and 5 mM of phosphate and ammonium. Consequently, it could be helpful to carry out more research on the combination application of these nutrients to see if it can result in even greater biomass production and plant development.

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Conflict of Interests

The authors declared no conflict of interest.

Statement of author's credit

The program was entirely imagined and designed by all the authors. Nargis Sultana wrote the main article and carried out the experiments, including the statistical analyses. Subroto K. Das and Shawon Mitra supervised the experiments and offered advice on how to write the manuscript. The final draft of the manuscript was approved by all the authors.

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