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GROWTH AND BIOCHEMICAL RESPONSE OF *AZOLLA CAROLINIANA* WILLD TO SOLUBLE NPK FERTILIZERS

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Abstract: Azolla is an aquatic fern, which grows quickly on the surface of the water. Which holds the promise of providing sustainable food for livestock, fish, and poultry because it contains tremendous amounts of protein, amino acids, vitamins. Besides, it can also be used in farming as biofertilizer. The objective of this work is to study the effect of different fertilizer units of NPK plus trace elements (0-0-0; 15-15-15 and 20-20-20) on biomass, Relative growth rates (RGR), doubling time and the chlorophyll content of *Azolla caroliniana* under controlled conditions. The test was carried out in an automated greenhouse. The azolla was cultivated in 0.016 m^3 tanks in different culture solutions, the control constitutes only water, the second batch contains a solution enriched with NPK 15-15-15 plus TE and the last batch constitutes a mixture of water plus NPK 20-20-20 plus TE. All these batches are in four repetitions dispositive. The results obtained show that the biomass, growth index, as well as the chlorophyll content of *Azolla caroliniana*, were important in the culture solution enriched with NPK 15-15-15 compared to that found in the medium pure water and NPK 20-20-20. On the other hand, the azolla doubling time increased in the control groups.

Keywords: *Azolla caroliniana*, biomass, chlorophyll, doubling time, NPK fertilizer.

INTRODUCTION

Azolla is an aquatic free-floating fern belonging to the family Salviniaceae. It doubles its biomass in 3-10 days (Hasan et al,2009). This fern exhibits high relative growth rates (RGRs) when grown individually on an open water surface, i.e. not competing for light. RGRs of over 0.5 d^{-1}, or biomass doubling times of less than 2 days have been reported – much higher than generally encountered in land plants (Maejima, 2001; van der Werf, 1998). However, Azolla does have several unquestionable agronomic qualities: the capacity to fix atmospheric nitrogen, very high productivity in the right environment (Van Hove et al., 1996). It can fix N in large quantities, approximately twice that of Rhizobia living in soybean nodules (Hung et al., 2013; Brouwer et al., 2017). It is also a probable source of nitrogen as well as a feeding ingredient for livestock (Lumpkin, 1984).In addition, The use of aquatic macrophytes, such as *Azolla* with hyperaccumulating ability is known to be an environmentally friendly option to restore polluted aquatic resources (Sood, 2012) . The dead or pretreated Azolla biomass has been used by various workers for the biosorption of heavy metals (Cohen-Shoel et al, 2002; Rakhshaee et al., 2006; Umali et al., 2006; Nedumaran and Velan, 2008; Mashkani and Ghazvini, 2009).

Looking for alternatives to (concentrates, fodder, feed) to various species of animals, magnificent plant called Azolla was revealed, it is expected to provide sustainable feed for livestock. Since Azolla contains most of the nutrients necessary for all livestock including livestock and fish (Gouri, 2012). Nutritive value of Azolla is well documented which shows that it is a good source of high protein yields with almost all essential amino acids (especially lysine) required for animal nutrition **(**Hossiny et al., 2008; Brouwer, 2018). and provitamins for poultry nutrition (Lejeunea et al., 1999 Lumpkin, 1984**).** To achieve the goal of increasing livestock productivity; an important step is to simplify the cultivation of Azolla in livestock breeding areas (Singh, 2008).

Like many other photosynthetic aquatic organisms, Phosphorus (P) is the main nutrient limiting the growth of Azolla spp (Sadeghi et al., 2013; Temmink et al., 2018), The effects of N, P, and K on the growth of *A. caroliniana* depended upon the growing season, the characters of different species of *Azolla*, the environmental conditions, and the amount of fertilizers. The application of P was showed a positive effect in all the seasons. Under low temperature the application of K was effective. Nitrogen fertilizers generally inhibited the growth of healthy Azolla under suitable environmental condition except in winter. The N, P and K were comparatively effective under the weak light intensity or for weak Azolla. A small amount of N could promote the propagation of *A. caroliniana* (Linhuo, 1986). Doubling time (Dt) defined by Stewart and Boyd (1999) as the time required for a relative amount to increase in value from one to two, undoubtedly affects biomass production in Azolla. The aim of this trial is to promote

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the hydroponics of *Azolla caroliniana* in greenhouses as a potential alternative source of food for broilers and goats in the arid areas of Algeria. Thus, the objectives of this study were to assess the effects of NPK fertilization levels on biomass, water content, and the amount of *Azolla caroliniana* chlorophyll.

MATERIALS AND METHODS

The trial was shared between biology laboratory and environmentally controlled greenhouse located at Naâma University Center, Algeria (33°16'36.67"N, 0°19'8.54"O). During the period from January 2020 to April 2020.

Plant material

The vegetable material used is an Azolla. Before starting the experiment, *A. caroliniana* maintained in an aquaculture pond, was acclimated to experimental conditions by inoculating 20 g fresh weight (FW) into the 16 L drums. Ponds were drained and cleaned, and freshwater was added to a depth of 0.2 m. Thus, the volume of added water was 16 L. Then, we divided our experimental device into three batches, the first constitutes test plants (fresh water) called FU00, the second batch was enriched with NPK fertilizer plus trace elements, i.e. FU15 and the last batch of the culture of *Azolla* contains NPK 20-20-20 plus trace elements named FU20. The applied dose of NPK (FU15 and FU 20) in the culture solution is 5 g / L. pH of the solution is maintained in the value 6.8 as well as the water has been changed every three days with the same filling and emptying flow to ensure good oxygenation of environment culture of *Azolla*. Fertilizer addition to the fresh pond water was according to treatment,

The experimental design was adopted at one factor of NPK fertilizer with three rates (figure 1) 0-0-0 (FU00) ; 15-15-15 plus TE (FU15) and 20-20-20 plus TE (FU20).

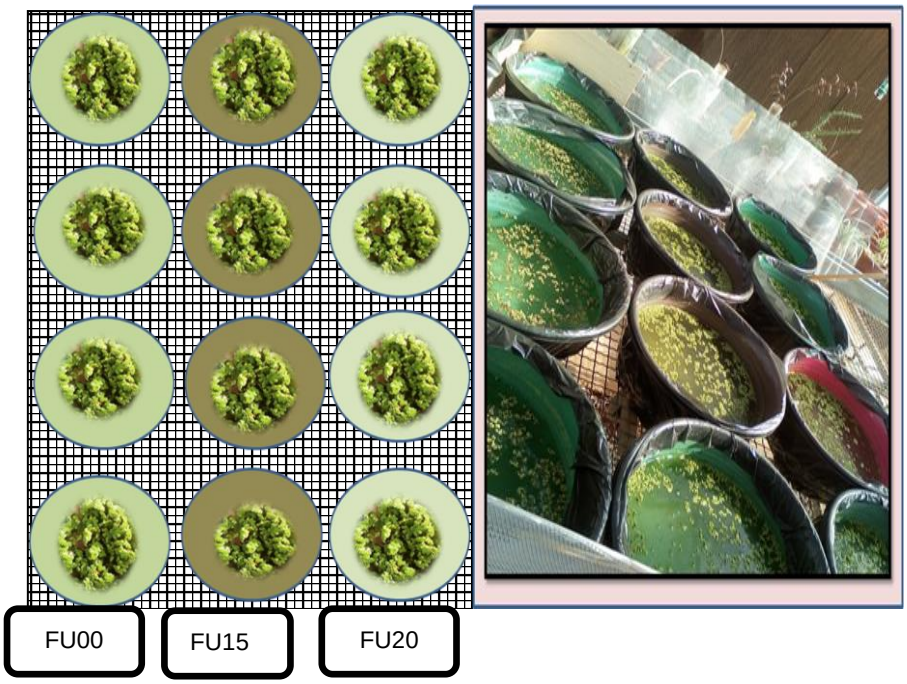


Fig. 1 Experimental design under green housse of Naâma university center.

Fresh biomass weight was determined by taking the plants out of the ponds and carefully blotting them dry on a paper towel before weighing them in (g).

Doubling time (d) and Relative growth rate (g/g/d) were calculated using the formula reported by Badayos (1989) and Hechler and Dawson (1995), respectively.

$$\mathbf{Dt} = \frac{0.69\ t}{\ln Af/A0}$$

where: Af: final biomass Ao: initial biomass t = growth period

RGR1-2 = ln mass2 – ln mass1/T2 – T1

Chlorophyll content was determined according to the method developed by Coombs *et al* (1985). It is carried out in the mixture of acetone and ethanol (75% and 25%) in volume and 80% and 20% in concentration. An amount of 0.01 g of fresh leaf samples is added 10 ml of a mixture of acetone and ethanol of volumes Respectively 75 and 25% with two concentrations of 80 and 20%. After 10 min of centrifugation at 5000 rpm at 4 ° C., Spectrophotometric reading was taken at 645 and 663 nm wavelengths. Data were assessed in formulate:

Chl a = 12,7x DO (663) – 2,59x DO (645) x V/ (1000x W).

Chl b = 22,9 x DO (645) – 4,68 x DO (663) x V/ (1000x W). Total Chl = Chl a + Chl b

V: volume extracted solution, and W the weight of fresh material of the sample. The Unit of Chlorophyll is µg/g FW.

The experiment was arranged in a completely randomized design with four replications. The level of significance for ANOVA was set at $p< 0.05$. The Data were subjected to analyses using STATISTICA 8. Unless otherwise stated, values shown are means ± SD.

RESULTS

Biomass

The illustration of results in figure 2a reveals that the biomass was important in the plants which are in the cultivation solutions FU 15 and FU 20 with values 39.68 and 37.93 g progressively. However, the lowest biomass values of *Azolla caroliniana* Willd were recorded in plants grown in a hydroponic solution of FU00, with 40 g.

Doubling time and relative growth rate

The results obtained are illustrated in figure 2 b and c, showing that the highest value of the maximum relative growth rate reached 0.10 g/g/d which corresponded to 7.35 days of doubling and in most of the *Azolla caroliniana* cultivated in the solution of FU15, As the plants grew, the growth rate slowed down more severely in FU00 with 0.07 g/g/d corresponding a doubling time of 11.12 days.

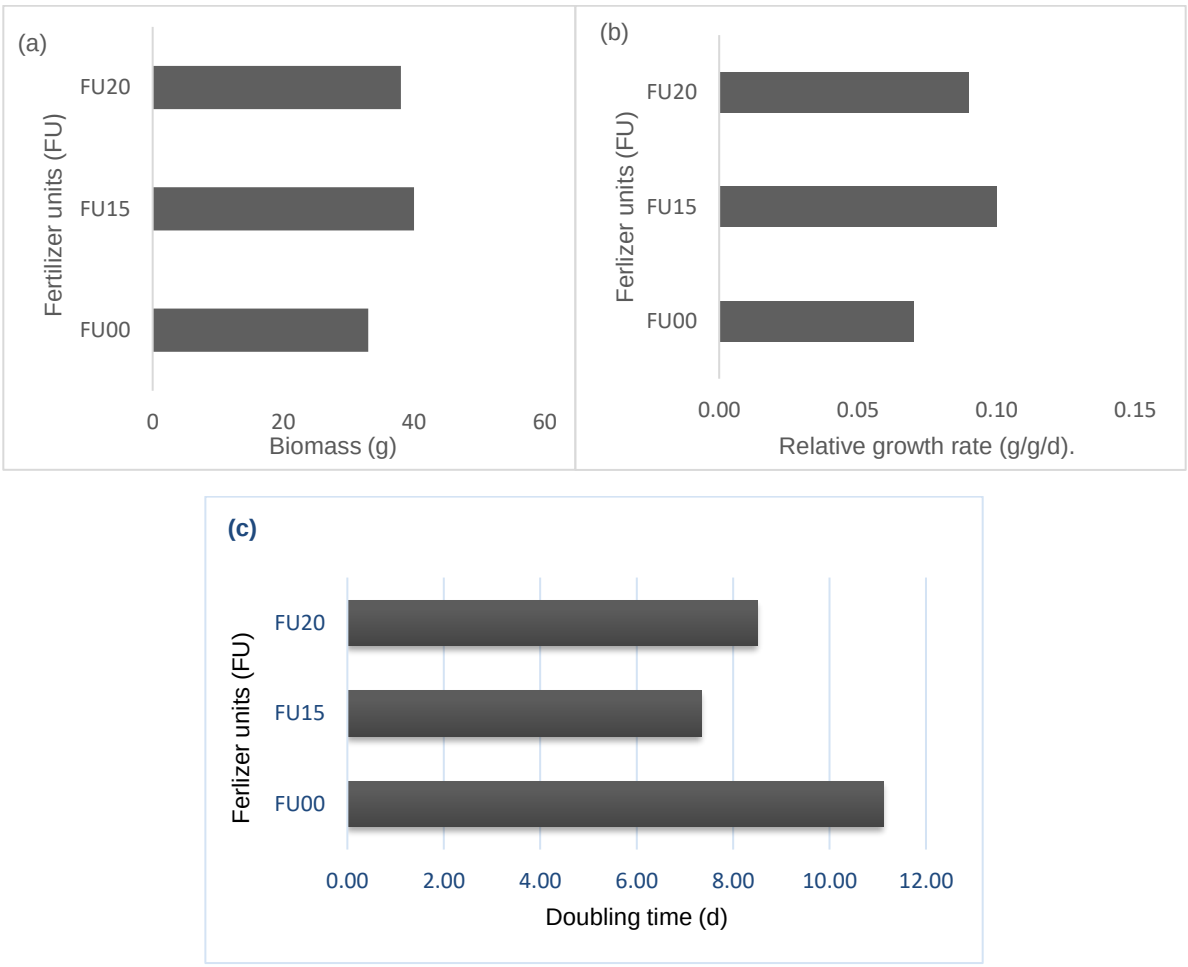


Fig. 2 Effect of fertilizer units (FU) on growth parametrs of *Azolla caroliniana* Willd. (a) Biomass ; (b) Relative growth rate (RGR) and (c) Doubling time.

Chlorophyll content

The analysis of the ANOVA at the threshold of a probability $p<0.05$ shows a significant effect of NPK fertilizer (FU) on the content of chlorophyll (a) in the leaves of *Azolla caroliniana* ($p=0.0012$). In fact, the highest content of this pigment was recorded in the subjects cultivated in the solution enriched by FU15 with an average of 6.4 µg/g FW. While the plants which are in the culture mixture FU00 and FU20 reached 3 and 4.88 µg/g FW of chlorophyll (a) in the leaves of *Azolla* (figure.3a).

The applied fertilizers FU15 and FU20 reported significant effects ($p<0.05$) on chlorophyll (b) in the leaves of Azolla. Indeed, the chlorophyll b amount of the Azolla was increased in the FU15 and FU20. The highest values were observed in the plants found in the solution treated with FU20 compared to control batch (figure.3b).

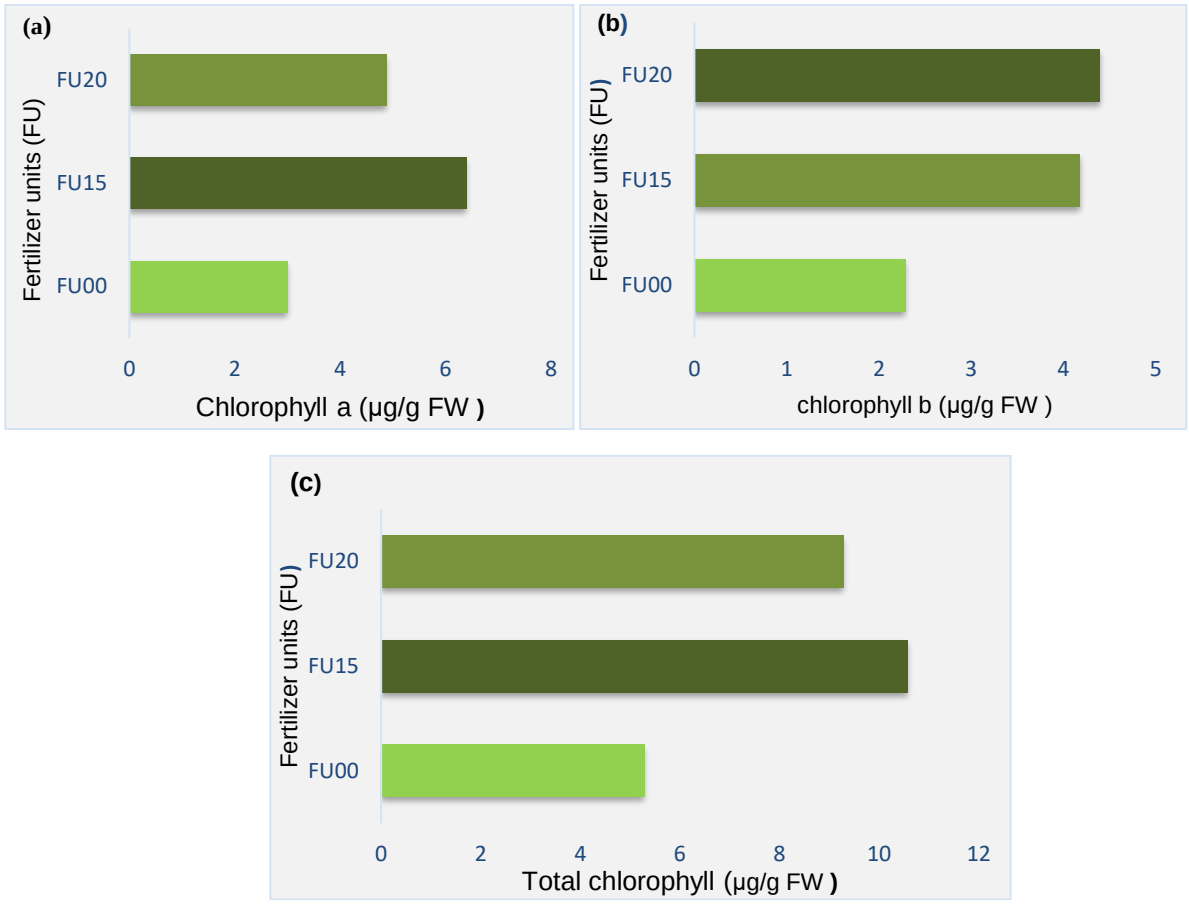
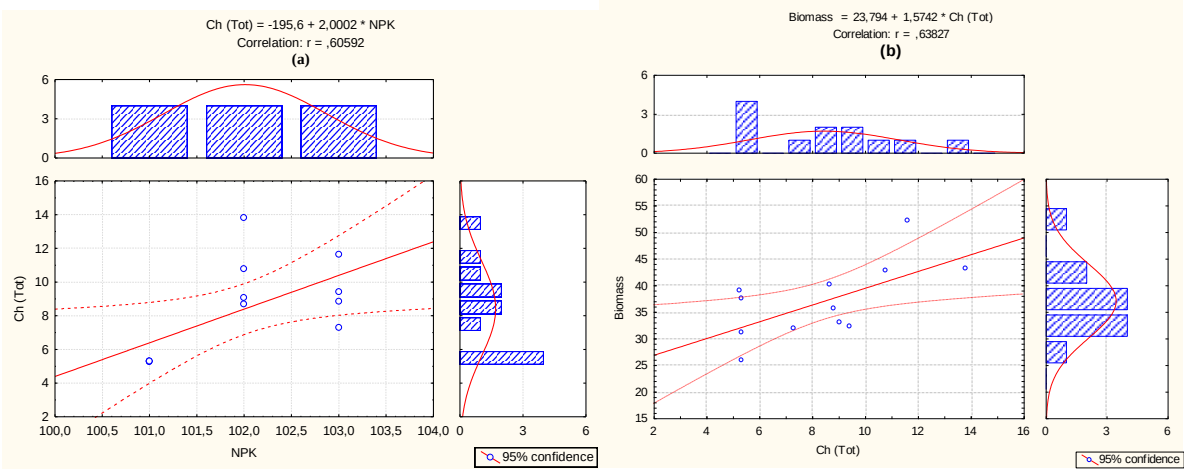


Fig. 3 Effect of Fertilizer units (FU) on chlorophyll of *Azolla caroliniana* Willd. Chlorophyll (a) content; (b): Chlorophyll (b) content; (c): Total chlorophyll content.

The analysis of the variance at the threshold of an error $p < 0.05$ of the effect of fertilizer units (FU) on the total chlorophyll in the leaves of the *Azolla caroliniana* Willd shows a highly significant effect (P = 0, 00449), this pigment of plants was proportional to the fertilizers. Indeed, the FU00 (the control plants) caused a significant decrease in total chlorophyll compared to Azolla cultivated in FU15 where the pigment reached its minimum of 10.58 µg/g FW (figure 3c).

Correlation between parameters and factors

Table 1 and the illustration of figures 4 (a, b, c and d), shows the correlations at $p < 0.05$ between the parameters studied under the effect of the fertilizer units NPK (FU). We note positive and significant correlations between total chlorophyll and RGR and biomass with r = 0.62 and 0.5 respectively. However, the doubling is negatively and significantly correlated with RGR and Azolla biomass, ie r = 0.93 and r = 0.86 progressively. On the other hand, the NPK (FU) factor is positively correlated with chlorophyll b and total with the values of r ie 0.63 and 0.59 respectively.



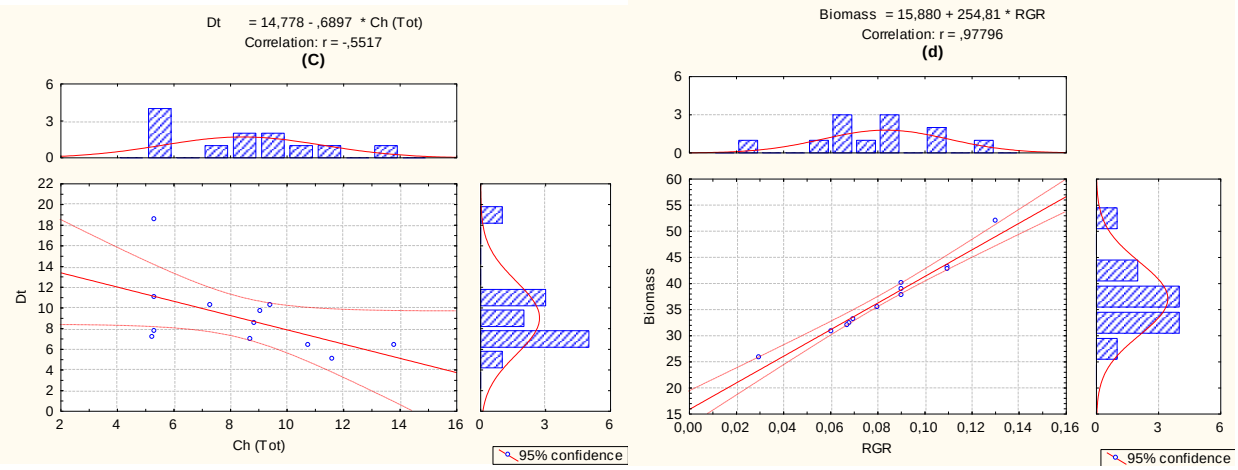


Fig. 4 Correlation between pair of variables.
a :Correlation between chlorophyll and NPK (FU) ; b : Correlation between biomass and chlorophyll ; c :Correlation between doubling time and chlorophyll ; d : Correlation between biomass and relative growth rate. Dt : doubling time ; RGR : relative growth rate, Ch(tot) : Total chlorophyll ; r : correlation coefficient.

Table 1

Correlations between each pair of variables

Variable	StDev.	NPK	Ch(a)	Ch(b)	Ch(tot)	Dt	RGR	Biomass
NPK	0.85	1.00	0.43	**0.63**	**0.59**	-0.32	0.30	0.2
Ch (a)	0.15	0.43	1.00	0.49	**0.92**	-0.44	0.51	0.4
Ch (b)	0.10	**0.63**	0.49	1.00	**0.79**	-0044	**0.59**	**0.6**
Ch (Tot)	0.22	**0.59**	**0.92**	**0.79**	1.00	-0.51	**0.62**	**0.5**
Dt	3.52	0.32	-0.44	-0.44	-0.51	1.00	**-0.93**	**-0.86**
RGR	0.03	0.30	0.51	**0.59**	**0.62**	**-0.93**	1.00	**0.98**
Biomass	6.94	0.28	0.46	**0.60**	**0.59**	**-0.86**	**0.98**	1.00

Significant values at : $p < 0.05$, ($n = 36$). Data showed the correlation coefficients (r) and probability (p) between each pair of variables. In bold significant correlation ; RGR : relative growth rate ; Dt : doubling time, Ch(tot) : Total chlorophyll.

DISCUSSIONS

The result of this study showed that fertilizer unit FU 15 significantly increased the biomass and relative growth rate of *Azolla caroliniana Willd* compared to batches of plants. Application of phosphorus increased the growth, biomass and Nz-fixation of Azolla (Singh et al., 1990). The phosphorus-enriched Azolla maintained higher plant phosphorus content and produced a greater biomass and nitrogen yield than the unenriched Azolla. Application of phosphorus during intercropping significantly increased the dry weight (Singh, 2008). Liu (1985) showed that 1g Azolla biomass is able to take up about 70% of K from 800 ml of culture solution containing 0.85 ppm K 2 O in 1 d. This may be considered the physiological critical point of Azolla's K requirement. Ehab and Abdel-fatah (2020) reported that the fertilized treatments (organic et inorganic) were associated with an increase in biomass compared with the control group, with the highest values occurring with the organic treatment. growth increased following treatment with organic and inorganic fertilizers of Azolla Sp. Against, we noted an increase the doubling time in control group but when enrich of culture solution with NPK, this parameter decreases significantly.

The maximum content of chlorophyll in *Azolla caroliniana Willd* samples occurred in the lots grown in the hydroponic solution enriched with NPK 15-15-15 (FU15), while the minimum value of this pigment was found in the control treatment group and NPK 20-20-20 (FU20) respectively. Ren et al. (2017) showed that with an increase in plant density, chlorophyll (a) and (b) contents significantly decreased, leading to a decreased photosynthetic rate during plant growth. These results are similar to those obtained for this study. Fertilization increases chlorophyll, nutrient contents (N and P), biochemical composition, and growth rate of Azolla species. Adding, fertilizers to Azolla sp. enhances its nutrition value (Temmink et al., 2018).

CONCLUSION

From the results, it is concluded that *Azolla caroliniana Willd* can grow quite well in treated NPK 15-15-15, having chlorophyll content and growth rates and biomass great enough to encourage its utilization. Indeed, as *Azolla caroliniana* presents some interest for its use as an economical and sustainable crop given its high biomass under the effect of NPK 15-15-15 in hydroponics. Moreover, fodder rich in proteins, it would be more advantageous to carry out an in-depth research on the association of this fern with other fodder steppe plants to reach the ideal and balanced ration in particular in the Sheep, and the dairy cattle breeding.

AUTHORS CONTRIBUTIONS

Nouri Tayeb: Supervisor and Laboratory manipulation, methodology construction; Bouyahia

Hadj: Proposition of the studied species and problematic; Amari Zahira and Arbaoui: Laboratory manipulation and monitoring the evolution of the species "Azolla" in the greenhouse; Mekhloufi Moulay Brahim and Badji Abderrezak: Data validation, statistical analyzes and proofreading.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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