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NUTRIENT SOLUTION NO_3^-:NH_4^+, CONCENTRATION AND pH IN GROWTH OF *Digitalis purpurea* L.

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ABSTRACT

Fox glove (*Digitalis purpurea* L.) is a biannual herbaceous plant native to Europe. It is used as an ornamental and has medicinal uses in treatment of cardiac insufficiency. In this study, we evaluated the effect of NO_3^-:NH_4^+ ratio, concentration and pH of the nutrient solution on plant growth. The experiment was conducted in the Colegio of Postgraduados, Campus Montecillo, from January to May, 2016. The design was completely randomized with a 2^3 factorial: NO_3^-:NH_4^+ ratio (100:0, 80:20), nutrient solution concentration (50%, 100%) and pH (5, 7). Eight treatments with six replications were obtained. We assessed number of leaves, SPAD readings, NO_3^- concentration, Ca^{2+} and K^+ in petiole extract, plant height, number of shoots, stem diameter, and plant dry weight. The NO_3^-:NH_4^+ ratio 80:20 significantly increased SPAD readings, Ca^{2+} concentration, plant height and stem dry weight. The 100% solution significantly increased the number of leaves, NO_3^-, Ca^{2+}, and K^+ concentrations. pH 5 increased significantly the number of leaves, SPAD readings, NO_3^- concentration, number of shoots, and root, stem and leaf dry weight. pH 7 significantly increased K^+ concentration. The treatment with the NO_3^-:NH_4^+ ratio of 80:20, 100% solution and pH 5 was that which produced significantly higher values of the variables evaluated.

Keywords: *Digitalis purpurea*, Hydroponics, SPAD readings

Introduction

Digitalis purpurea is a biannual herbaceous plant, native to Europe, but it can be found wild in disturbed areas of Mexico (Christenhusz, 2011). This species is one of the main sources of cardiac glycosides (Chong-Pérez *et al*., 2008) such as digitalina (Barquero, 2007), digoxin and digitoxin (Pérez-Alonso *et al*., 2014), which are used in the treatment of cardiac insufficiency (Ávalos and Pérez-Urria, 2009). González *et al*. (2003) mention that digitoxin is one of the drugs most widely used for problems of cardiac insufficiency and auricular tachyarrhythmias. The most accepted therapeutic interval oscillates between 0.8 and 1.9 ng mL^{-1} (Nogué *et al*., 2012).

These figures do not have an absolute value nor are they determinant at the moment of adjusting the dosage. This means that there is no standard dosage of digoxin, but rather the dosage must be adjusted for age, body area and renal function (González *et al*., 2003). *D. purpurea* is a very attractive plant, highly sought for its showy flowers (Vega, 1988), which are used to adorn temples for religious ceremonies (Pardo, 2008). It is also cultivated as an ornamental (Christenhusz, 2011) in patios and gardens with other seasonal plants or herbs. However, there are few studies on the nutrient requirements of this species. Some of these studies have looked into hydroponics as an alternative to achieve high yields per square meter (Sandoval *et al*., 2007), temperature control and pest and disease control (Lara, 1999) and efficient control of nutrient concentrations in plant nutrient solutions (Bugarin-Montoya *et al*., 2002). The objective of this study was to evaluate the effect of the $NO_3^-:NH_4^+$ ratio, concentration and pH of the nutrient solution on growth of *D. purpurea* in greenhouse hydroponics.

Materials and methods

Experimental site

The study was conducted at the Colegio de Postgraduados, Campus Montecillo, 19° 27’ 38’’ N and 98° 54’ 11’’ W, at an altitude of 2 250 m. The plants grew in hydroponics in a tunnel type greenhouse, 22.75 m^2, of the Area of Plant Nutrition.

Experimental design

The experimental design was completely randomized with a 2^3 factorial array: $NO_3^-:NH_4^+$ ratio (%), 100:0 and 80:20; nutrient solution concentration, 50% and 100%; and nutrient solution pH, 5 and 7. We obtained eight treatments with six replications. Each experimental unit consisted of one plant in a 30x45x30 cm black polyethylene bag with red volcanic sand (tezontle) as the substrate. There was a total of 48 experimental units.

Nutrient solution

Steiner (1964) Universal Solution was used to prepare the nutrient solutions in 20 L plastic containers. pH was adjusted to 5 and 7, depending on the respective solution, with NaOH or H_2SO_4. The treatments were applied to the plants as of five months of age. Irrigation was daily at 11:00 h applying 1.0 L of nutrient solution.

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Evaluated variables

Every two weeks, leaves per plant were counted and SPAD readings were carried out with SPAD-502 (Minolta). With portable ionometers (HORIBA®), concentrations of NO_3^-, Ca^{2+} and K^+ were determined by shredding the petioles of four newly mature leaves taken from the four cardinal points (Alcántar and Sandoval, 1999; Hard *et al*., 2003); these readings were obtained only 90 days after setting up the experiment between 7:00 and 8:00 h. At harvest (105 days after beginning the experiment), the number of shoots from each plant was counted. Stem diameter (mm) was measured with an electronic digital precision Vernier (Truper). Plant height (cm) from the base of the stem to the tip of the inflorescence was determined with a flexometer. To obtain dry weight, each plant organ was placed in paper bags, dried in an oven at 70 ºC for 72 h (Villar *et al*., 2005) and weighed on an analytical balance.

Statistical analysis

An analysis of variance of each variable was performed and means were compared with the Tukey test (α=0.05) using the NCSS (Number Cruncher Statistical System) demo software in the internet 2016.

Results and discussion

Analysis of variance

pH affected the number of leaves from 45 to 105 days. The concentration of the solution and the interaction between solution concentration and pH did not have an influence until 105 days. SPAD readings showed influence of the NO_3^-:NH_4^+ ratio 80:20 from 30 to 105 days, pH had an influence from 45 to 90 days, and the interaction NO_3^-:NH_4^+-solution concentration affected only from 30 to 60 days, respectively. The NO_3^-:NH_4^+ ratio affected Ca^{2+} concentration, plant height and stem dry weight. Solution concentration affected NO_3^-, Ca^{2+} and K^+ concentrations, and pH acted on the NO_3^- and K^+ concentrations, number of shoots, and root, leaf and stem dry weight. The interaction NO_3^-:NH_4^+-solution concentration affected Ca^{2+} and K^+ concentrations, number of shoots and stem diameter. The interaction solution concentration-pH had an effect on leaf and stem dry weight. The interaction NO_3^-:NH_4^+-pH affected Ca^{2+} and K^+ concentrations, and the triple interaction of the factors affected K^+ concentration, stem diameter, plant height and leaf dry weight (Table 1).

Table 1. Analysis of variance for concentrations of NO_3^-, Ca^{2+} and K^+ in petiole extract, plant height, number of shoots, stem diameter, and root, leaf and stem dry weight of *D. purpurea*, grown in hydroponics.

Source of variation	D.F	NO_3^-	Ca^{2+}	K^+	PH	NP	SD	RDW	LDW	SDW	FDW
$NO_3^-:NH_4^+$	1	ns	*	ns	*	ns	ns	ns	ns	*	ns
CS	1	*	*	*	ns	ns	ns	ns	ns	ns	ns
$NO_3:NH_4^+$-CS	1	ns	*	*	ns	*	*	ns	ns	ns	ns
pH	1	*	ns	*	ns	*	ns	*	*	*	ns
$NO_3^-:NH_4^+$-pH	1	ns	*	*	ns	ns	ns	ns	ns	ns	ns
CS-pH	1	ns	ns	ns	ns	ns	ns	ns	*	*	ns
$NO_3^-:NH_4^+$-CS-pH	1	ns	ns	*	*	ns	*	ns	*	ns	ns
Error	40										
Total	47										

CS=solution concentration, D.F=degrees of freedom, PH=plant height, NP=number of shoots, SD=stem diameter, RDW=root dry weight, LDW=leaf dry weight, SDW=stem dry weight, FDW=flower dry weight, ns=not significant, *=significant difference ($p \leq 0.05$)

Kinetics of leaf growth

The ratio $NO_3^-:NH_4^+$ 80:20 produced a larger number of leaves than the ratio $NO_3^-:NH_4^+$ 100:0 during the entire experiment. The number of leaves on plants in the 100% nutrient solution was higher (Tukey, $p \leq 0.05$) than those on plants in the 50% solution on the 105 of the experiment. With pH 5, the number of leaves was higher (Tukey, $p \leq 0.05$) than with pH 7 from 45 to 105 days (Table 2).

Table 2. Effect of $NO_3^-:NH_4^+$ ratio, concentration and pH of the solution on number of leaves during the 105 days of the experiment with *D. purpurea* cultivated in hydroponics.

Factor	Level	Days after treatment set up						
		15	30	45	60	75	90	105
		Number of leaves						
Ratio $NO_3^-:NH_4^+$ (%)	100:0	28.7 a	51.9 a	83.4 a	108.2 a	134.4 a	159.4 a	167.7 a
	80:20	29.7 a	53.3 a	86.4 a	113.7 a	140.3 a	168.0 a	177.1 a
Solution concentration (%)	50	29.4 a	52.2 a	82.9 a	107.5 a	130.5 a	155.7 a	161.6 b
	100	29.1 a	53.0 a	86.9 a	114.3 a	144.3 a	171.8 a	183.2 a
pH	5	32.4 a	56.3 a	91.6 a	119.5 a	149.1 a	179.7 a	189.2 a
	7	26.0 a	48.9 a	78.2 b	102.4 b	125.6 b	147.7 b	155.6 b
HSD		6.706	8.792	10.268	12.421	15.122	18.495	18.842
C.V (%)		39.272	28.625	20.725	19.186	18.860	19.356	18.728

Values with the same letter are significantly different (Tukey, $p \leq 0.05$) within each factor and on each date of data collection (days). HSD=honest significant difference, C.V=coefficient of variation.

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There were significant differences (Tukey, $p \leq 0.05$) in number of leaves from 75 to 105 days due to the different levels of the factors. These data were fit to second degree polynomial equations and showed constant increase during the 105 days of the experiment (Figure 1). At 105 days the accumulated number of leaves on plants with the factor pH 5 varied from 182.6 to 195.3 leaves (a difference of 12.5 leaves), with a standard deviation (S) of 6.30. With pH 7, the accumulated number of leaves was 124.8 to 185.3 (a difference of 60.5 leaves), with S=26.37. With pH 5 the plant had greater growth. Several authors (Jones, 2012; Andreau *et al*., 2015) have suggested that optimal pH for plant growth in hydroponics is 5.0, 5.5 and 6.0; that is, at lower pH there is more availability of most micronutrients.

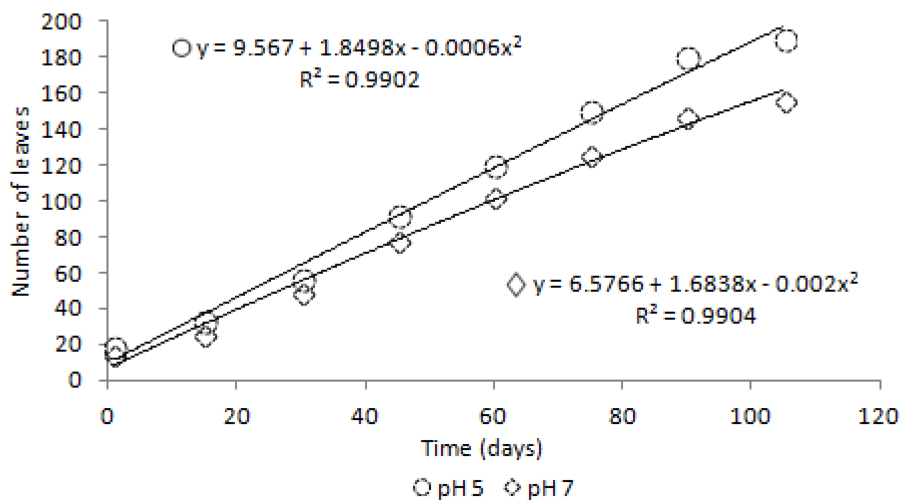

Figure 1. Trend in number of leaves during 105 days of the experiment on *D. Purpurea* with pH 5 and 7.

Kinetics of SPAD readings

SPAD readings (chlorophyll content) in the NO_3^-:NH_4^+ ratio 80:20 were higher (Tukey, $p \leq 0.05$) than in the 100:0 ratio from 30 to 105 days of the experiment. It has been demonstrated that plants supplied with part of the N as NH_4^+ produce greener leaves (Sandoval *et al*., 1999). The solution concentration (50%, 100%) did not affect SPAD readings. However, the readings were higher in plants grown in solution with pH 5 (Tukey, $p \leq 0.05$) from 45 to 90 days of the experiment (Table 3).

Table 3. Effect of the NO_3^-:NH_4^+ ratio, concentration and pH of the solution on SPAD readings during the 105 days of the experiment on *D. purpurea*, cultivated in hydroponics.

Factor	Level	Days after treatment setup						
		15	30	45	60	75	90	105
		SPAD readings						
Ratio NO_3^-:NH_4^+ (%)	100:0	37.34 a	40.50 b	42.69 b	43.70 b	40.37 b	37.76 b	35.69 b
	80:20	38.17 a	42.57 a	45.88 a	47.59 a	42.97 a	41.12 a	40.32 a
Solution concentration (%)	50	37.60 a	41.13 a	43.80 a	44.98 a	41.13 a	40.06 a	38.92 a
	100	37.90 a	41.94 a	44.77 a	46.31 a	42.21 a	38.96 a	37.10 a
pH	5	38.03 a	42.28 a	45.37 a	46.88 a	42.92 a	40.70 a	38.70 a
	7	37.47 a	40.79 a	43.19 b	44.41 b	40.42 b	38.32 b	37.32 a
HSD		1.782	1.499	1.477	1.475	1.775	2.015	2.067
C.V (%)		8.092	6.185	5.718	5.539	7.302	8.514	9.321

Values with the same letter are not significantly different (Tukey, $p \leq 0.05$) within each factor and level and on each data collection date (days). HSD=honest significant difference, C.V=coefficient of variation.

SPAD readings were significantly different (Tukey, $p \leq 0.05$) among the different levels of the factors from 30 to 105 days. These data were fit to second degree polynomial equations and showed that SPAD readings reached peak values 60 days after beginning the experiment, when the plant reached its maximum vegetative development and is in full photosynthetic process (Ribeiro *et al*., 2015). After this time (60 days), formation of floral buds began, the plant used the photosynthates, and SPAD readings showed decreasing values.

In the sampling on d 60, with pH 5, the SPAD readings varied from 44.26 to 49.33 (Figure 2), with S 2.08. With pH 7, they oscillated between 40.68 and 48.45 (Figure 2), with S 3.28. This was likely due to the greater absorption of N when the two forms of nitrogen (NO_3^-:NH_4^+) are present in the nutrient solution and to the better absorption of NO_3^- in acid pH (Mengel and Kirkby, 2000). Thus, concentrations of nitrogen and chlorophyll are higher.

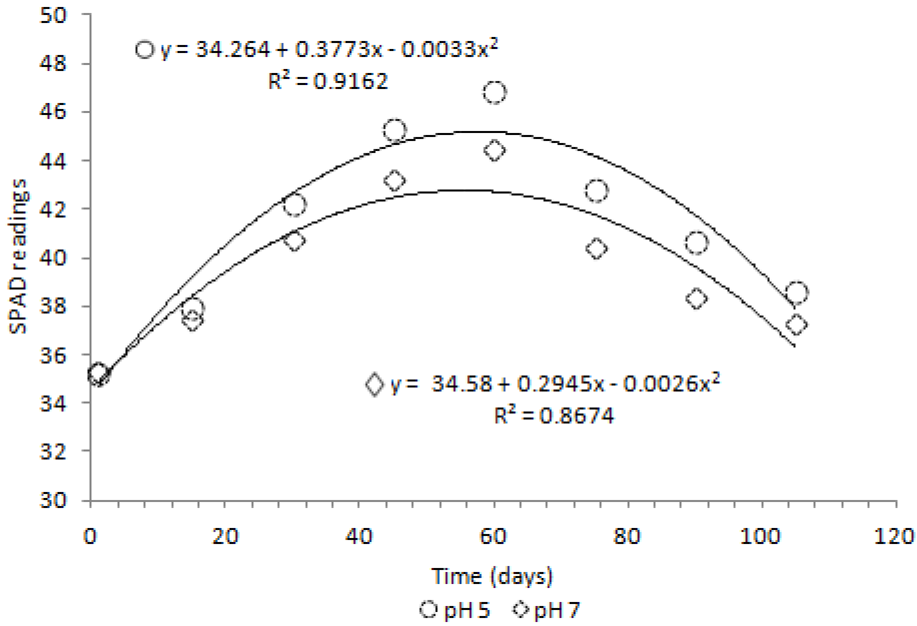

Figure 2. Trends in SPAD readings during 105 days of the experiment on *D. purpurea* with pH 5 and 7.

NO_3^-, Ca^{2+} and K^+ in petiole extract

The solution with an NO_3^-:NH_4^+ ratio of 80:20 produced a higher concentration of Ca^{2+} than that with a 100:0 ratio. Mengel and Kirkby (2000) point out that the manner and the level of the nitrogenized nutrient affects the balance of anions and cations of the plants; supplying NH_4^+ in low quantities favors absorption of Ca^{2+}. NO_3^-, Ca^{2+} and K^+ concentrations in the petiole were higher with the 100% solution (Tukey, $p \leq 0.05$) than with the 50% solution. This may be because nutrient absorption depends on a higher concentration of nutrients in the external solution (Rodríguez *et al*., 2007). In the solution with pH 5, the concentration of NO_3^- in the petiole was higher (Tukey, $p \leq 0.05$) than that found with pH 7. However, the concentration of K^+ was higher with pH 7 (Tukey, $p \leq 0.05$) than with pH 5 (Table 4). According to Mengel and Kirkby (2000), absorption of potassium is higher when pH increases.

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Table 4. Effect of NO_3^-:NH_4^+ ratio, concentration and pH of the solution on concentration of NO_3^-, Ca^{2+} and K^+ in petioles of *D. purpurea*, cultivated in hydroponics.

Factor	Level	NO_3^-	Ca^{2+}	K^+
		mg L^{-1}		
NO_3^-:NH_4^+ ratio (%)	100:0	10866.7 a	414.17 b	4779.2 a
	80:20	12158.3 a	496.67 a	4754.2 a
Solution concentration (%)	50	10558.3 b	367.50 b	4420.8 b
	100	12466.7 a	543.33 a	5112.5 a
pH	5	12358.3 a	440.83 a	4420.8 b
	7	10666.7 b	470.00 a	5112.5 a
HSD		24.39	54.498	270.79

Values with the same letter are not significantly different (Tukey, $p \leq 0.05$) within each factor and level. HSD=honest significant difference.

The treatment with the NO_3-:NH_4^+ ratio 80:20, 100% solution concentration and pH 5 had higher concentrations of NO_3^- and Ca^{2+}. The same was observed for K^+ concentration, but with pH 7 (Table 5).

Table 5. Effect of treatments on concentrations of NO_3^-, Ca^{2+} and K^+ in *D. purpurea*, cultivated in hydroponics.

Treatment	NO_3^-:NH_4^+ (%)	Solution concentration (%)	pH	NO_3^-	Ca^{2+}	K^+
				mg L^{-1}		
1	100:0	50	5	11217 ab	300.0 c	3683.3 e
2	80:20	50	5	12750 ab	405.0 bc	4600.0 cd
3	100:0	100	5	10833 ab	388.3 bc	4516.7 cde
4	80:20	100	5	14633 a	670.0 a	4883.3 bc
5	100:0	50	7	9050 b	410.0 bc	5616.7 ab
6	80:20	50	7	9217 b	355.0 c	3783.3 de
7	100:0	100	7	12367 ab	558.3 ab	5300.0 abc
8	80:20	100	7	12033 ab	556.6 ab	5750.0 a
HSD				5183	172.38	856.55
C.V (%)				1638.6	20.51	9.73

Means in the same column with the same letter are not significantly different (Tukey, $p \leq 0.05$). HSD=honest significant difference, C.V=coefficient of variation.

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Plant height, number of shoots and stem diameter

Plant height was higher with the NO_3^-:NH_4^+ ratio 80:20 (Tukey, $p \leq 0.05$) than with the 100:0 ratio (Table 6). González *et al*. (2009) reported similar behavior of basil (*Ocimum basilicum* L.), for which greater height was obtained with nutrient solutions of NO_3^-:NH_4^+ 80:20 than with NO_3^- or NH_4^+ applied separately. The higher number of shoots was obtained with pH 5 (Tukey, $p \leq 0.05$) than with pH 7 (Table 6). Stem diameter was not different (Tukey, $p \leq 0.05$) among the levels of the factors.

Table 6. Effect of NO_3^-:NH_4^+ ratio, concentration and pH of the nutrient solution on plant height, number of shoots and stem diameter of *D. purpurea*, cultivated in hydroponics

Factor	Level	Plant height (cm)	Number of shoots	Stem diameter (mm)
NO_3^-:NH_4^+	100:0	60.20 b	6.91 a	24.87 a
ratio (%)	80:20	76.83 a	6.50 a	25.20 a
Solution	50	70.16 a	6.70 a	25.20 a
concentration (%)	100	66.87 a	6.70 a	24.87 a
pH	5	68.25 a	7.37 a	24.70 a
	7	68.79 a	6.04 b	25.37 a
HSD		13.192	0.8424	1.4989

Values with the same letter are not significantly different (Tukey, $p \leq 0.05$) within each factor and level. HSD=honest significant difference.

The treatments with pH 5 resulted in higher values in plant height, number of shoots and stem diameter (Table 7).

Table 7. Effect of treatments on plant height, number of shoots and stem diameter of *D. purpurea*, cultivated in hydroponics.

Treatment	$NO_3^-:NH_4^+$ (%)	Solution concentration (%)	pH	Plant height (cm)	Number of shoots	Stem diameter (mm)
1	100:0	50	5	69.50 a	7.16 ab	27.33 a
2	80:20	50	5	72.33 a	8.33 a	22.50 b
3	100:0	100	5	47.33 a	8.00 ab	21.66 b
4	80:20	100	5	83.83 a	6.00 ab	27.33 a
5	100:0	50	7	55.17 a	5.50 b	25.50 ab
6	80:20	50	7	83.67 a	5.83 ab	25.50 ab
7	100:0	100	7	68.83 a	7.00 ab	25.00 ab
8	80:20	100	7	67.50 a	5.83 ab	25.50 ab
HSD				41.728	2.663	4.741
C.V (%)				32.998	21.516	10.259

Means in the same column with the same letter are not significantly different (Tukey, $p \leq 0.05$). HSD=honest significant difference, C.V=coefficient of variation

Plant dry weight

Plant dry weight was higher in the $NO_3^-:NH_4^+$ ratio 80:20 than in the 100:0 ratio. It has been found that ammonium in small quantities improves plant growth. Rivera-Espejel *et al.*, (2014) obtained greater plant height and stem diameter in tomatoes (*Solanum lycopersicum* L.) by using both sources of nitrogen (NO_3^- and NH_4^+) in the nutrient solution. The concentration of the solution (50%, 100%) did not affect (Tukey, $p \leq 0.05$) plant dry weight. The solution with pH 5 resulted in higher (Tukey, $p \leq 0.05$) dry weight of stem, leaf, root and total plant than that with pH 7 (Table 8).

Table 8. Effect of NO_3^-:NH_4^+ ratio, concentration and pH of the solution on root, stem, leaf and flower dry weight of *D. purpurea*, cultivated in hydroponics

Factor	Level	Root	Leaf	Stem	Flower	Total weight
		----------	----------	(g)	----------	----------
NO_3^-:NH_4^+ ratio (%)	100:0	10.17 a	83.89 a	8.35 b	2.55 a	104.97 a
	80:20	9.68 a	89.28 a	13.11 a	2.55 a	114.64 a
Solution concentration (%)	50	10.35 a	83.02 a	12.04 a	2.55 a	107.97 a
	100	9.51 a	90.15 a	9.42 a	2.55 a	111.64 a
pH	5	10.73 a	94.61 a	12.32 a	2.90 a	120.57 a
	7	9.12 b	78.56 b	9.14 b	2.20 a	99.03 b
HSD		1.210	10.04	2.814	0.761	10.587

Within each factor and level, values with the same letter are not significantly different (Tukey, $p \leq 0.05$). HSD=honest significant difference.

The treatment with the NO_3^-:NH_4^+ ratio 100:0, 50% solution concentration and pH 5 had higher root dry weight. The treatment with the NO_3^-:NH_4^+ ratio 80:20, 50% solution concentration and pH 5 produced the highest dry weight of leaf, stem, flower and total plant (Table 9).

Table 9. Effect of treatments on dry weight of root, leaf, stem and flower of *D. purpurea*, cultivated in hydroponics.

Treatment	NO_3^-:NH_4^+ (%)	CS (%)	pH	Root	Leaf	Stem	Flower	Total weight
				----------	----------	(g)	----------	----------
1	100:0	50	5	11.97 a	89.91 ab	12.26 ab	2.21 a	116.36 ab
2	80:20	50	5	11.29 ab	102.49 a	18.47 a	3.53 a	135.81 a
3	100:0	100	5	9.83 ab	95.13 ab	5.52 b	3.46 a	113.96 ab
4	80:20	100	5	9.83 ab	90.89 ab	13.04 ab	2.41 a	116.19 ab
5	100:0	50	7	10.15 ab	72.39 ab	5.68 b	2.36 a	90.60 b
6	80:20	50	7	7.97 b	67.30 b	11.76 ab	2.08 a	89.12 b
7	100:0	100	7	8.73 ab	78.12 ab	9.95 ab	2.15 a	98.97 b
8	80:20	100	7	9.64 ab	96.44 ab	9.17 b	2.20 a	117.46 ab
HSD				3.8299	31.757	8.9039	2.4084	33.489
C.V (%)				20.897	19.872	44.943	51.092	16.525

Means in the same column with the same letter are not significantly different (Tukey, $p \leq 0.05$). CS=concentration of the solution, HSD=honest significant difference, C.V=coefficient of variation.

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Conclusions

The NO_3^-:NH_4^+ ratio 80:20 increased (Tukey, $p \leq 0.05$) SPAD readings, Ca^{2+} concentration, stem length and stem dry weight. The 100% nutrient solution concentration increased (Tukey, $p \leq 0.05$) the number of leaves, SPAD readings, NO_3^- concentration, number of shoots, and root, stem and leaf dry weight. pH 7 increased (Tukey, $p \leq 0.05$) K^+ concentration. Supplying ammonium at low quantities (NO_3^-:NH_4^+ 80:20), 100% concentration and pH 5 of the nutrient solution favored growth of *Digitalis purpurea.*

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