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# Agronomic traits of hydroponically grown nettle under conditions of two irrigation intervals

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## Abstract

The ebb and flow system is a hydroponic technique for growing plants that allows rational water use and management of water stress to obtain plant material rich in bioactive compounds. The risk with this technique is the lower yield and the deterioration of the morphological characteristics of the cultivated plants that can occur. The aim of this study was to determine the effect of 2 intervals of irrigation intervals on the agronomic traits of stinging nettle in 2 harvest periods. The combination of 48 hours of water stress and the 2<sup>nd</sup> harvest period gave the highest yield (1.21 kg m<sup>-2</sup>), while the combination of 72 hours of water stress and the 1<sup>st</sup> harvest period resulted with the lowest nettle yield (0.65 kg m<sup>-2</sup>).

**Keywords:** ebb and flow technique, irrigation frequency, yield, morphological characteristics, *Urtica dioica* L.

## Introduction

Pronounced climate changes in the form of extreme weather conditions such as drought, environmental pollution with emphasis on groundwater contamination, and the general decrease in the availability of water suitable for growing food are among the major challenges facing modern agriculture (Kheradmand et al., 2014.). For this reason, the demand for sustainable production methods and management of important resources, such as water, is increasing, and growers are turning to hydroponic cultivation in greenhouses. Various hydroponic techniques allow the control and management of abiotic factors that directly affect the success of agricultural production. Through appropriate agrotechnical measures, higher yields and products with distinct nutrient quality can be obtained, all with precise and balanced plant nutrition and rationalized water use (Resh, 2013.).

For hydroponic cultivation of medicinal and aromatic plants, the nutrient film technique, floating hydroponics, aeroponics and the ebb and flow system are recommended. Various authors (Ferrarezi et al., 2015.; Yang et al., 2018.) point to ebb and flow as a water-saving technique suitable for rationalized fertilizer consumption and precise adjustment of nutrient solution rations to the needs of the cultivated species and the targeted end product. The mentioned technique works on the principle of time intervals in which the nutrient solution is available for the plants. In the period between two fertigations, the plants are exposed to water stress, which can affect the morphological and nutritional characteristics of the plants (Gent and McAvoy, 2011.; Opačić et al., 2022a). Water stress positively affects the amount of bioactive compounds produced by plants, and such plant material is rich in various phytochemicals. Since even controlled water stress can lead to yield loss, it is extremely important to find the right balance between induced stress depending on the plant species, which gives the plant material a richer content of bioactive compounds, and satisfactory yield (Ahanger et al., 2017.).

Stinging nettle (*Urtica dioica* L.) is considered a functional food rich in essential minerals, vitamins, phytochemicals, and other specialized metabolites and has been successfully grown using the floating hydroponics technique (Pagliarulo et al., 2004.; Hayden, 2006.; Radman et al., 2014.; Radman et al., 2021.; Opačić et al., 2022b). There is a lack of studies on the morphological characteristics, yield, and nutrient quality of stinging nettle under conditions of controlled water stress in the ebb and flow system. For this reason, a research was carried out with the aim of determining the agronomic traits of hydroponically grown nettle under conditions of different irrigation intervals during two harvest periods.

## Material and methods

The study was conducted in an unheated greenhouse at the University of Zagreb Faculty of Agriculture experimental station during the winter-spring growing season of 2022. Nettle was grown using the ebb-and-flow technique. The influence of 48- and 72-hour irrigation intervals during the first and second harvest periods on the agronomic traits of nettle plants was studied. The experiment was laid out according to randomized complete block design in three replicates with water stress and harvest as factors. Seeds were sown on January 20, 2022, in polystyrene containers (dimensions 31 cm × 53 cm) containing 40 pots filled with commercial substrate (Klasman Potgrond H) and placed on ebb and flow benches (dimensions 2 m × 3 m; 33 containers per bench) after sowing and irrigated regularly. Nettle seeds (B&T World Seeds, France) were sown at a rate of 10 seeds per pot and thinned to 3 plants after germination to achieve uniformity in the experiment. Complete emergence was recorded on February 14, and thinning of the plants occurred on March 14. From sowing until April 10 (when it was estimated that they had reached the appropriate size and crop density), the plants were watered as needed according to temperature and relative humidity (RH) in the greenhouse. On April 11 (81 days after sowing), treatments with nutrient solution (NS) according to the recipe of Johnson (Table 1; Lorenz and Maynard, 1988.), began with different irrigation intervals according to the following schedule:

Bench 1 - the interval for ebb and flow of NS was set to every 48 hours.

Bench 2 - the interval for ebb and flow of the NS was set to every 72 hours.

Table 1. Composition of the nutrient solution in mg L<sup>-1</sup>

Macroelements	
KNO <sub>3</sub>	251.0
KH <sub>2</sub> PO <sub>4</sub>	142.7
Ca(NO <sub>3</sub> ) <sub>2</sub> × 4H <sub>2</sub> O	501.5
MgSO <sub>4</sub> × 7H <sub>2</sub> O	256.3
Microelements	
FeEDTA 13%	12.8
H <sub>3</sub> BO <sub>3</sub>	1.32
CuSO <sub>4</sub> × 5H <sub>2</sub> O	0.03
MnSO <sub>4</sub> × 4H <sub>2</sub> O	0.79
ZnSO <sub>4</sub> × 7H <sub>2</sub> O	0.11
Na <sub>2</sub> MoO <sub>4</sub> × 2 H <sub>2</sub> O	0.02
Acid	
HNO <sub>3</sub>	concentration – 56 %
EC (mS cm <sup>-1</sup> )	1.5
pH	5.8 – 6.2

The nutrient solution was brought to the plants in a volume of 120 L and remained on the bench for 1 hour, after which it was drained. This was repeated every 48 (bench 1) and 72 (bench 2) hours throughout the experiment. During cultivation, the abiotic factors of the greenhouse (air temperature, RH) and the nutrient solution (pH and EC values) were monitored regularly using a tabletop thermohygrometer (Agrologistika d.o.o., Croatia) and multiparameter meter (Hanna instruments HI98194, Romania).

Harvests of nettle herb were made in the pre-flowering stage on May 10 and June 8, and the morphological characteristics of 30 representative plants per treatment were analyzed. These included the following measurements: plant height (cm), number of leaves, and leaf width and length (cm), from which leaf area index (LAI) was calculated using the formula leaf length × width (cm<sup>2</sup>), and total yield (kg m<sup>-2</sup>) was also measured. To ensure retrovegetation,

nettle plants were cut above the first two nodules.

Statistical data processing was performed in SAS software v. 14.3 (2017.) using the PROC GLM (general linear model) procedure. The results were subjected to two-factor analysis of variance (ANOVA), and the differences obtained between the means were compared using the t-test (LSD) at the significance level  $p \leq 0.05$ .

### Results and discussion

The abiotic factors of the greenhouse and NS are shown in Table 2 as decade values for the period from the second decade in February (greenhouse) i.e. the second decade in April (NS) to the first decade in June.

Table 2. Abiotic factors of the greenhouse and nutrient solution during nettle cultivation in the ebb and flow system

Period	Greenhouse			Nutrient solution		
	Temperature (°C)			RH (%)	pH	EC (mS cm <sup>-1</sup> )
	Min.	Max.	Mean			
February II	4.5	29.9	17.2	53		
February III	8.7	29.7	19.1	47		
March I	8.0	23.1	15.6	35		
March II	10.6	32.1	21.3	32		
March III	13.3	35.1	24.1	33		
April I	14.8	32.0	23.4	42		
April II	16.8	32.9	24.8	36	6.8	1.5
April III	12.9	32.0	22.5	48	6.6	1.5
May I	17.7	35.2	26.4	46	6.4	1.4
May II	17.2	40.6	28.9	42	6.8	1.4
May III	16.7	40.1	28.4	48	6.4	1.4
June I	16.2	38.2	27.2	52	6.4	1.5
Average	13.1	33.4	23.2	43	6.6	1.5

Minimum greenhouse air temperature during nettle cultivation ranged from 4.5°C (February II) to 17.7°C (May I), and maximum temperature ranged from 23.1°C (March I) to 40.6°C (May II). Average air temperature span was from 19.6°C (April II) to 24.9°C (May III) with an average of 23.2°C, which according to Opačić et al. (2022a) is within the range of optimal temperatures for nettle growth and development (20 to 25°C).

The average minimum temperature was 13.1°C, while the average maximum temperature reached 33.4°C, which is above the optimal range and can cause reduced growth of nettle plants (Opačić et al., 2022b). The average value of RH varied between 32 and 53%, which corresponds to the values recommended by Toth et al. (2012.) for RH during the cultivation of rocket in unheated greenhouse. The same authors recommend a solution pH of 5.8 to 6.2, but the value of 6.6 in this study was slightly higher. The average EC value was 1.5 mS cm<sup>-1</sup>, which is the recommended value for the Johnson nutrient solution according to Lorenz and Maynard (1988.).

Table 3 shows the influence of irrigation intervals and harvests and their interaction on the agronomic traits of nettle grown in the ebb and flow system. A justified influence of irrigation intervals as the main factor on plant height, number of leaves, and yield is evident, while a statistically justified influence on LAI was not found. Depending on the harvest period, there were statistically justified differences in the number of leaves and yield, while no justified influence of this factor on plant height and LAI was detected.

Table 3. Influence of irrigation intervals, harvests and their interaction on agronomic traits of hydroponically grown nettle

		Plant height (cm)	Leaf number/ plant	LAI (cm <sup>2</sup> )	Yield (kg m <sup>-2</sup> )
Irrigation interval	48 h	33.2 ± 0.62 <sup>a</sup>	11 ± 0.84 <sup>a</sup>	23.5 ± 2.07 <sup>ns</sup>	1.06 ± 0.16 <sup>a</sup>
	72 h	19.6 ± 0.41 <sup>b</sup>	10 ± 1.19 <sup>b</sup>	20.0 ± 5.73	0.66 ± 0.09 <sup>b</sup>
Harvest	1 <sup>st</sup> (1)	25.9 ± 3.18 <sup>ns</sup>	11 ± 0.34 <sup>a</sup>	22.5 ± 4.51 <sup>ns</sup>	0.76 ± 0.14 <sup>b</sup>
	2 <sup>nd</sup> (2)	26.8 ± 2.99	10 ± 1.15 <sup>b</sup>	21.0 ± 4.77	0.96 ± 0.09 <sup>a</sup>
Interaction	48 h1	33.0 ± 1.14 <sup>a</sup>	11 ± 0.20 <sup>a</sup>	24.1 ± 1.18 <sup>ns</sup>	0.91 ± 0.05 <sup>b</sup>
	48 h2	33.4 ± 2.08 <sup>a</sup>	10 ± 1.11 <sup>a</sup>	22.9 ± 2.86	1.21 ± 0.09 <sup>a</sup>
	72 h1	18.8 ± 0.22 <sup>b</sup>	11 ± 0.42 <sup>a</sup>	20.9 ± 6.47	0.62 ± 0.03 <sup>d</sup>
	72 h2	20.3 ± 0.98 <sup>b</sup>	9 ± 0.40 <sup>b</sup>	19.0 ± 6.11	0.71 ± 0.02 <sup>c</sup>

Results are expressed as the mean value of triplicate ± standard deviation. Mean values followed by the same letter within each column do not differ significantly according to LSD test ( $p \leq 0.05$ ); ns – non significant

The interaction between different irrigation intervals and harvest periods had a significant influence on most of the observed traits (plant height, number of leaves and yield), while for LAI the influence was not statistically significant. An interval of 48 hours irrigation interval resulted in statistically significant taller nettle plants (33.2 cm) compared to an interval of 72 hours (19.6 cm). Considering only the harvest period, relatively taller plants were measured after the 2<sup>nd</sup> harvest (26.8 cm) compared to the 1<sup>st</sup> harvest (25.9 cm), but this difference was not statistically justified. Considering the irrigation interval and harvest period, the tallest plants were measured at 48 hours interval in both harvests (48 h×1= 33.0 cm, 48 h×2= 33.4 cm). The combination 72 h×1 resulted in the lowest plants (18.8 cm), but statistically not different from the 72 h×2 combination (20.3 cm). Radman et al. (2014.) found that the height of nettle plants grown in floating hydroponics in two different substrates at 3 different seeding densities was greater on average after the first harvest, which is contrary to the results of this study.

According to Chidiac (2017.), the height of lettuce shoots depends on the water stress interval, and a longer interval has a negative effect on growth, which is consistent with this study, where plants exposed to a longer period without nutrient solution were shorter than those that received nutrient solution more frequently. Osakabe et al. (2014.) found that water stress reduces plant growth by decreasing the opening of stomata, which reduces CO<sub>2</sub> uptake and thus photosynthetic activity.

When considering each factor, the highest number of leaves (11) was determined on plants grown under shorter irrigation interval, while irrigation interval of 72 hours resulted in plants with an average of 10 leaves. An identical result was obtained with harvest as the second factor. In the interaction of these two factors, the combinations 48 h×1 (n=11), 48 h×2 (n=10), and 72 h×1 (n=11) belonged to the highest statistical rank, while the lowest number of leaves was counted in plants in the combination 72 h×2 (n=9). Radman et al. (2014.) reported higher average number of nettle leaves after the second harvest. Mahmood et al. (2004.) found that water stress in the form of withholding irrigation until the lower 2-3 leaves of the plants wilted reduced the number of leaves in arugula and canola plants during 6 treatment cycles. In this study, a significantly lower number of leaves was seen only in the second harvest in combination with 72-h irrigation interval, which could be due to the temperature and RH in the greenhouse (Table 2), i.e., water stress did not occur in plants in the other treatments. According to Ncise et al. (2020.), different intervals of water stress (5, 14 and 21 days) in hydroponic cultivation of *Tulbaghia violacea* significantly affect the number of leaves, with a higher number of leaves recorded at intervals of 5 and 14 days compared to 21 days.

Regarding the tested factors and their interaction, no statistically significant differences were found, but there is still evidence of some trend. Plants exposed to 48 hours irrigation interval and plants after 1<sup>st</sup> harvest were found to have relatively higher LAI (23.5 cm<sup>2</sup> and 22.5 cm<sup>2</sup>, respectively). As expected, the 48 h×1 interaction resulted in plants with the relatively highest LAI (24.1 cm<sup>2</sup>), while the 72 h×2 combination resulted in the relatively lowest LAI (19.0 cm<sup>2</sup>). Leaf surface area, on which the intensity of photosynthesis and transpiration largely depends, can be smaller to adapt plants to water stress, as a reduction in leaf surface area may prevent water loss through transpiration (Mahmood et al., 2004.). The same authors note that the lowest LAI value of arugula plants was recorded during the

longest drought period to which the plants were exposed, but there was no significant difference compared to the control.

The main factor, irrigation interval, had a significant effect on nettle yield in the ebb and flow system, and the highest yield was recorded in plants subjected to 48 hours irrigation interval ( $1.06 \text{ kg m}^{-2}$ ), while the yield was 38% lower in plants subjected to 72 hours irrigation interval. Considering only the harvest, the 1<sup>st</sup> harvest yielded 21% less than the 2<sup>nd</sup> harvest ( $0.96 \text{ kg m}^{-2}$ ). In the interaction of the tested factors, the highest yield was determined at the combination 48 h×2 ( $1.21 \text{ kg m}^{-2}$ ), while the yield was almost two times lower at combination 72 h×1 ( $0.65 \text{ kg m}^{-2}$ ). In Leskovar and Piccinni (2005.) study, deficit irrigation based on evapotranspiration affected the yield of spinach grown in the soil, with the lowest yield obtained at the highest water stress. Ors and Suarez (2017.) reached a similar conclusion when growing spinach in the open field under water and salt stress. The results of Radman et al. (2021.) indicated that nettle yield in floating hydroponics was higher after the 2<sup>nd</sup> harvest than after the 1<sup>st</sup> harvest. In the study by Radman et al. (2014.), the highest yield of nettle grown in floating hydroponics was obtained after the third harvest. The yield obtained after each harvest and number of harvests depend, among other factors, on the growing season and abiotic factors during cultivation.

### **Conclusion**

A longer irrigation interval (72 h) negatively affected plant height, number of leaves, and yield. Plants had a greater number of leaves in the first harvest period, but a higher yield was observed in the second harvest. As expected, the irrigation interval had a greater effect on nettle agronomic traits than the harvest subfactor. The interaction between the irrigation interval of 48 hours and the 2<sup>nd</sup> harvest period resulted in the highest yield. Considering that water stress affects not only the agronomic traits but also the nutritional value of the plant material, future research should be focussed on the effect of different irrigation intervals on the nutritional quality of fresh nettle leaves.

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