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ARTEMISIA SEEDLINGS QUALITY PRODUCED IN GREENHOUSE UNDER DIFFERENT IRRIGATION SYSTEM AND FERTILIZER DOSES

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1 ABSTRACT

The *Artemisia annua* L. is a source of essential oils used in perfumery and cosmetics industry, and the yield of oil is affected by the quality of the seedlings used. The present study was carried out in order to evaluate the production of *Artemisia annua* L. seedlings grown in greenhouse, under two irrigation systems and four levels of commercial fertilizer of high solubility, in a completely randomized design in a 2x4 factorial arrangement and four replications. Germination and growth of roots and shoots seedlings (stems and leaves) were assessed. It was observed at 36 days of sowing (DAS), that plant height was 743% higher on floating system than plants under micro sprinkler system, and that the relationship between root's and shoot's dry matter is inversely proportional to the doses of nutrient solution applied. Seedlings showed Dickson quality index greater than 1.3 for all the doses of fertilizer under the floating system. On the other hand, values less than 0.6 were observed for plants under micro sprinkler system.

Keywords: seedling production; floating system; medicinal plants irrigation

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QUALIDADES DE MUDAS DE ARTEMISIA PRODUZIDAS EM CASA DE VEGETAÇÃO SUBMETIDAS A DIFERENTES SISTEMAS DE IRRIGAÇAO E DOSES DE FERTILIZANTES

2 RESUMO

A *Artemisia annua* L. é uma fonte de óleos essenciais utilizados na indústria de perfumaria e cosméticos, e o rendimento do óleo é afetado pela qualidade das mudas. O presente estudo foi realizado para avaliar a produção de mudas de *Artemisia annua* L. cultivadas em casa de vegetação, sob dois sistemas de irrigação e quatro níveis de fertilizante comercial de alta solubilidade, em um delineamento experimental inteiramente aleatorizado, em esquema fatorial 2x4 e quatro repetições. Foram avaliados a germinação e o crescimento das mudas de raízes e

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parte aérea (caule e folhas). Foi observado aos 36 dias após a semeadura (DAS) que a altura das plantas era 743% maior no sistema de flutuação do que das plantas sob microaspersão, e que a relação entre massa seca da raiz/massa seca da parte aérea foi inversamente proporcional às doses de solução nutritiva aplicada. As mudas apresentaram índice de qualidade de Dickson superior a 1,3 para todas as doses de fertilizante no sistema flutuante. Por outro lado, valores inferiores a 0,6 foram observados para as plantas sob microaspersão.

Palavras-chave: produção de mudas; floating system; irrigação de plantas medicinais

3 INTRODUCTION

Artemisia annua L. belongs to the class Angiospermae, family Asteraceae. It is an annual plant, presenting upright aromatic stalk with about 80-150 cm height and native of Asia, probably originated from China. The leaves of this species contain artemisinin, sesquiterpene present in the leaves and considered as the main chemical component of the plant, its properties can be used in the cure of malaria, a disease caused by *Plasmodium falciparum sporozoan*. Since the discovery of its antimalarial properties, interests in this plant have increased worldwide, especially in searches for alternatives to improve the production of this substance in large scale (MARCHESE et al., 2005; WHO, 2006).

The success of commercial production of *Artemisia annua* L. begins with the production of high quality seedlings. According to Fachinello, Hoffmann e Kluge (1995), seedlings that do not achieve desirable requirements can compromise more than 50% of the commercial production. The Artemisia's seedlings production is not yet fully established and needs researches regarding the type of substratum (MARQUES et al., 2003), fertilization, size and shape of the vessel (BARBOSA et al., 2010) and irrigation, among other factors supposedly treated as determinants for the success or failure of the process.

According to Minami (1995), seedling's irrigation consists in one of the most important factors for its production in the emergence and seedling formation. During this period, it is essential to have water in adequate quantity and quality. Thus, lack of water can lead to water stress, and reflect in the decreased nutrients absorption. On the other hand, water in excess may favor the leaching of nutrients as well as provide a favorable microclimate for the development of diseases in addition to the environmental issues regarding water saving and the accumulation of leached salts in the soil (LOPES; GUERRINI; SAAD, 2007).

The nutrition of seedlings is another important factor because it exerts a marked influence on the root system and the nutritional status of plants, profoundly affecting seedling's quality. The amount of nutrients in the substrate, the type of fertilizer and proper doses upon administration are points that need to be taken in consideration.

Considering this, the objective was to evaluate the production of *Artemisia annua* L. seedlings grown in greenhouse.

4 MATERIAL AND METHODS

The experiment was conducted between October and December 2012, at the Experimental Area of the Biosystems Engineering Department, belonging to the Escola Superior de Agricultura "Luiz de Queiroz", located in Piracicaba-SP, at the geographical coordinates of 22° 42' 40" S; 47° 37' 45" W and 547 meters of altitude. According to the Köppen climate classification, Piracicaba has Cwa climate, in other words, humid subtropical, with dry and cold winters and rainy and hot summers, mean temperature of the warmest month of 22ºC and the coldest 18ºC, average relative humidity of 74% and average wind speed of 2.2 m s^{-1}.

This experiment was conducted in a greenhouse with galvanized steel structure, measuring 6.4 m high in the central part, 12.8 m wide and 22.5 m long, presenting front windows. The cover consisted of a transparent low-density polyethylene 0.15 mm thick film. The weather was monitored with sensors of global radiation model CM3 Kipp & Zonen®, temperature and relative humidity - the thermo hygrometer HMP45C Vaisala® model. The data from these sensors was used to calculate the reference evapotranspiration (ET_0) daily by Penman-Monteith method (ALLEN et al., 1998).

The experimental design was completely randomized factorial 2 x 4 with four replications (32 units or trays), using 10 plants per plot. The experimental unit consisted of two thirds of the tray containing 162 cells with a volume of 31 cm³, totaling 108 seedlings per experimental unit. The treatments were of the combination of two irrigation systems (micro sprinkler and capillarity or "floating") and four levels of commercial fertilizer with high solubility the concentrations in solution were 1.5; 3.0; 4.5 to 6.0 g L^{-1}.

The floating system was designed by placing black plastic over a wooden structure, to form small reservoirs, which made possible to the water level rise in the cells at the daily immersion of the trays. The water application was made by capillarity and consisted of immersion in water to cover trays until 90% of the height of the cells from the base for a time of 10 minutes. This operation was performed daily at 8 a.m. with all trays referring to the system by floating system treatments, providing the average application of 0.88 L d^{-1}, an amount determined by measuring the volume of water initially placed in the installed canvas over timber structure, from which we quantified by the difference of the volume absorbed and the substrate after irrigation.

The micro sprinkler system consisted of manual application of known volume of water through a garden sprinkler so, that each tray received a daily average volume of 1.19 L, divided into two applications (at 8 a.m. and 5 p.m.), except on cloudy and rainy days or when there was high air relative humidity. The whole structure (floating system and micro sprinkler system) was installed and fixed on metal benches 1 m tall, allocated inside the greenhouse.

Trays were previously disinfected with bleach solution 5% (SANTIN et al., 2005). After rinsed, cells were filled with Plantmax®-HT commercial substratum, whose nutrient availability and physical characteristics are presented in Table 1.

Table 1. Amount of available nutrients and physical characteristics of the substrate prior to the establishment of the experiment.

Substrate	C/N	N	P	K	ASO	SOH_2O	MO total	SD	pH	CE
		--------g g^{-1}--------			-------------%-----------			g cm^{-3}		mS m^{-1}
Plantmax®-HT	40/1	0.42	0.31	0.12	19	17	25	0.51	5.8	1.2

ASO – air space occupied; SOH_2O – space occupied by water; SD – substrate density; MO total – total organic matter and C/N – total carbon nitrogen relation. Source: Paulino et al. (2011).

The seeds were sown at a depth of 5 mm into the substrate. After emergence, thinning was realized, leaving only one seedling per cell, keeping the one with greater strength. At 15 and 25 days after sowing (DAS), fertigation was performed within the irrigation treatments.

The fertilizer solution was prepared with commercial compost with high solubility in concentrations of 1.5; 3.0; 4.5 to 6.0 g L^{-1}. The chemical composition of the fertilizer source used in the experiment is described in Table 2.

Table 2. Chemical constitution of commercial fertilizer solubility and high concentrations of fertilizer solutions.

Water soluble nutrients (%)		Fertilizer solution (g L^{-1})			
		1.5	3.0	4.5	6.0
N	30	0.45	1.35	9.0	18
P_2O_5	10	0.15	0.45	3.0	6.0
K_2O	10	0.15	0.45	3.0	6.0
Mg	0.5	0.0075	0.0225	0.15	0.3
B	0.02	0.0003	0.0009	0.006	0.012
Cu	0.05	0.00075	0.00225	0.015	0.03
Mn	0.1	0.0015	0.0045	0.03	0.06
Mo	0.02	0.0003	0.0009	0.006	0.012
Zn	0.1	0.0015	0.0045	0.03	0.06

Nutrient solution preparation consisted of weighing 45 g (1.5 g L^{-1}) and dilution of fertilizer in 30 L of water. Subsequently, half of this solution was used for immersion of 3 trays containing 162 seedlings at 15 DAS. The other half of the solution was applied by micro sprinkler for garden watering, being the same amount consumed by the immersion process, in other words, 2.7 L per tray of 162 cells.

The fertigation applied 25 DAS followed the same criteria and concentrations of nutrient solution. The treatments of higher levels of fertilization were established with the same criteria of application and preparation of the solution.

The variables evaluated were divided into two groups regarding characteristics of germination and growth, respectively. Emergence time was monitored and determined the speed of emergence index (SEI) through eq. (1). Assessments occurred at 5, 8, 9, 10, 11 and 12 DAS, also determining the percentage of cells emerged from the tray in which at least one plant (PCEP).

$$SEI = \frac{E1}{N1} + \frac{E2}{N2} + \frac{En}{Nn} \quad (1)$$

In which:
SEI: speed of emergence index (plants day^{-1});
E1, E2...En: Number of emerged plants in the first, second and final assessment (plant);
N1, N2...Nn: Number of days from sowing to first, second and last evaluation (day).

The final assessment at 36 DAS was the final assessment was made by selecting 20 plants from the center of the tray in a systematic and randomized way. The selected plants were removed from the trays and sectioned in the cervical region, separating the root part of the shoot. The root system was washed in a 2 mm sieve mesh, aiming to remove it from the substratum. Subsequently, the roots were placed in plastic trays for 2 hours to remove excess water.

The aerial and root plant parts were placed separately in packs of paper, identified and dried in an oven, preheated to 75°C, where the material was maintained until constant dry weight, further measured in digital scale with accuracy of ± 0.01 g.

The variables analyzed were as follows: Root system length (RSL); Plant height (HGT); Stalk diameter (DIAM), measured at 0.01 m height from the substrate level, in mm, by using a high precision digital pachymeter; Number of leaves (NL); shoots' fresh matter weight (WFS); root's fresh matter weight (WFR), according to the procedure described by Paulino et al. (2010). The dry weight of shoots and roots were determined were a precision balance of ± 0.01 g after being dried in a forced ventilated oven at 65 °C for 60 hours. Total fresh (TFW) and dry weight (TDW) were obtained by the sum of fresh and dry weight of shoots and roots, respectively. Dickson's quality index (DQI), given by Eq. (2), was obtained as well.

$$DIQ = \frac{TDW}{HDR+DMR} \quad (2)$$

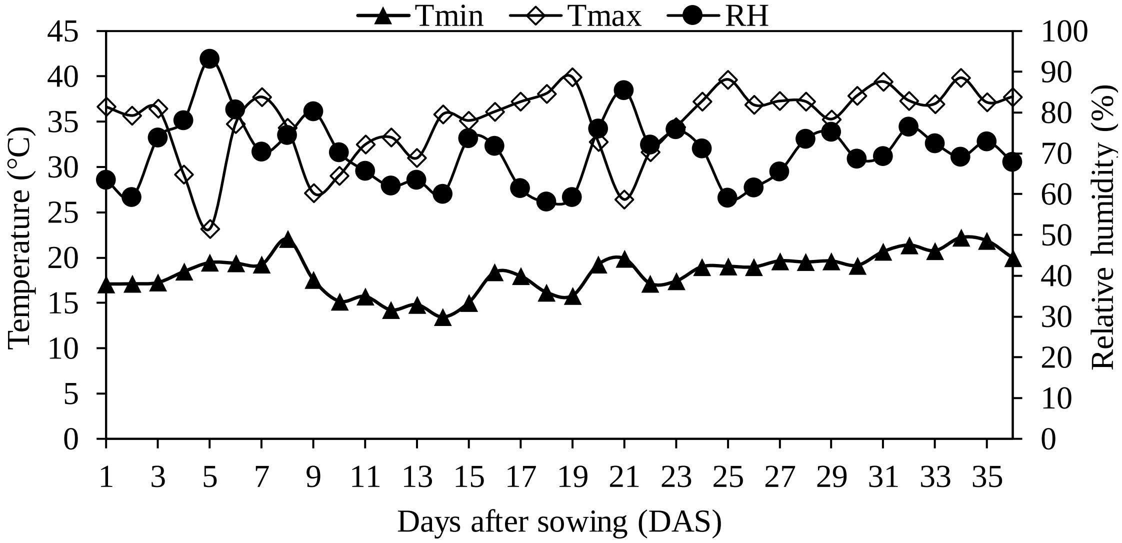
In which:
DIQ: Dickson's quality index;
TDW: Total dry weight, g;
HDR: Ratio Height-diameter, cm mm^{-1};
DMR: Aerial dry mater/root dry mater, dimensionless.

Initially, the experimental data were subjected to the Shapiro-Wilk test (SHAPIRO; WILK, 1965) ($P > 0.01$) and Levene ($P > 0.01$) for verification of residual normality and homoscedasticity, respectively after, the analysis of variance with the results were compared by regression analysis of quantitative variables and by the Scott-Knott test ($P < 0.05$) for qualitative variables. For non-parametric data, it was used nonparametric analysis of variance by the Kruskal-Wallis test ($P < 0.05$). The statistical software R, version 2.2.1 (R DEVELOPMENT CORE TEAM, 2010) was used in order to submit data to statistics tests.

5 RESULTS AND DISCUSSION

The maximum and minimum temperatures recorded during the experiment were 39.91°C, at 19 DAS, and 13.45°C, at 14 DAS, respectively, as presented in the Figure 1. *Artemisia annua* L. seedlings grow best in warm weather with temperature range of 7°C and maximum and minimum temperatures of 18 and 25°C, respectively (MARCHESE et al., 2005). During experiment, average values of 26.65°C and 70% for temperature and relative air humidity were observed, respectively. The initial development phase of *Artemisia annua* L. is critical because high temperatures promote significant increase in seedling mortality, making the irrigation system a key for the success in the production of high quality seedlings factor.

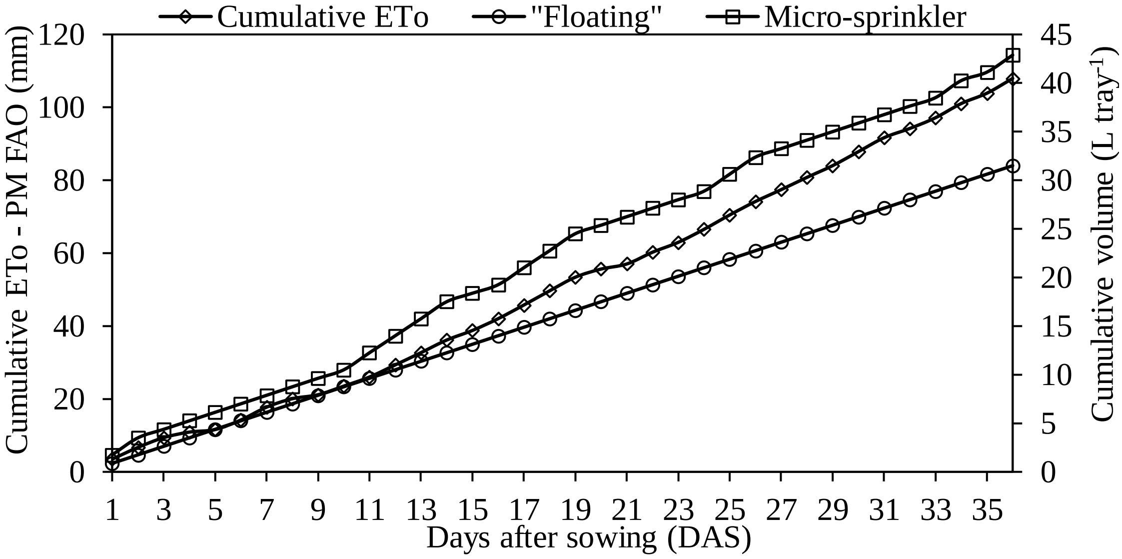
Figure 1. Variation of minimum temperature (Tmin), maximum temperature (Tmax) and mean relative humidity (RH) inside the greenhouse.



Some plant species require specific conditions for successful germination being directly influenced by environmental conditions (SOUZA et al., 2007). Temperature is one of these factors, reported by Marcos (2005) and it is essential for germination. According to Marcos (2005) this variable has a specific value, but can be expressed in terms of cardinal temperatures, in other words, maximum, minimum and optimum, and the last set to one in which the maximum germination occurs in a minimum period of time.

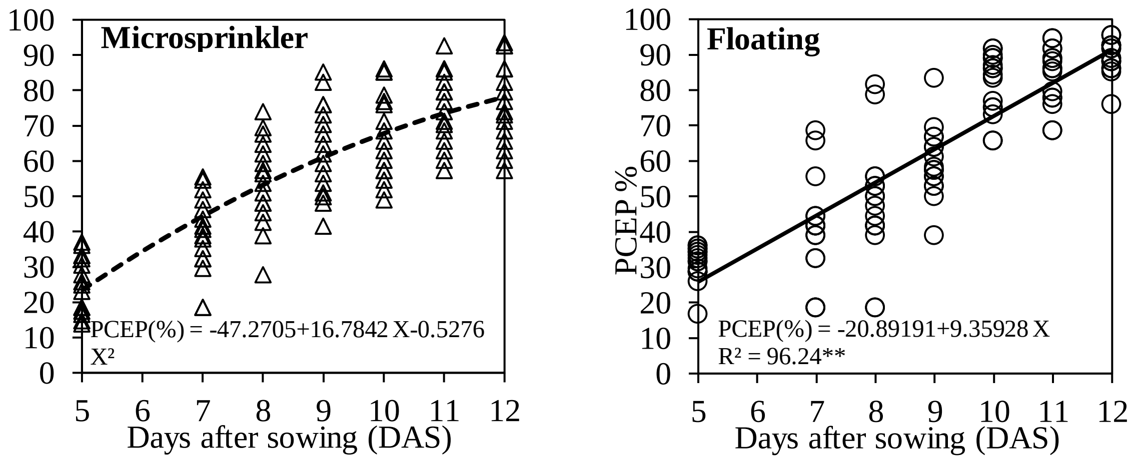
The volumes of water applied by both irrigation systems and the accumulated reference evapotranspiration are shown in Figure 2. The accumulated evapotranspiration was 107.93 mm, measured inside the greenhouse during the growth of the seedlings, the average ETo was 3 mm d^{-1} and the mean volume of water applied daily was 0.88 and 1.19 L $tray^{-1}$ day^{-1}, respectively for the "floating" system and for the microsprinkler system. This difference may be associated to the greater uniformity of water distribution in the substratum and better application efficiency when considering the system of "floating". The possibility of loss by percolation occurs in the microsprinkler system, which demands higher volumes of water applied per tray, to enable the development of seedlings under similar levels of moisture in the substratum. The "floating" system provided a decrease of 27% in cumulative volume applied to the trays at the end of the seedlings growing period, which leads to greater efficiency of water use at the adoption of this system.

Figure 2. Volume applied to both systems and reference evapotranspiration accumulated inside the greenhouse for the training period of the seedlings of *Artemisia annua* L.



The results of the analysis of variance revealed significant differences (P> 0.01) between the two irrigation systems used and among the fertilization doses on the early development of seedlings of *Artemisia annua* L. In Figure 3, it is shown the regression curves for the percentage of cells that emerged at least one plant in the “floating” and microsprinkler systems. The beginning of the emergence occurred five days after sowing (DAS), with more than 20% of the seeds arising to 40 and 50% in the third and fourth DAS, respectively. It was observed that the two irrigation systems maintained the same trend emergence until 9 DAS, when the plots irrigated by microsprinkler system started to present lower values. There was a significant difference in the percentage of germination at 12 DAS between the "floating" system and microsprinkler system, with observed values of 89 and 76%, respectively.

Figure 3. Regression analysis of emergence of at least one plant per cell (PCEP) as a function of the time lapsed from sowing (DAS), for both irrigation systems.



There was no significant difference among the irrigation systems about the speed emergence index (SEI) 12 DAS (Table 3). Shaferet al. (2004) found similar results in an experiment that evaluated the systems of irrigation by capillary rise and microsprinkler in citrus seedlings, observing that the two systems were capable in providing enough substratum moisture and high level of emergence of plants, not identifying statistical differences between both systems.

Table 3. Influence of irrigation systems on the percentage of cells that emerged from a seedling (PCEP) and the speed emergence index (SEI) both DAS 12.

Irrigation System	PCEP**(%)	SEI^{ns} (plants day^{-1})
Floating	89 a	185.15
Microsprinkler	76 b	180.60

** Means followed by different letters differ by the Scott-Knott test at 0.05 level of probability

ns not significant at 0.05 level of probability by the Scott-Knott test

Regarding growth parameters, there was significant interaction between the different irrigation systems and nutrient doses. The higher average height, number of leaves and root system length were found in the "floating" system, differing among levels of fertilization. Compared to microsprinkler, which one system provided higher values for plant height in the order of 270% for the fertilizer solution of 1.5 g L^{-1}, 367% to 3,0 g L^{-1}, 506% to 4.5g L^{-1} and 481% to 6.0 g L^{-1}. In Figure 4, there are evident differences in plant development for the irrigation systems in relation to fertilizer doses. This difference can be explained by the fact that at the "floating" system, the water was more uniformly distributed, increasing the efficiency of fertilizer application and providing increments in the variables height and number of leaves; reflecting concomitant increase of 72% in total dry matter. It is also observed that most seedling height should not be because of shading, since the weights of dry matter per plant were higher for all treatments (Figure 4).

Figure 4. Seedlings of *Artemisia annua* L. under fertilizer solutions at concentrations of 1.5 (a) 3.0 (b) 4.5 (c) and 6.0 (d) g L^{-1} under microsprinkler system and 1.5 (e), 3.0 (f), 4.5 (g), and 6.0 (h) g L^{-1} under the floating system, both at 36 DAS.

Magalhães et al. (2012) reported that seedlings of *Artemisia annua* L. can be transferred to the field when presenting a height of 100 mm, roughly at 60 DAS. In the present experiment, it was observed plant heights of 150.5 and 20.27 mm for “floating” and microsprinkler systems, respectively, at 36 DAS (Table 4).

According to Lopes, Guerrini e Saad (2007) amounts of water above the holding capacity of the substrate favor the leaching of nutrients, impairing the morphological development of the plant, which may have influenced the treatment of the microsprinkler system.

The “floating” system with 3.0, 4.5 and 6 g L^{-1} doses did not stimulate root growth, given that non-temporary restriction or limitation on the supply of water and nutrients can reduce root growth, disfavoring the fixation and resumption of growth after transplanting the seedlings.

According to Marques, Cripa, e Martinez (2013) a well-developed root system can provide better supply conditions of water demand for the plant, especially in the first weeks, when adverse conditions may compromise its survival.

The relationship between root dry matter and aerial part was inversely proportional to applied doses, with observed values of 1.57, 0.68, 0.44 and 0.23 for the treatments of 1.5, 3.0, 4.5 to 6.0 g L^{-1} in the “floating” system (Figure 5). The two irrigation systems have provided the formation of a divot at the moment of withdrawing the seedling from the trays, regardless of dose levels.

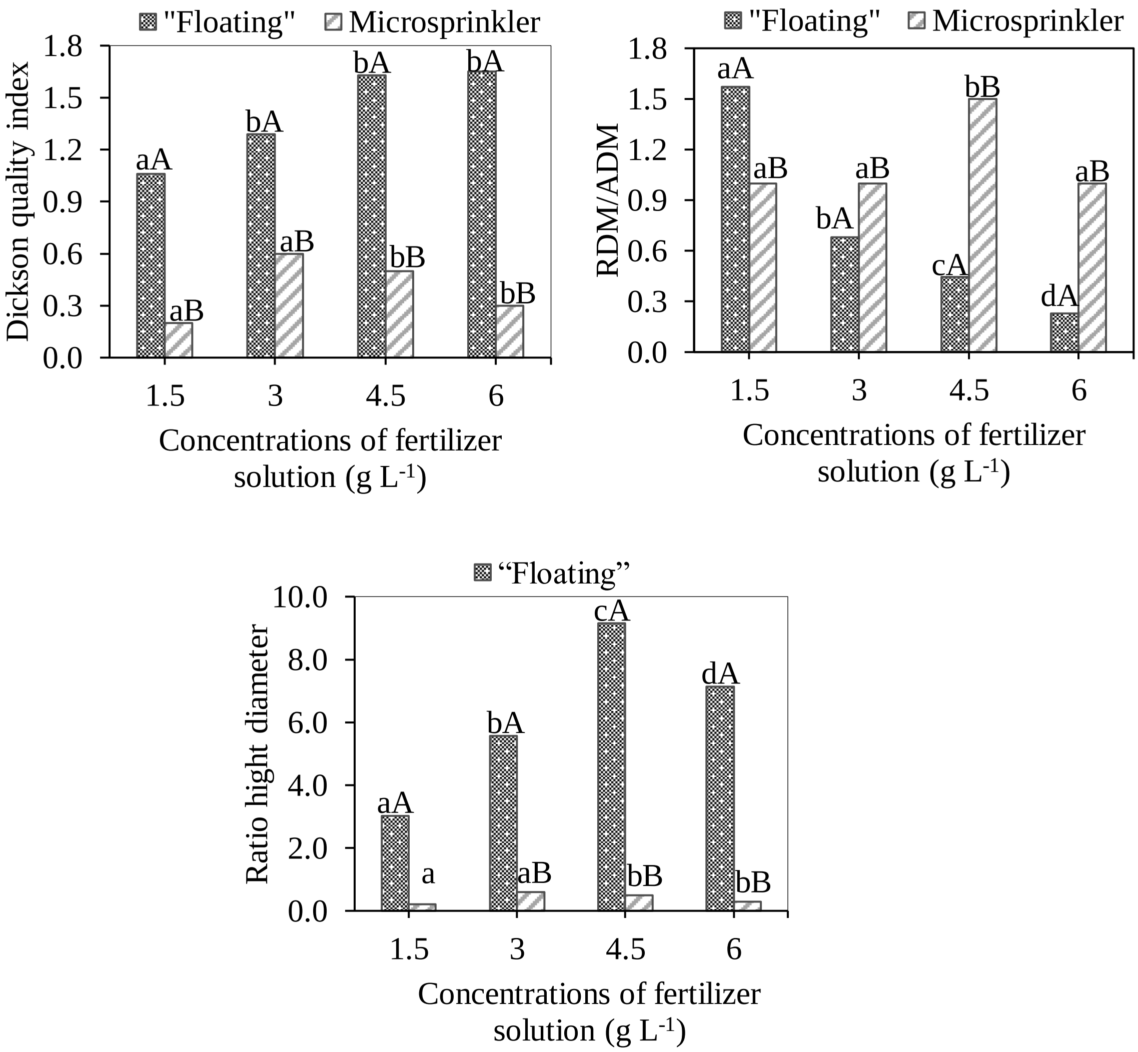
Table 4. Effect of irrigation and doses of nutrient solution system in plant height (HGT), number of leaves (NL), length of the root system (RSL), stem diameter (DIAM), total fresh matter (TFM) and total dry matter (TDM) of seedlings of *Artemisia annual* L. at 36 days after sowing.

Fertilizer Solution (g L^{-1})	HGT (mm)		NL		RSL (mm)	
	Floating	Microsprinkler	Floating	Microsprinkler	Floating	Microsprinkler
1.5	46.47 aA	17.20 aB	6.85 aA	4.97 aB	114.97 aA	83.52 abB
3.0	123.02 bA	33.47 bB	12.85 bA	6.42 bB	100.1 bA	91.37 caB
4.5	166.15 cA	32.80 cB	12.92 bA	5.65 cB	97.52 bA	95.65 cA
6.0	167.42 cA	34.80 bB	17.20 cA	5.97 cB	94.3 bA	81.52 bB
Irrigation System	150.51 A	20.27 B	12.45 A	5.75 B	101.73 A	88.02 B
Fertilizer Solution (g L^{-1})	DIAM (mm)		TFM (gramas)		TDM (gramas)	
	Floating	Microsprinkler	Floating	Microsprinkler	Floating	Microsprinkler
1.5	1.59 aA	1.11 aB	1.29 aA	0.12 aB	0.18 aA	0.02 aB
3.0	2.32 bA	1.51 bB	2.88 bA	0.41 bB	0.38 bA	0.06 bB
4.5	1.87 aA	1.41 cB	2.00 cA	0.37 bB	0.27 cA	0.05 bB
6.0	2.41 cA	1.33 cB	2.49 cA	0.35 cB	0.33 cA	0.04 cB
Irrigation System	2.05 A	1.34 B	2.17 A	0.31 B	0.29 A	0.04 B

Means followed by the same lower case letter in the column and capital letter in the line not differ by Kruskal-Wallis test at 5% level of probability.

The height diameter ratio and the Dickson quality index by, both parameters indicators of quality seedlings are shown in Figure 5. The average quality score of Dickson for the irrigation systems has shown statistical differences. It was noticed values greater than 1.3 for the “floating” system and values below 0.6 for microsprinkler system (Table 4). Seedlings with DQI lower than 0.2 indicate poor final quality be transfer to the to the field however, the higher the value of DQI, higher will be the quality of the seedlings. According Fonseca et al. (2002) the Dickson quality index is a good indicator of quality because it takes into account the balance of the distribution of biomass, being an important parameter to be used in assessing the quality of seedlings.

Figure 5. Dickson quality index, RDM/ADM e Ratio height diameter for two irrigation systems under different concentrations of fertilizer solution.



Therefore, the system most suitable for production of seedlings of Artemisia irrigation is floating, because it keeps the moisture and the nutrient solution on the substrate evenly. Similar results were obtained by Andriolo, Boemo e Bonini (2001), who observed that the “floating” system was superior to conventional production of seedlings of melon and tomato. Likewise, Teramoto, Kano, e Minami (2006) studied the production of tomato seedlings under different irrigation systems, observing that irrigated by “floating” system presented higher production compared to microsprinkler system. Santin et al. (2005) observed that seedlings of beets that received nutritional conditioning through the “floating” system showed higher growth compared to seedlings developed under the conventional microsprinkler.

However these results do not make treatment microsprinkler contraindicated for the intended purpose of this work, because differences in seedling growth can be recovered after transplanting, as noted by Marques et al. (2003), who studied lettuce seedlings produced in trays with different dimensional characteristics. Barbosa et al. (2010) experimented the cultivation of marigold under two different substratum types and sizes of tray cell, observing that improved seedling development where equivalent to those less developed, after transplanting, resulting in plants with similar yield.

According to these results, the A. annua high quality seedlings can be commercially produced with the use of floating system, allowing success in biomass production and essential oil yield.

6 CONCLUSIONS

The “floating” system was more suitable for seedlings production of *Artemisia annua* L., because it allowed uniformity of distribution of moisture and nutrient solution in the substrate. All doses of fertilizers in nutrient solution resulted in Dickson quality index greater than 1.3 under floating system, while in the micro sprinkler system, for all doses, those did not exceeded 0.6. The floating system allowed a 27% saving in the total amount of water applied.

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