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CULTIVATION OF *Rosmarinus officinalis* IN HYDROPONIC SYSTEM

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KEYWORDS

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Hydroponics

Population density

Growth curve

Biomass

ABSTRACT

This research was conducted to establish the relationship between population density to total dry biomass production and estimate the nutrient absorption curve in a hydroponics system for *Rosmarinus officinalis* L. Study was carried out at the Center for Research and Development of Hydroponics in Faculty of Agronomy at the Autonomous University of Nuevo Leon. Three population densities viz., 8, 16 and 24 plants per square meter were evaluated in a hydroponic system, using volcanic rock with grain diameter of 20-40 mm as an inert substrate and a standard hydroponic nutrient solution. Among tested three plant densities, population density of 8 plants m^{-2}, total dry biomass production produced highest, total dry biomass and it shows superiority over the plant density with 16 to 24 plants m^{-2} populations. There were no significant differences in plant height. The data obtained were fitted to linear growth models, which were used to estimate nutrient absorption curves.

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1 Introduction

Crop population density is a determining factor in biomass production; it is related with the number of individuals or plants per unit of surface area. Nutrient absorption curves is a possible way to determine preliminary nutrient requirement and to design a suitable fertilization programs for a crop which can allowing the efficient use of chemical fertilizers with a consequent reduction in pollution and avoidance of unnecessary expenditure of crop production (Molina et al., 1993; Jiménez-García et al., 2009). Nutrient absorption curve estimations reflect the changes in the plant phenology which can be associated with maximum nutrient absorption points at key development stages of plant such as flowering and fruiting.

In perennial species for maintaining their ability to survive and higher yield, proper management and optimal conditions should provide to maintain a defined yield level. In relation to traditional soil culture production systems, hydroponics methods applied in greenhouses offer a greater level of control over plants. Hydroponics is a technique which normally used to estimate growth curves and nutrient absorption for any plant species (Rodríguez & Leihner, 2006; Almaguer-Sierra et al., 2009; Jiménez-García et al., 2009; Rodríguez-Fuentes et al., 2009b). This is comparable to modern industrial production systems in which automation and control concepts are applied on a tactical and operational basis that supports decision making at the level of management.

Mathematical crop models can also be generated to predict nutritional and environmental requirements for better crop production. Assessing nutrient extraction by the plant during its life-cycle over time intervals can build an absorption curve and in some cases allow for mathematical modeling (Roose et al., 2001). Nutrient removal depends on both internal and external factors, in this former consisting the genetic potential of the plant and its phenological development stage, while external factors relate to the environment where the plant is grown such as soil texture, pH, electrical conductivity air temperature, light and relative humidity (Prusinkiewicz, 1998; Gary et al., 1998).

The population density of the culture refers to the number of individuals or plants on a unit of surface area. Cruz-Huerta et al. (2009) pointed out that population density is one of the factor which influences the amount of biomass generated; additionally, there is a relationship between the number of individuals in a defined area and biomass produced. They determined that in sweet pepper (*Capsicum frutescens*) fruit per plant load were decreased with the increasing population density, but overall fruit production per unit area increased. Similar type of findings was also reported in banana (Rodrigo et al., 1997) and potato (Flores-Lopez et al., 2009) and these researchers suggested that higher population density decreased the amount of total biomass per individual but overall production per unit area increased (Flores-Lopez et al., 2009).

Martinez-Fernandez et al. (1996) mentioned that the wild rosemary is found in average population density of 1-2 plants m^{-2} and produces an aerial biomass from 266.4 to 836 g m^{-2}, depending on water compensation mechanisms. Contradictory observation was reported by Mishra et al. (2009) when they conducted a two-year on rosemary cultivation under dry conditions with three densities (6, 8 and 16 plants m^{-2}). These researchers reported that higher population density led to increased production of dry biomass and essential oils as compared to lower densities in which yield per individual was higher. In Spain this species is found naturally in population densities of 1.0 to 2.0 plants m^{-2} and producing on average 551 g m^{-2} of dry biomass (Martinez-Fernandez et al., 1996).

Besides, Sardans & Peñuelas (2005) reported that rosemary production was reported 200-300 g m^{-2} at population densities of 1.5 to 2.0 plants m^{-2}. They also reported that addition of nitrogen and phosphorus to the hydroponics solution increased biomass production and the concentration of these elements in leaves of in rosemary (*Rosmarinus officinalis* L.). SAGARPA (2012) reported that in 2011 total 50.75 ha were under rosemary cultivation in Mexico and the states of Baja California Sur (11.75 ha) and Estado de Mexico (39 ha) are the major rosemary cultivated area with mean annual biomass production (not indicated whether it was wet or dry) 7 and 6 t ha^{-1} respectively. However, there is little information regarding crop management, nutritional needs and hydroponics production for *R. officinalis*.

In this study, effect of population density on biomass production and absorption of N, P, K, Fe and Mn was evaluated in the hydroponic cultivation of rosemary (*R. officinalis*). Further, growth curves and absorption of N, P, K, Fe and Mn for hydroponically grown.

2 Material and Methods

2.1 Cultural conditions and setup of study

This study was conducted from the 30th October 2011 to 30 May 2012 at the Center for Research and Development for Hydroponics of Marin Campus Faculty of Agriculture, Autonomous University, Nuevo Leon, which is located in the municipality of Marin, Mexico, at the geographical coordinates: L 25º 23” N and L 100º 12” W with 393 m altitude. Maximum rainfall was reported in the month of October 2011 (110 mm) and January 2012 (334.6 mm) (INIFAP, 2011; INIFAP 2012). The wind direction from north to south with an average annual temperature of 24 °C; maximum temperature of 38 °C and minimum of 7°C; the warmer months are June, July and August (INIFAP, 2012).

A closed hydroponic system was developed on the terraces which built up by of concrete blocks with dimensions of 14m long and 1.10m wide (inside), 0.20 m in height and with a polished concrete floor and sealed finish, was used in this study. The terrace consists of two parts, the body and head allowing the nutrient solution to be drained through a collector below the floor connected below the floor to a 2.5 m 3 tanks. Lava rock substrate (20-40 mm in diameter) was used to anchor the plants and to help provide nutrients to the plant roots. The substrate was previously cleaned and disinfected with a solution of industrial-grade sulfuric acid buffered at pH = 3.0, with this solution the terrace was flooded for a period of three hours and subsequently washed twice by tap water.

The volume of HNS was prepared 2000 L and completely renewed every 10 day intervals. The pH of HNS solution was adjusted to 5.0-5.5. Irrigation with HNS was performed every after third day. A 0.373 kW centrifugal pump of 3.81 cm in diameter, located in the outlet, was used to saturate the substrate contained in the terrace. The excess HNS solution was drained (recycled) immediately into the tank by gravity. To estimate the amount of water retained in the substrate, the moisture holding capacity was determined by the known volume method (Ramachandra et al., 2016). For transplanting, about 30 cm of a rosemary landrace plant was used. To prevent fungal attack, the plant roots and root source substrate (leaf mulch) were immersed in a fungicide solution (30% cymoxanil, 72% chlorothalonil) by dosing 90 g L^{-1}. The plants were subsequently inserted into volcanic rock to a depth of 15 cm.

2.2 Experimental design

A randomized complete block design was used with 3 treatments (T1 - 8 plants m^{-2}; T2 – 16 plants m^{-2} and T3 -24 plants m^{-2}) and 4 replications.

2.3 Sampling before transplanting

Before transplanting, 10 plants were randomly selected to be used in determination of initial dry biomass of plant aerial parts (DBPA), root dry biomass (RDB), total dry biomass (TDB= DBPA + RDB).

2.4 Assay I

Once the crop established and acclimatized, four plants for each treatment were harvested every 30 days from 30 October 2011 until 30 May 2012. These harvested plants consisted of a whole plant (aerial part + root). Once the plants were removed from each treatment, they were identified and labeled. These plants were placed in a container with clean water to remove substrate residues and then washed under a water jet prior to transfer to the laboratory. For each replication, estimation of TDB, DBPA, RDB, plant height and concentrations of N, P, K, Fe and Mn (Paech & Tracey, 2013). To estimate the moisture content, the samples were placed in identified brown paper bags and then dried in a forced convection oven (Brand Riossa, Model H-62, Mexico), maintained at a temperature of 70 to 80 °C to constant weight. Information regarding TDB, DBPA and RDB were determined for all three trials using the formula described in Equation 1.

$$H = P_f - P_s \quad (1)$$

whereas, H= moisture, g; P_f= Fresh weight (g); P_s= Dry weight (g)

Total dry biomass (TDB) for all trials were estimated by ground the samples in a Willey stainless steel mill, sieved with a mesh of 20 microns, and then placed in a muffle furnace at 450-550ºC for 4 h. The Kjeldahl method (Labconco, 2016) was used to determine total nitrogen content while the total P was determined by optical spectroscopy (Spectronic 21D, Milton Roy) according to the Vanadate/molybdate or yellow method. Further level of K, Fe and Mn were determined by atomic absorption spectroscopy (Rodriguez-Fuentes & Rodriguez-Absi, 2015).

Table 1 Micro and macro nutrient concentration of hydroponic nutrient solution (SNH) used in this study.

Element	Concentration (mg/L)	Source
N	200	---
P	60	KH_2PO_4
K	250	KNO_3
Ca	200	$Ca(NO_3)_2.4H_2O$
Mg	50	$Mg(NO_3)_2$
S	100	H_2SO_4
Fe	0.50	$FeSO_4.7H_2O$
Mn	0.25	$MNSO_4.H_2O$
B	0.25	H_3BO_3
Cu	0.02	$CuSO_4.5H_2O$
Zn	0.25	$ZnSO_4.H_2O$
Mo	0.01	$Na_2MoO_4.2H_2O$

Source: Rodríguez-Fuentes et al. (2011).

2.5Assay II

For II assay 10 plants were established in field under natural condition, from 30 October 2011 to the completion of the study which is May 30, 2012 in order to measure plants height per treatment every 10 days. On the other hand, a total 10 whole plants (aerial and root) were harvested per treatment on every 10 days intervals; these were identified and washed with water. From these plants, 500 g samples of fresh material per treatment were collected to determine the TDB of each treatment.

2.6 Statistical analysis

To run variance analysis and mean comparisons, a software on Design of Experiments (Olivares, 2012) and SPSS 17.0 (2008) were used. To estimate the growth curves and nutrient absorption, Sigma Plot software 10TM (Systat Inc., 2010) was used.

Table 2 Monthly average of TDB (g/plant) reported in assay I.

Treatments	Dec.	Jan.	Feb.	Mar.	Apr.	May.
T1	85.68±3.53[a]	123.76±3.02[a]	117.00±6.56[a]	209.50±5.87[a]	229.50±7.98[a]	267.25±8.99[a]
T2	55.60±2.36[ab]	67.01±1.89[b]	117.00±5.66[a]	133.50±4.87[b]	174.25±7.06[b]	159.25±7.89[b]
T3	46.79±2.03[b]	68.45±1.91[b]	67.50±3.65[b]	76.00±4.65[c]	144.50±6.15[b]	176.25±7.01[b]

Different letters in same column show significant difference ($p \leq 0.05$)

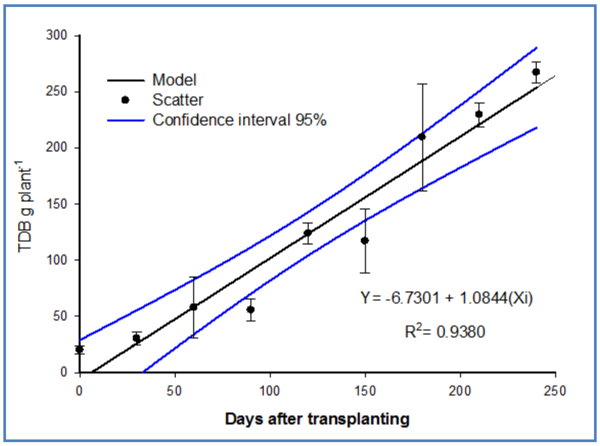

Figure 1 Model of linear adjust for production of TDB in Treatment1. TDB= Total dry biomass (g). Vertical bars in each point represent standard deviation of the mean.

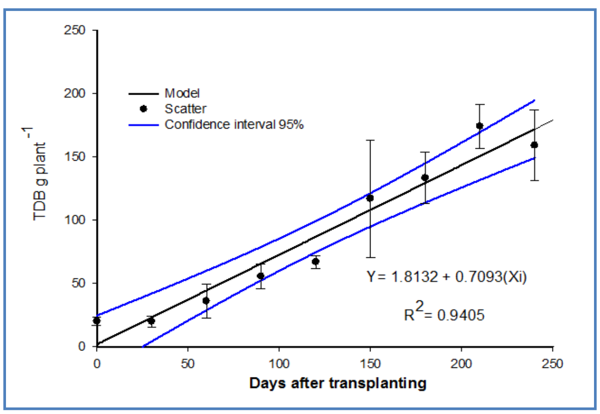

Figure 2 Model of linear adjust for production of TDB in Treatment 2. TDB= Total dry biomass (g). Vertical bars in each point represent standard deviation of the mean.

3 Results and Discussion

3.1 Assay I

Effect of plant density on TDB was reported in Table 1 and a significant difference was reported between various treatments T1, T2 and T3 (g plant^{-1}) during the trial period (December 2011 to May 2012) ($P \leq 0.05$). Among various treatments, T1 showed the highest TDB production for each month (Table 2).

Scattering data of TDB (g plant^{-1}) were obtained for treatments 1, 2 and 3, with their respective standard deviations. It was reported that all three treatments followed a similar growth pattern (Figure 1, 2 & 3).

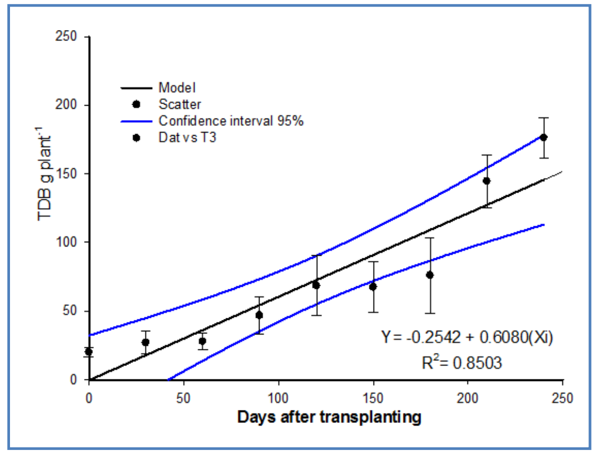

Figure 3 Model of linear adjust for production of TDB in Treatment 3. TDB= Total dry biomass (g). Vertical bars in each point represent standard deviation of the mean.

Based on the concentration of N, P, K, Fe and Mn in the TDB, the extraction over time was estimated. For this, the linear model was used. Table 3 showed the extraction curves for each nutrient per treatment and the estimated model. Results of this assay are coincided with results reported by Mishra et al. (2009), who used more space between rows and plants (0.60 m x 0.30 m). Treatment with 6 plants m^{-2} produced the greatest amount of aerial parts of plants compared with the other treatment dimensions evaluated: 0.30 x 0.20 m (17 plants m^{-2}) and 0.40 x 0.30 m (8 plants m^{-2}) between rows and plants, respectively. Experiment was conducted in India, using rosemary plants under dry conditions. Moreover, Escalante-Estrada & Linzaga-Elizalde (2008) evaluated the total dry weight of sunflower plants that were set to 7.5, 10, 12.5 and 15 plants m^{-2} and concluded that lowest density (7.5 plants m^{-2}) was the one with the highest dried biomass production.

The relationship between rosemary TDB production and sampling time was adjusted to linear models ($P \leq 0.05$); the determination coefficients (R^2) were 0.9380, 0.9405, 0.8503 for treatment 1, 2 and 3 respectively. It is considered that linear equations adequately estimate growth (Rodas-Gaitan et al., 2012).

Table 3 The extraction curves for each nutrient per treatment and the estimated model. Where: x= elapsed time (in days); y= g of absorbed nutriment per plant (TDB) for Assay I.

	Treatment	Model	Determination coefficient (R^2)
Nitrogen	T1	y = 0.2607+0.0188x	0.9813
	T2	y = 0.0364+0.0149x	0.9596
	T3	y = 0.0109+0.0116x	0.8996
Phosphorus	T1	y = 0.0196+0.0029x	0.7699
	T2	y = 0.0350+0.0022x	0.7581
	T3	y = 0.0351+0.0019x	0.8540
Potassium	T1	y = 0.0665+0.0190x	0.9415
	T2	y = 0.1043+0.0125x	0.8848
	T3	y = 0.0493+0.0109x	0.9100
Iron	T1	y = 15.1179+0.3537x	0.7744
	T2	y = 6.9924+0.2539x	0.8047
	T3	y = 5.4824+0.2161x	0.8558
Manganese	T1	y = 1.6682+0.0726x	0.9462
	T2	y = 1.1717+0.0530x	0.8650
	T3	y = 10.5213+0.0457x	0.8561

The linear fit may be due to the critical period of the trial and due to the perennial nature of the species; this can be explained by Rodriguez & Leihner (2006) study those who point out that plants generally have a growth pattern which is represented by a sigmoidal model, however through segmenting the model can be separated into linear models. The sigmoidal model and the determination coefficient values were also similar to the estimated linear models; so it was decided to use a linear relationship in order to make an easier calculation of the nutrient extraction and to estimate the hydroponic nutrient solution to be used as a first approximation in the nutritional management in future studies.

Similar types of results were also reported by Mishra et al. (2009) those who attributed these results to plants having an improved ability to spread and grow better because the level of competition for light, water and nutrients is lower when plants have wider spacing. Also, Pakrasa et al. (1999) reported increased TDB production in rosemary, when it grown under irrigation and nitrogen fertilizer at a density of 3 plants m^{-2} with spacing of 60 x 60 cm. Both authors are agreeing that the total production per unit area (and not per plant) is lower with these densities.

3.2 Assay II

Comparison of means ($p \leq 0.05$) for production TDB $plant^{-1}$ is shown in Table 4, and it was observed that treatment 1 was statistically superior to 2 and 3. Figure 4 represent that plant height did not vary significantly between treatments ($p \leq 0.05$).

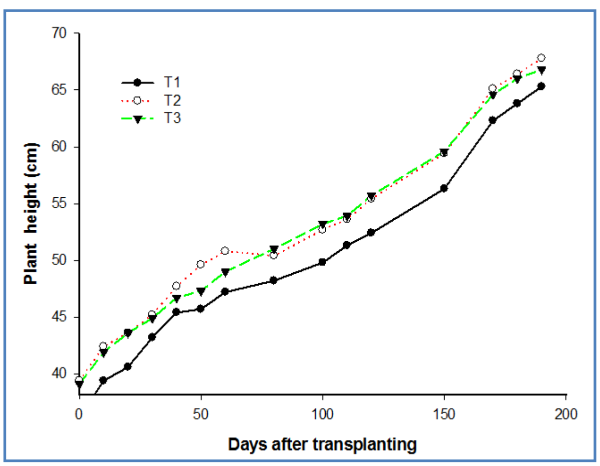

Figure 4 Trend in plant height (cm) between the three treatments (Assay II).

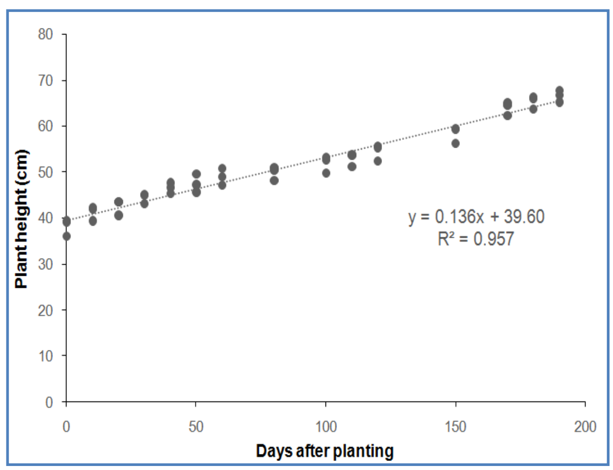

Figure 5 Trend between treatments and linear model to predict crop height.

Table 4 Comparison of means for production of TDB plant^{-1} (Assay II).

Treatment	Mean	($p\leq0.05$)
T1	253.090±11.05	a
T2	167.556±8.55	b
T3	154.134±7.77	b

Different letters in the same column indicate significant difference (Tukey $P\leq0.05$)

In present study, production of TDB (g plant^{-1}) in treatment 1 was superior to treatment 2 and 3 (Tukey $p\leq0.05$); these results were similar to the assay 1and also are agreement with Misrah et al. (2009) and Pakrasa et al. (1999). Further, similar results in the cultivation of rosemary was reported by Escalante-Estrada & Linzaga-Elizalde (2008), Cruz-Huerta et al. (2005) and Vega et al. (2001) in studies of population density in other plant species have concluded that increasing the population density per individual production decreases but increases per unit area.

About the plant height there were not significant differences between treatments. Figure 5 shows trend between treatments and linear model to predict crop height under these conditions. Therefore, plant height sometimes has not relation to plant growth (accumulated TDB) (Bidwell, 2002; Saldívar, 2010).

Conclusions

The highest dry biomass production per plant at the end of assay II occurred in treatment 1 (8 plants m^{-2}) and corresponded to 253.09g ($P\leq0.05$). Total dry biomass production per plant was fitted to linear models for treatments 1, 2 and 3 with R^2 values of 0.9380, 0.9405 and 0.8503 respectively. Plant height was not significant ($P \leq 0.05$) for different population densities, after 240 days after transplanting and plant heights ranged between 65.30 cm and 67.80 cm. For the last month of crop sampling, the concentration (mg kg^{-1}) in all plant nutrients was not significant ($P \leq 0.05$).

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Abbreviations

HNS	Hydroponic nutrient solution
DBPA	Dry biomass of plant aerial parts
RDB	Root dry biomass
TDB	Total dry biomass

Conflict of interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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