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(Organizadores)

Ciência em Foco

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Apresentação

O avanço da Ciência tem promovido o desenvolvimento de inúmeras tecnologias que tende a proporcionar o incremento da produção de alimentos, a melhoria da qualidade de vida da população, a preservação e sustentabilidade do planeta. Todavia, além da geração de novos conhecimentos é necessário a dispersão para o público alvo. Algo que geralmente é negligenciado por muitos autores, pois, se limitam apenas em publicar um artigo científico.

Nesse aspecto, a "Pantanal Editora" surgiu com a missão de "publicação de trabalhos de pós-doutorado, teses, dissertações, monografias, trabalhos de conclusão de curso, ensaios e artigos científicos" com o lema "Ciência com consciência". Nossos valores são construídos sob esse alicerce. Qualidade, ética, relevância acadêmica e impacto social, norteiam nossos trabalhos. Diferentemente de outras editoras, nós procuramos pesquisadores que estejam dispostos a fazerem capítulos que passaram por revisões criteriosas e não somente aplicar o binômio pagou-publicou.

Além disso, tem como visão "A ciência é vital para o desenvolvimento humano, e seu progresso somente é possível quando apoiado sobre o conhecimento científico passado. Por isso a divulgação dos trabalhos científicos é essencial para que a ciência possa alcançar a todos, transformando nossa sociedade."

Com base nesses pilares, a "Pantanal Editora" orgulhosamente apresenta em seu primeiro livro "Ciência em Foco", em seus 22 capítulos, avanços nas áreas de Ciências Agrárias e da Engenharia. Conhecimento estes, que irá agregar muito aos seus leitores, entre os assuntos, adubação nitrogenada na soja, diversidade genética de cultivares de mandioca, produção de mudas, magnetismo na agricultura, técnicas de avaliação do sistema radicular das plantas, percepção ambiental de alunos, análise de gestão de resíduo sólidos, conservação de estradas, sustentabilidade e responsabilidade social. Portanto, fica evidente que essas pesquisas procuram promover melhorias quantitativas e qualitativas na produção de alimentos e, ou melhorar a qualidade de vida da sociedade. Sempre em busca da sustentabilidade do planeta.

Aos autores dos diversos capítulos, pela dedicação e esforços sem limites, que viabilizaram esta obra que retrata os recentes avanços científicos e tecnológicos, os agradecimentos dos Organizadores e da Pantanal Editora.

Por fim, esperamos que este livro possa colaborar e instigar mais estudantes e pesquisadores na constante busca de novas tecnologias. Assim, garantir uma difusão de conhecimento fácil, rápido para a sociedade.

Alan Mario Zuffo
Jorge González Aguilera
Bruno Rodrigues de Oliveira

SUMÁRIO

Ciências Agrárias

Engenharias

Capítulo 9

Stimulation of physiological parameters of *Rosmarinus officinalis* L. with the use of magnetically treated water

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INTRODUCTION

Phytotherapy studies the extractive use of medicinal plants, or their derivatives, for therapeutic purposes, including, for example, those used to treat or prevent cardiovascular disease, cancer, neurodegenerative diseases, and oxidative stress. The discovery of new plant species has sparked renewed interest in understanding the therapeutic properties of plants. New investigations are undertaken every day in anticipation of finding and confirming new pharmacological agents (Falzon; Balabanova, 2017).

Rosmarinus officinalis L. (rosemary), *Lamiaceae*, has been used since ancient times as a spice and in traditional medicine, owing to its many properties. This plant can usually be found on coastal lands surrounding the Mediterranean Sea, extending eastward to the Caucasus. It is currently cultivated in much of the world. However, interest in this plant today arises from the potent antioxidant effect of some of its components (Ladeiras, 2015; Cutillas et al., 2018). Recent studies have reported its strong therapeutic potential in the treatment and prevention of many diseases, such as asthma, liver disorders, peptic ulcers, anti-inflammatory diseases, ischemic cardiovascular diseases, atherosclerosis and poor sperm motility (Ngo et al., 2011; Rašković et al., 2014).

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It is currently withdrawn from the National Formulary of Phytopharmaceuticals for availability in Cuba, and in recent years, this species has been on the list of prioritized plants for the development of natural and traditional medicine in Brazil (MINSAP, 2014). Although the plant is well documented in the literature, its medicinal properties are far from homogeneous (Ladeiras, 2015). In particular, terpenoids (volatiles) and polyphenolic compounds are those that have strong antioxidant effect (Boix et al., 2011; Cutillas et al., 2018). Indeed, with its daily use among the population, such properties are the main theme of the present research.

Within the technology applied for its conventional propagation, the use of organic agriculture, compost-enriched soils and substrates, such as zeolite, are used, as well as growth regulators to stimulate rooting (Lemes et al., 2001). Magnetically treated water is an alternative technology to improve plant growth variables, as well as increase the corresponding secondary metabolites of pharmacological interest (Boix et al., 2008; 2018; 2019). With the application and use of this technology, plants can be obtained in less cultivation time and with better quality of the raw material necessary for the preparation of antioxidant biopreparations. In particular, then, this study aims to evaluate physiological parameters, including mass, pigments and mineral contents of *R. officinalis* cultivated with magnetically treated water (MTW) under nursery conditions.

MATERIAL AND METHODS

The experimental work was carried out at the National Center for Applied Electromagnetism (CNEA), part of the University of Oriente (UO) in Santiago de Cuba, Cuba. As initial material leaves of six-month-old adult *R. officinalis* measuring 50 cm in length were used and were grown in the beds of the experimental plot of the CNEA.

Substrate characteristics

A substrate composed of a mixture of soil and organic matter (3:1) was used. Three soil samples were chosen and analyzed in the "Elio Trincado" Laboratories of the Geominera Oriente Company in Santiago de Cuba, and their chemical properties were presented in Table 1.

Table 1. Chemical properties of soil used in the experimental plot.

Characteristics	Media
Electrical conductivity (µS cm^{-1})	397
Potassium oxide (%)	0.30
Phosphorus (%)	0.94
Calcium oxide (%)	7.29
Ferric (%)	3.9
Aluminum (%)	12.67
Magnesium oxide (%)	8,06
Cobalt (%)	<0.005
pH	7.84
Manganese (%)	0.20

Irrigation scheme employed

Irrigation was carried out twice a day through a sprinkler system for 30 minutes, which has, among its accessories, an ITUR Pump and a distributor system controlled by valves that guarantee irrigation will be carried out in sections.

Characteristics of the irrigation water used were as follows: water speed of 1.4 – 1.6 m s^{-1}, pump flow rate of 2.54 – 2.91 $m^3 h^{-1}$, and electrical conductivity of 0.25 µS cm^{-1}.

Chemical analysis of the irrigation water was carried out in the laboratories of the LabTur Company, and the properties were shown in Table 2.

Table 2. Chemical composition of irrigation water employed.

Mineral composition	mg mL^{-1}	Mineral composition	mg mL^{-1}
HCO^-	130.58	Na^+	11.31
PO_4^{3-}	1.33	CI^{-1}	33.18
Ca^{2+}	39.88	$S0_4^{-2}$	31.20
Mg^{2+}	8.88	NH_4^+	0.28
K^+	2.54		
Physical properties			
Conductivity (mS cm^{-1})	pH	Total hardness (mg L^{-1})	
0.28	7.50	158.5	

Description of the treatments

Three employed treatments included Treatment 1: (control, MTW1) plants grown in water without magnetic treatment; Treatment 2: (MTW 2) plants grown in magnetically treated water at an induction range between 60 and 100 mT; and Treatment 3: (MTW 3) plants grown in magnetically treated water with an induction range between 110 and 160 mT) (Figure 1). Magnetic treatment was achieved with a permanent magnet. The design, construction and calibration were carried out at CNEA, according to the protocol of Gilart et al. (2013).

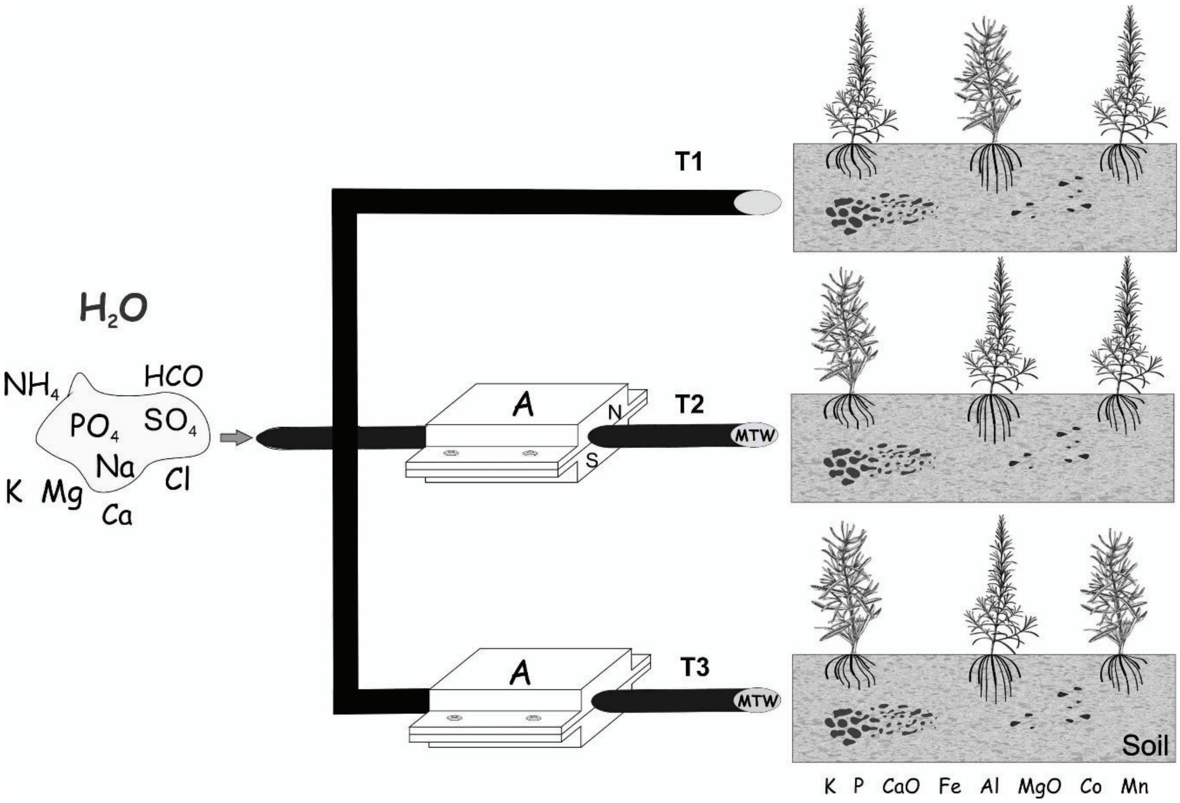

Figure 1. Cultures of *Rosmarinus officinalis* L. subjected to irrigation using water treated with static magnetic field (MTW): T1 (control), T2 (60-100 mT) and T3 (110-160 mT). A. Magnetizer dispositive showing their N – North and S- South Poles (A), the direction of magnetic field generated, and water flow between the two permanent magnets in the PVC pipe. Mineral composition may be seen in water and soil.

Determination of photosynthetic pigments of *Rosmarinus officinalis* L.

One way to know the water status of plants is through the determination of water content and loss in tissues. Fresh weight is used as a basis for calculating water content, and the percentage of water content (PWC) was calculated, taking into account the ratio of fresh mass/dry mass (Ortega; R, 1986). A Sartorious® digital analytical balance (BS 124S, China; with 0.1 mg accuracy) was used. The results are expressed in grams.

$$PWC = \frac{FW0 - FW}{FW0} * 100 ,$$

where FW_0 is the fresh mass, and FW is the dry mass.

In the determination of chlorophyll and carotenoid contents, adsorption readings at different wavelengths were taken, and the following formulas were employed:

$$C_a = 0.0127\, A_{663} - 0.00269\, A_{643}$$

$$C_b = 0.0229\, A_{645} - 0.00468\, A_{663}$$

$$C_{carot} = 4.695\, A_{440.5} - 0.268\, C_{a+b}.$$

Determination of ions in the leaves of *Rosmarinus officinalis* L.

The chemical characterization of *R. officinalis* leaves cultured with magnetically treated water was performed through ionomic studies by the Inductively Coupled Plasma Atomic Spectrometry (ICP-AES) in the laboratories of the Geominera Oriente Company, according to MINSAP regulations by Benzie and Szeto (1999).

Two grams of dried drug were digested in a mixture of concentrated nitric/hydrochloric acids, and the resulting material was filtered and transferred to a volume of 25 mL for subsequent analysis by Inductively Coupled Plasma Spectrometry (ICP) using the SPECRO ARCOS device (Germany) in axial view. All reagents used were spectral quality.

In the preparation of the solutions, deionized water with a conductivity of less than 0.1 $\mu\Omega\ cm^{-1}$ was used (Table 3).

Table 3. Experimental conditions of Inductively Coupled Plasma Atomic Spectrometry.

Parameters	Conditions	Parameters	Conditions
Frequency	27 MHz	Nebulizer type	Meinhard (concentric)
Plasma energy	1.4 KW	Type of torch	One piece
Integration time	24 seconds	Carrier gas flow	0.4-1.5 L min^{-1} (optimized)
Background correction	Dynamic	Flux of auxiliary gas	1 L min^{-1}
Slow preflux	2.28 rpm (3 rpm)	Plasma Ar gas	12 L min^{-1}
Number of measurements	3	Fast preflux	4.20 mL min^{-1} (63 rpm)
Input Slot	25 µm	Observation distance	3-10 mm (optimized)
Dispersion	0.55 mm min^{-1}	Resolution of monochromator	0.0137 mm
Time factor	100%	Focal distance of spectrum	750 mm

Statistical analysis

It was considered a completely randomized experimental design with three replicas, using the Kolmogorov-Smirnov normality test to corroborate the normality of the parametric data. For the analysis of the variable dry mass and content of photosynthetic pigments, a One-way ANOVA analysis was used, and then a Tukey test was applied for multiple media comparison. For analysis of the different ions used, a One-way ANOVA Analysis was used, along with the Student's -*t* test. The value of statistical significance was established as $p<0.05$.

RESULTS AND DISCUSSION

The determination of dry mass is used to measure growth. This is an indispensable parameter with which to assess the quality of dry material from a medicinal plant. Significant differences were observed between control plants that weighed 4.31 g in the MTW 1 and MTW 2 treatments. The highest values were obtained in plants irrigated with magnetically treated water, weighing 4.89 g as a result of the wider range of magnetic induction used in irrigation relative to MTW 1 which was 4.55 g (Table 4).

Table 4. Effect of magnetically treated water (MTW) on dry mass and concentration of photosynthetic pigments in leaves of *Rosmarinus officinalis* L..

Treatments	Dry mass (g)	Concentration of photosynthetic pigments (mg L^{-1})		
		Chlorophyll $_a$	Chlorophyll $_{b + carotenes}$	Carotene
Control	4.31 ± 0.02 ^{b}	0.72 ± 0.03 ^{b}	0.31 ± 0.03 ^{b}	0.20 ± 0.03 ^{b}
MTW 2	4.55 ± 0.06 ^{a}	0.82 ± 0.02 ^{ab}	0,49 ± 0.03 ^{ab}	0.29 ± 0.03 ^{ab}
MTW 3	4.89 ± 0.09 ^{a}	0.92 ± 0.04^a	0.75 ± 0.04 ^{a}	0.32 ± 0.03 ^{a}

Legend: control (MTW1): plants grown in water without magnetic treatment; MTW2: plants cultivated with magnetically treated water (MTW) in a range of magnetic indication between 60 and 100 mT; MTW3: plants grown in water treated magnetically with a range of magnetic induction between 110 and 160 mT. The values represent the means ± standard error (n= 3). Different letters in the same column mean statistically significant differences ($p<0.05$) for the Tukey test

Table 4 shows the values obtained for each of the photosynthetic pigments. The highest values of chlorophyll$_a$ > chlorophyll$_{(b + carot)}$> carotenes were in plants grown with magnetically treated water, while Control had the lowest concentration of pigments.

For the same species cultivated with magnetically treated water within a range of magnetic induction from 110 to 160 mT in spring, Boix et al. (2008) demonstrated similar results. The plants presented increases in the concentration of chlorophyll a relative to plants without magnetic field irrigation. Hozayn et al. (2016) obtained higher concentration values of photosynthetic pigments in canola (*Brassica napus* L. var. Serw-6) plants irrigated with magnetically treated water.

Thus, the use of magnetic field irrigation can facilitate better water absorption by the plant, compared to control. Pang and Deng (2008) demonstrated that this type of disturbed water presents changes in physicochemical properties, such as solubility, pH, and electrical conductivity. Magnetically treated water for irrigation can provide plants with better water absorption, which affects various metabolic functions and all biochemical processes. These results indicate greater growth in plants with magnetic treatment, obtaining even higher values of dry mass and concentration of photosynthetic pigments in a correspondingly higher range of magnetic induction (MTW3), compared to MTW2 and control (Table 4). It is apparent that the effect of magnetically treated water favored the structural formation and growth of tissues where stimulation of metabolic processes could be aimed at vegetative development.

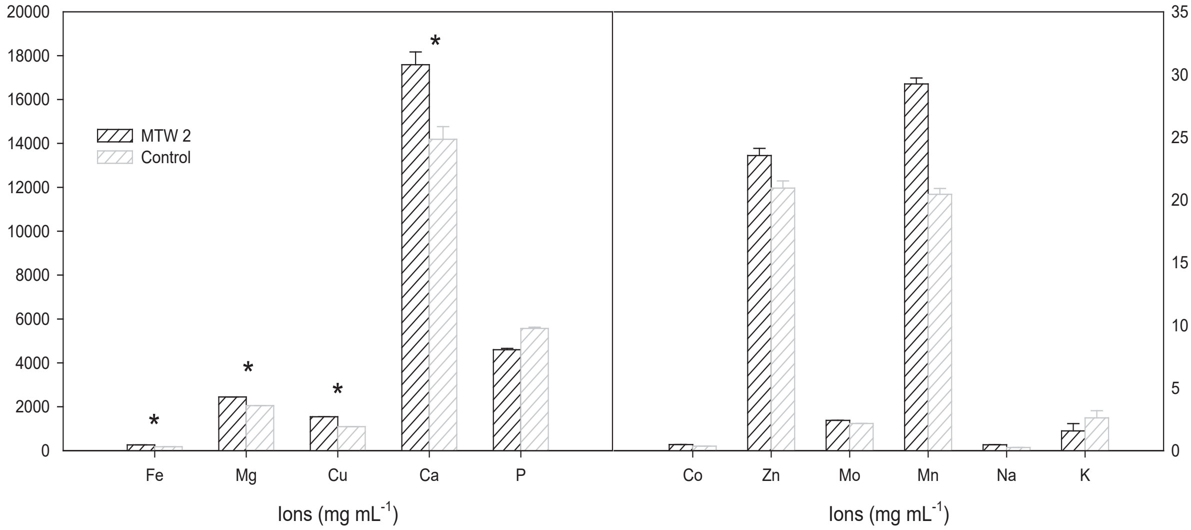

Figure 2. Leaf concentration of ions in *Rosmarinus officinalis* grown under irrigation with magnetically treated water (MTW). (*) Statistically significant differences ($p < 0.05$), n= 3.

Figure 2 shows the ions determined in *R. officinalis* sheets of MTW 2 treatment, which had the highest concentration of dry mass values and photosynthetic pigments. In the leaves from MTW 2 plants, statistically significant differences were obtained among the values of ions, such as iron (Fe), magnesium (Mg), copper (Cu) and calcium (Ca) relative to control.

Plants accumulate calcium mainly in the leaves. This ion is used in the synthesis of new cell walls, and it appears mainly in the middle sheet, thus providing leaves with permeability, stability, expansion and integrity (Iqbal et al., 2013; Thor, 2019). It can also be present in vacuoles in the form of oxalates and contributes to the transport of minerals, as well as their retention. Leaves are involved in the formation of proteins which prevent plants from early aging, and protein is vital to counteract the effects of alkaline salts and organic acids. In addition to its effects as a macro and structural nutrient element, it is fully recognized as a signaling molecule, acting as a second messenger in signal transduction pathways and usually its concentration increases in response to the stimuli including stress signals (Tuteja; Supory, 2008). It is also used in the mitotic spindle during cell division, and it is required for normal functioning of cell membranes. As a second messenger, it can bind to calmodulin, a protein found in the cytosol of plant cells, forming a complex which regulates many metabolic processes (Taiz; Zeiger, 2014). It is required as a cofactor for some enzymes involved in the hydrolysis of ATP. Studies have revealed that the Static Magnetic Field (SMF) can influence Ca ion channels in the endoplasmic/sarcoplasmic reticulum that results in the emptying of intracellular Ca stores, thereby increasing its concentration in the cytosol (Maffei, 2014).

In plant cells, magnesium is part of the ring structure of the chlorophyll molecule; therefore, it is an essential nutrient for photosynthesis involved in the growth and development of plants through hormonal activation. Magnesium has a specific role in the activation of several enzymes involved in the respiration and synthesis of DNA and RNA, and Mg is involved in phosphate transfer. Also, Mg is important to maintain structural stabilization of tissues (Guo et al., 2016).

Copper, like iron, is associated with certain enzymes involved in redox reactions with reversible oxidation from Cu^{+} to Cu^{2+}. An example of such enzyme is plastocyanine, which is involved in electron transport during light reactions of photosynthesis. It is part of the chlorophyll formation process, helping in root metabolism, as well as helping plants make better use of proteins. It is a component of ascorbic acid oxidase, tyrosinase, monoamine oxidase, uricase, cytochrome oxidase and phenolase. The initial symptom of copper deficiency is the production of dark green leaves, which may contain necrotic spots. Necrotic spots appear first on the tips of young leaves and then extend to the base of the leaf along the margins. The leaves can also be crooked or malformed. Copper is an important element of ethylene receptors and their functionality (Iqbal et al., 2013). Under extreme copper deficiency, the leaves may have premature abscission. Variable ethylene production has been reported from a wide range of plant species in response to Cd, Cu, Fe and Zn (Iqbal et al., 2013; Taiz et al., 2014).

Iron plays a key role as a component of enzymes that participate in the transfer of electrons, such as cytochromes. Since copper reversibly oxidizes Fe^{2+} to Fe^{3+} in electron transfer, it participates in the fixation of N_2, respiration, and it is a constituent of photosynthetic proteins. Fe is a limiting factor for biomass production and for plant product quality (Briat et al., 2015). The low mobility of iron probably results from its precipitation in old leaves, such as insoluble oxides, or from phosphates or the formation of complexes with phytoferritin, an iron-binding protein found in the leaf and other parts of the plant (Navarro et al., 2013). The precipitation of iron decreases the subsequent mobilization of the metal in the phloem.

All of our results are similar to those of (Hozayn et al., 2013; 2015; 2016) with other species (*Beta vulgaris* L., *B. napus* and *Allium cepa* L.) cultivated with magnetic irrigation, which was found to have a positive effect on growth, development, and yield. Shahin et al. (2016) found that irrigation with magnetized water and seeds of cucumber at (40 mT) significantly improved nitrogen, phosphorus and potassium content in cucumber shoots. The values of N, P and K% were (1.55, 0.145, and 0.081) as compared to control without magnetized irrigation where recorded values of N, P and K % were 1.388, 0.123, and 0.072 for N, P, and K, respectively, throughout the growing season. Also, they were obtained for iron, manganese, zinc and copper content (ppm) in shoots of cucumber plants.

Results of the current study demonstrated beneficial effects of magnetically treated water. Magnetic treatment of water may be influencing desorption of P and N from soil adsorbed on colloidal complex, and thus increase their availability to plants. This results in improved plant growth and productivity. In this study, we demonstrated that *R. officinalis* could be cultivated with magnetically treated water to obtain the highest crop yield for plants grown in propagation systems for this species.

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