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VEGETATIVE GROWTH OF *HYPERICUM PERFORATUM* L. PLANTS TREATED WITH HIGH DYNAMIZED DILUTIONS OVER DIFFERENT GROWING SEASONS

CRECIMIENTO VEGETATIVO DE PLANTAS DE *HYPERICUM PERFORATUM* L. TRATADAS CON DILUCIONES ALTAMENTE DINAMIZADAS DURANTE DIFERENTES TEMPORADAS DE CRECIMIENTO

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ABSTRACT

Contextualization: The species *Hypericum perforatum* is widely used as a treatment for several diseases, especially depression. This plant is not native to Brazil and, therefore, it is not planted in the country. Brazil has high rates of occurrence of depression, same as the whole Latin America, being considered a public health problem, so its cultivation is considered as a potential treatment tool.

Knowledge gap: The cultivation of the species in the country is still inefficient, as the plant does not reach an adequate stage for flowering. Thus, further studies regarding the vegetative growth and the establishment of *H. perforatum* are needed, as it can provide great economic and health autonomy by addressing a public interest, using a treatment with practically no side effects, reducing drug costs for the country.

Purpose: Evaluate the effects of high dynamized dilutions and the influence of the seasons on vegetative growth and contents of bioactive compounds in *Hypericum perforatum* plants.

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Methodology: Experiments were performed in two seasons: Spring/Summer and Summer/Autumn. The experimental plot consisted of 12 plants and 4 repetitions, totaling 48 plants per treatment. Five treatments were used consisting of homeopathic preparations of *Kali carbonicum*, *Natrum muriaticum, Phosphorus*, and *Silicea terra* at 12CH, and distilled water as control. The height of the longest branch, the total number of branches, shoot dry weight, and a number of dark glands were evaluated, as well as the amounts of phenolic compounds.

Results and conclusions: Homeopathic preparations affected *H. perforatum* plants differently over the cultivated seasons. In the Spring/Summer experiment, the *Silicea terra* treatment promoted higher plant growth than *Phosphorus*, but a similar rate to that of the other treatments. In the Summer/Autumn experiment, the homeopathies *Kali carbonicum*, *Natrum muriaticum*, and *Phosphorus* increased the vegetative growth in comparison to control. It was observed that in the experiment carried out in the Spring/Autumn, the plants of *H. perforatum* had difficulty in development. The formation of dark glands was not stimulated by the use of homeopathic preparations. The hypericin compound was not detected in any sample of *H. perforatum* leaves. This suggests the need for an extended cultivation time for the naphthodianthrone compound to accumulate in the dark glands.

Keywords: Hypericin; Homeopathy; Medicinal plant; St. John's wort.

RESUMEN

Contextualización: la especie *Hypericum perforatum* se usa ampliamente como tratamiento para varias enfermedades, especialmente la depresión. Esta planta no es originaria de Brasil y, por lo tanto, no se planta en el país. Brasil tiene altas tasas de ocurrencia de depresión, al igual que toda América Latina, siendo considerado un problema de salud pública, por lo que su cultivo se considera una posible herramienta de tratamiento.

Vacío de conocimiento: el cultivo de la especie en el país aún es ineficiente, ya que la planta no alcanza una etapa adecuada para la floración. Por lo tanto, es necesario llevar a cabo estudios adicionales sobre el crecimiento vegetativo y el establecimiento de *H. perforatum*, ya que puede proporcionar una gran autonomía económica y sanitaria, al atender un interés público usando un tratamiento que prácticamente no tenga efectos secundarios, lo que reduce los costos de los medicamentos para el país.

Propósito del estudio: evaluar los efectos de las diluciones altamente dinamizadas y la influencia de las estaciones sobre el crecimiento vegetativo y el contenido de compuestos bioactivos en plantas de *Hypericum perforatum*.

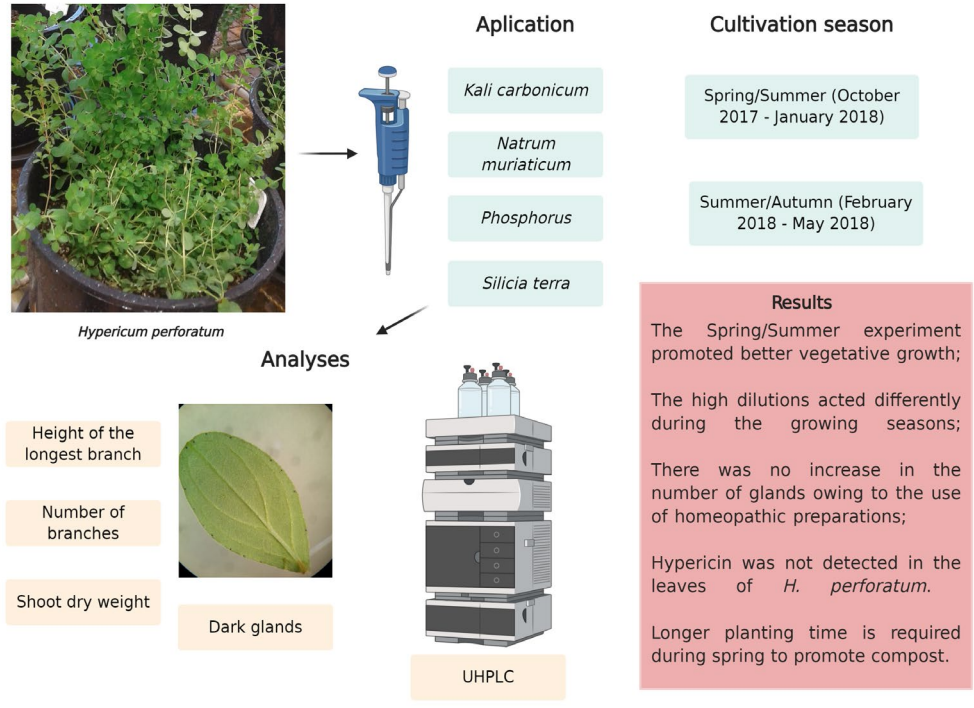
Metodología: los experimentos se realizaron en dos estaciones: Primavera/Verano y Verano/Otoño. La parcela experimental constó de 12 plantas y 4 repeticiones, para un total de 48 plantas por tratamiento. Se utilizaron cinco tratamientos que consistieron en preparaciones homeopáticas de *Kali carbonicum*, *Natrum muriaticum*, *Phosphorus* y *Silicea terra* a 12CH y agua destilada como control. Se evaluó la altura de la rama más larga, número de ramas, peso seco del brote y número de glándulas oscuras, así como las cantidades de compuestos fenólicos.

Resultados y conclusiones: las preparaciones homeopáticas afectaron a las plantas de *H. perforatum* de manera diferente durante las temporadas de cultivo. En el experimento de Primavera/Verano, el tratamiento con *Silicea terra* promovió un mayor crecimiento de las plantas que el *Phosphorus*, pero a una tasa similar a la de los otros tratamientos. En el experimento

Verano/Otoño, las homeopatías *Kali carbonicum*, *Natrum Muriaticum* y *Phosphorus* aumentaron el crecimiento vegetativo en comparación con el control. Se observó que en el experimento realizado en la Primavera/Otoño, las plantas de *H. perforatum* tuvieron dificultad de desarrollo. La formación de glándulas oscuras no fue estimulada por el uso de preparaciones homeopáticas. El compuesto de hipericina no se detectó en ninguna muestra de hojas de *H. perforatum*. Esto sugiere la necesidad de un tiempo de cultivo prolongado para que el compuesto de naftodiantrona se acumule en las glándulas oscuras.

Palabras clave: Hipericina; Homeopatía; Planta medicinal; Hierba de San Juan.

GRAPHIC SUMMARY



Source: authors

1. INTRODUCTION

Hypericum perforatum, known as St. John's wort, has been used as a medicinal plant to treat different human diseases, mainly mild and moderate depression (Ng et al., 2017). The biological activity of *H. perforatum* is attributed to more than ten classes of secondary metabolites, including anthraquinones/naphthodianthrones, phloroglucinol, flavonoids, xanthones, volatile oils, vitamin C, tannins, proteins, carotenoids, and coumarins. However, hypericin and hyperforin have been the main compounds studied in this medicinal plant because of their well-known antidepressant effects (Mullaicharam and Halligudi, 2018).

Hypericin, an anthraquinone derivative, is naturally found in the yellow flowers of *H. perforatum*. It has antidepressant activity, resulting from an inhibitory effect on the neuronal uptake of norepinephrine, dopamine, γ-amino

butyric acid and L-glutamate. It is accumulated in specialized morphological secretory structures know as dark glands (Gaid et al., 2016). Previous studies have reported that hypericin concentrations will depend on different factors, such as planting and harvesting time, the phenological stage of plants at harvest and use of appropriate treatments for phytosanitary maintenance (Southwell and Bourke, 2001).

Native from Europe, Asia, and North Africa, the species *H. perforatum* can abundantly grow up on pastures, roadsides, and environments modified by human activity (Crompton et al. 1998). However, the adaptation and cultivation of *H. perforatum* in Brazil is still ineffective, because the plant does not reach a proper size (~ 60 cm) and does not reach an adequate stage to flower. Considering the high therapeutic potential of *H. perforatum*, it needs to be grown in ecological systems, so that there are no negative changes in the content of medicinal compounds that provide efficiency against diseases, such as depression (Faron et al., 2004).

Regulated by Normative Instruction No. 17/2014 by the Ministry of Agriculture, Cattle and Supplying for organic production (Ministério da Saúde, 2014b), homeopathic preparations have proven to be an effective and residue-free technology for use in agriculture (Teixeira and Carneiro, 2017; Sen et al., 2018). The application of homeopathic preparations can help the cultivation of medicinal plants on a more sustainable basis, eventually improving plant growth, and secondary metabolites biosynthesis and accumulation (Pereira et al., 2019).

In addition to the choice of residual-free treatments in medicinal plants (Ministério da Saúde, 2014a), the period of cultivation of the species needs to be determined, as this factor will also be determinant for the production of biomass and the biosynthesis of bioactive compounds. According to Soni et al. (2015), the growing season influences the availability and the amounts of bioactive compounds in medicinal plants, determining their phytotherapeutical potential. Planting and/or harvesting at the wrong time may impair the yield of secondary metabolites pharmacologically relevant, so it is very important to identify the best seasons for cultivation. In this sense, this study aimed to evaluate the use of high dynamized dilutions and the influence of seasons on the vegetative growth and content of phenolic compounds in *Hypericum perforatum* plants.

2. MATERIALS AND METHODS

Cultivation of *Hypericum perforatum:* The experiments were carried out in a culture room with controlled temperature and light at the Laboratory of Plant Health and Homeopathy and also in a greenhouse at the Epagri Experimental Station, located in the city of Lages (50º 19'46.93" W, 27º 48'28.746" S), Santa Catarina state, southern Brazil.

Two experiments were performed as follows: The Spring/Summer experiment, from October 2017 to January 2018 and the Summer/Autumn experiment, from February 2018 to May 2018. *H. perforatum* seeds were acquired from Feltrin Seeds®, showing a 64% germination rate, according to the manufacturer.

For production of the seedlings, the seeds were sown in a sowing tray filled with vermiculite and black earth, in a 2:1 ratio. The experiment used a randomized block design and the sowing trays were separated into blocks and transferred to a growth room at 25 ºC and 16h/8h photoperiod, under luminous intensity set up at 2.338 LUX provided by LED lamps. The sowing trays were placed on a plastic tray containing 200 mL until the seeds' emergence, 15 days. Five treatments were used,

consisting of homeopathic preparations in the 12CH (twelfth order of the Hahnemannian centesimal dilution) of *Kali carbonicum*, *Natrum muriaticum*, *Phosphorus*, and *Silicea terra* and distilled water as a control. The matrices of the homeopathic preparations were acquired in a compounding pharmacy at 6CH. The preparations at 12CH were made according to the Brazilian Homeopathic Pharmacopeia (Ministério da Saúde, 2011).

The selection of homeopathic preparations was carried out using repertory language and in consultation with medical sources. The main characteristics of the species *H. perforatum* were analyzed, such as: sensitivity to cold, need for constant water and light, photosensitivity, and fragility. Using the materials listed, and according to the Homeopro® *software, it was determined which homeopathic best approached the level of similarity.*

All experiments were performed in a double-blind analysis, namely, the operator was unaware of the treatment to be used. *H. perforatum* seedlings were treated twice a week, dispensing 1 mL of homeopathic preparations per sowing cell directly in the soil, totaling eight applications in a 30-day experimental period.

Six weeks after sowing, the treated seedlings, with approximately 6 cm in height, were transplanted into pots (four plants per pot) and taken to a greenhouse. The experimental plot consisted of 12 plants and 4 repetitions, totaling 48 plants per treatment. 8.7 liters pots containing vermiculite, black soil, and sheep manure were used (1:1:1, v/v/v). 195 g natural phosphate per 360 liters of compost were used. In the greenhouse, homeopathic treatments were applied twice a week again and extended for one month until the end of the experiment, at 60 days after sowing.

After seed germination (15 days), two assessments per week were performed to measure the height of the main branch. The measurements were done from the base of the stem up to the highest leaf, and 17 evaluations of the main branch were carried out. At the end of the experiment (75 days), plant height, total number of branches and shoot dry weight were evaluated.

Sample collection and dark gland count: All the samples of the Spring/Summer experiment were collected in February 2019; whereas, for the Summer/Autumn experiment samples were harvested in June 2019. Dark glands were counted from a destructive sample of five leaves from each plant, totaling 240 leaves per treatment. The counting of dark glands was performed using a stereoscopic microscope (25x) on the adaxial face of the leaves. The remaining materials (leaves and roots) were placed in a force-air drying oven for 48 h at 50 ºC.

Preparation of hydroalcoholic extracts: After comparing the data obtained in the general average of the dark gland count, the samples treated with the homeopathic preparation *Silicea terra* 12CH and the control ones were selected for further chromatographic analysis. The extracts were obtained by maceration using commercial ethyl alcohol 92% (v/v) and 36g and 29g dry shoot samples of the control plants and *Silicea terra*-treated plants, respectively, from the Spring/Summer experiment. Similarly, for the Summer/Autumn experiment, 1.05 g and 1.39g for plants treated with water and *Silicea terra,* respectively. Grinding was performed with 48 plants that were divided into four repetitions. The material was kept under maceration at room temperature and protected from light for seven days. After filtration, the solvent was removed by rotary evaporation at 45 ºC to obtain the