



Hydropriming and substrates affect the emergence and production of *Calendula officinalis* L. seedlings

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ABSTRACT: In order to establish protocols that can contribute to the propagation of medicinal plants, this study evaluated the effect of hydropriming and substrates on the emergence and production of *Calendula officinalis* L. seedlings. Seeds were submitted or not to hydropriming for 24 hours, and subsequent sowing in four substrates: S1) 100% Dystroferric Red Latosol – DRL, S2) DRL + coarse sand (1:1 v/v), S3) DRL + Tropstrato® commercial substrate (1:1 v/v) and S4) 100% Tropstrato®, and kept in greenhouse with 50% shading. Higher emergence percentage in substrates with the presence of DRL was observed. Seed hydropriming impaired seedling emergence, but contributed to root growth, biomass production and seedling quality. The 100% Tropstrato® substrate favored higher dry mass production and seedling quality. Positive linear correlation (≥ 0.70) was observed between shoot and root growth characteristics with biomass production and Dickson's quality index. Principal component analysis explained 78.95% of the experimental variance, excluding the first count and the shoot/root ratio with low representativeness. In the cluster analysis of Euclidean distances, seedlings with the greatest similarity were those produced with 100% DRL and DRL + Tropstrato® with and without hydropriming, respectively. Dystroferric Red Latosol is recommended pure or combined with Tropstrato® for the production of *C. officinalis* L seedlings, and the hydropriming impaired seedling emergence characteristics.

Key words: calendula, Dickson quality index, pre-soaking, Tropstrato®.

Hidrocondicionamento e substratos afetam a emergência e a produção de mudas de *Calendula officinalis* L.

RESUMO: Com a finalidade de estabelecer protocolos que possam contribuir na propagação de plantas medicinais, objetivamos, com este estudo, avaliar o efeito do hidrocondicionamento e substratos na emergência e produção de mudas de *Calendula officinalis* L. As sementes foram submetidas ou não ao hidrocondicionamento durante 24 horas, e posterior semeadura em quatro substratos: S1) 100% Latossolo Vermelho Distroférico – LVD, S2) LVD + areia grossa (1:1, v/v), S3) LVD + substrato comercial Tropstrato® (1:1, v/v) e S4) 100% Tropstrato®, e mantidas em viveiro com 50% de sombreamento. Observamos maior percentagem de emergência nos substratos com presença de LVD. O hidrocondicionamento das sementes prejudicou a emergência das plântulas, mas contribuiu no crescimento de raiz, produção de biomassa e qualidade das mudas. O substrato 100% Tropstrato® favoreceu maior produção de massa seca e qualidade das mudas. Observamos correlação linear positiva ($\geq 0,70$) entre as características de crescimento da parte aérea e raiz com produção de biomassa e o índice de qualidade de Dickson. A análise de componentes principais explicou 78,95% da variância experimental, excluindo a primeira contagem e a relação parte aérea/raiz com baixa representatividade. Na análise de agrupamento das distâncias Euclidianas, as mudas com maior similaridade foram aquelas produzidas no 100% LVD e LVD + Tropstrato® com e sem hidrocondicionamento, respectivamente. Recomenda-se o Latossolo Vermelho Distroférico puro ou combinado com Tropstrato® para a produção de mudas de *C. officinalis* L., e o hidrocondicionamento prejudicou as características de emergência das plântulas.

Palavras-chave: calêndula, índice de qualidade de Dickson, pré-embebição, Tropstrato®.

INTRODUCTION

Calendula officinalis L. (Asteraceae), popularly known as 'calendula' or 'bem me quer', is a species of agroeconomic interest due to its applicability in gardening and landscaping, in addition to the flow of capitula due to its uses in the pharmaceutical and cosmetic industries (LEITE et al., 2005; BORBA et

al., 2012; SIMÕES et al., 2020), making it a good choice for cultivation in agroecological systems. In order to obtain uniform plants, the propagation phase is one of the most important stages, as the quality of seedlings will generally be reflected in their development in the field.

In nursery, hydropriming can be a promising technique, as seeds are immersed in water

solution for a certain period before sowing, promoting pre-soaking. According to ARAÚJO et al. (2011) and PAIVA et al. (2012), in addition to low cost, hydropriming promotes the activation of metabolic processes, protein synthesis and hormonal signaling, contributing to the speed of seed germination and seedling emergence, and thus seedlings can be obtained more quickly for commercialization or formation of production fields.

Another important factor for obtaining high quality seedlings is the selection of the substrate. This is because the substrate must have characteristics such as: porosity, water retention and fertility (SANTOS et al., 2020; SCHAFER et al., 2022; SOUSA et al., 2023), which favor nutrition, water balance and seedling growth. Furthermore, the substrate formulation varies according to the reality of the producer, especially regarding materials available in the rural property or region, in addition to the financial resources for the acquisition of external inputs.

Given this scenario, knowing the possibilities of substrates that optimize cost reduction for the producer and/or nurseryman and contribute to the production of seedlings is of paramount importance. Considering that the propagation information of the species is insufficient, this study evaluated the effect of hydropriming and substrates on the emergence and production of *C. officinalis* seedlings.

MATERIALS AND METHODS

Seed collection and processing

Ripe *C. officinalis* fruits were collected from reproductive plants cultivated under agroecological management at 'Horto de Plantas Mediciniais' – HPM (22°11'43.7" S and 54°56'08.5" W, 452 m a.s.l.), at the Faculty of Agricultural Sciences, Federal University of Grande Dourados – UFGD, Dourados – MS, Brazil. Processing was manually performed, selecting those with dark brown color, without morphological damage and adequate firmness. Seeds were immersed in 2% sodium hypochlorite solution for 5 minutes for sanitization and washed in running water. Subsequently, seeds were placed on a bench at the Laboratory of Vegetables and Medicinal Plants at room temperature (± 25 °C) until sowing, which occurred the next day, with an initial water content of 6.1%.

General conditions, treatments and experimental design

The experiment was carried out under agricultural screen with upper and side coverage of black nylon screen brand Sombrite® with 50% luminosity retention and additional upper protection

of a 150 μm transparent plastic cover, with average temperature and humidity during the experiment of 27.8°C and 61%, respectively, which were determined on alternate days at 9 am, using a thermohydrometer. Initially, seeds were divided into two groups: i) seeds submitted to hydropriming, immersing them in container with distilled water for 24 hours, and ii) seeds without hydropriming, which were kept at room temperature until sowing.

Subsequently, one seed per cell was sown in expanded polystyrene trays with 128 cells previously filled with four substrates: S1) 100% Dystroferic Red Latosol – DRL (SANTOS et al., 2018) of clay texture, corresponding to Oxisols in the USDA classification, S2) DRL + coarse sand, S3) DRL + Tropstrato® commercial substrate, and S4) 100% Tropstrato®. The substrates containing a mixture of DRL with coarse sand and Tropstrato® in proportion from a 1:1 (v/v), and were homogenized for later filling of the trays. The experimental design used was randomized blocks, and treatments were arranged in a 2 x 4 factorial scheme, with four replicates, and each experimental unit consisted of 32 cells.

The Dystroferic Red Latosol used presented the following chemical attributes: pH $\text{CaCl}_2 = 5.24$; $\text{P} = 0.94 \text{ mg dm}^{-3}$; $\text{Ca} = 1.04 \text{ cmol}_c \text{ dm}^{-3}$; $\text{K} = 0.50 \text{ cmol}_c \text{ dm}^{-3}$; $\text{Mg} = 0.20 \text{ cmol}_c \text{ dm}^{-3}$; $\text{Al} = 1.00 \text{ cmol}_c \text{ dm}^{-3}$; $\text{H} + \text{Al} = 5.23 \text{ cmol}_c \text{ dm}^{-3}$; sum of bases = $1.22 \text{ cmol}_c \text{ dm}^{-3}$; cation exchange capacity = $6.93 \text{ cmol}_c \text{ dm}^{-3}$ and base saturation ($\text{V}\%$) = 40.70. The Tropstrato® commercial substrate presented the following characteristics according to manufacturer's data: pH $\text{CaCl}_2 = 5.75$; $\text{P} = 65.70 \text{ mg dm}^{-3}$; $\text{K} = 1.60 \text{ cmol}_c \text{ dm}^{-3}$; $\text{Ca} = 23.80 \text{ cmol}_c \text{ dm}^{-3}$; $\text{Mg} = 12.40 \text{ cmol}_c \text{ dm}^{-3}$; $\text{Al} = 0.00 \text{ cmol}_c \text{ dm}^{-3}$; $\text{H} + \text{Al} = 4.20 \text{ cmol}_c \text{ dm}^{-3}$; sum of bases = $39.80 \text{ cmol}_c \text{ dm}^{-3}$; cation exchange capacity = $42.10 \text{ cmol}_c \text{ dm}^{-3}$; and base saturation ($\text{V}\%$) = 64.80. The wetting of seedlings was carried out with manual watering can in two daily watering shifts.

Evaluated characteristics

At 7 and 30 days after sowing (DAS), the first count (FC) and the final emergence percentage (E) were calculated according to BRASIL (2009) and MAGUIRE (1962), considering normal seedlings as those with hypocotyl appearance and expanded leaves.

At 35 DAS, seedlings were removed from substrates, washing roots in running water to remove excess substrate, and then the shoot length – SL (distance from the stem to the inflection of the highest leaf) and root length (RL) were measured

using graduated ruler. Stem diameter (SD) was measured with digital caliper (inserted 1.0 cm above the substrate level) and the number of leaves (NL) was manually counted.

The fresh material from the shoot and root system was placed in Kraft® paper bags and dried in an oven with forced air circulation ($60\text{ }^{\circ}\text{C} \pm 5$) for 72 hours and weighed on a precision scale (0.0001 g) to obtain of shoot and root dry mass (SDM and RDM, respectively), and the total dry mass (TDM) and the shoot/root ratio (SRR) were calculated. The quality standard by Dickson quality index (DQI) was calculated as proposed by DICKSON et al. (1960).

Data analysis

First count and emergence data were submitted to the Shapiro-Wilk normality distribution test. All data were submitted to analysis of variance, and when significant by the F test ($P \leq 0.05$), means were compared by the Bonferroni's t test for hydropriming, and Tukey's test for substrates, both at $P \leq 0.05 \pm$ standard deviation (SD). Subsequently, Pearson's linear correlation ($P \leq 0.05$) and principal component analyses (PCA) were performed, eliminating features with factorial score ≤ 0.30 , the criterion adopted with the aim of selecting features with experimental representativeness. The similarity index among factors under study was determined using the Euclidean distance with cophenetic correlation coefficient ≥ 0.60 , using the UPGMA clustering method.

RESULTS AND DISCUSSION

It was observed that the first emergence count of *C. officinalis* seedlings was influenced by the interaction between hydropriming and substrates, while the emergence percentage was influenced by factors alone (Table 1). Shoot length, number of leaves, stem diameter and shoot/root ratio were not influenced by factors under study ($P > 0.05$). The root length, biomass production characteristics of the different organs and the Dickson quality index (DQI) were influenced by the interaction between hydropriming and substrates (Table 1).

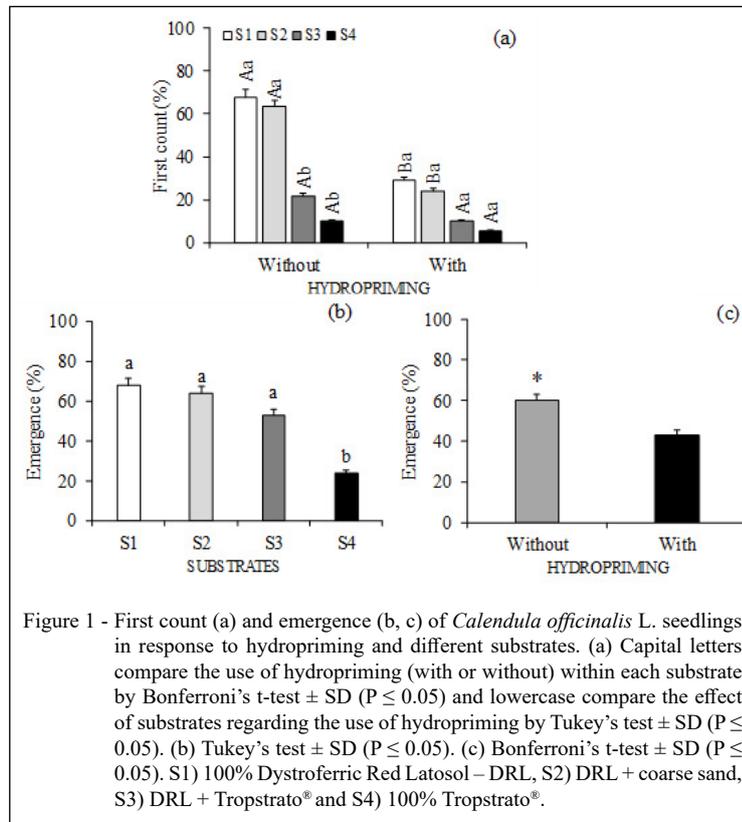
The highest values (67.75 and 63.28%) of the first count occurred when using seeds in DRL and DRL + coarse sand substrate, both not submitted to hydropriming, respectively, statistically differing from those observed with hydropriming in these same substrates (Figure 1a). Conversely, the FC values in the DRL + Tropstrato® and 100% Tropstrato® substrate were lower, regardless of hydropriming. Regarding the emergence percentage, the lowest value (24 and 43%) occurred in the 100% Tropstrato® substrate (Figure 1b) and with hydropriming, respectively (Figure 1c).

Although, hydropriming is a beneficial technique for some species (GAO & YAN, 2020; NASCIMENTO et al., 2021; BENADJAOU et al., 2022), this study verified that this procedure was not beneficial to *C. officinalis*. We suggested that this response is associated with possible damage caused by hydropriming due to the low water content in the

Table 1 - Results of the analysis of variance of the effect of hydropriming, substrates and the interaction on the characteristics evaluated in *Calendula officinalis* L. seedlings.

Evaluated characteristics	-----Hydropriming (H)-----		-----Substrates (S)-----		-----Interaction H x S-----		C.V. (%)
	F	P -value	F	P -value	F	P -value	
FC	24.128	0.0001	17.486	< 0.0001	3.509	0.0332	26.85
E	13.871	0.0013	24.834	< 0.0001	1.538	0.2340	20.91
SL	2.897	0.1035	0.272	0.8448	1.471	0.2511	16.53
RL	2.787	0.1099	1.830	0.1720	5.123	0.0081	11.57
SD	0.812	0.3776	2.699	0.0792	0.952	0.4334	21.11
NL	0.374	0.5621	1.591	0.2215	1.263	0.3126	13.77
SDM	0.183	0.6729	2.385	0.0980	5.070	0.0085	29.03
RDM	0.438	0.5151	1.700	0.1976	4.795	0.0107	29.49
TDM	0.315	0.5804	2.803	0.0632	6.906	0.0020	33.79
SRR	0.791	0.3839	1.867	0.1661	0.744	0.5378	10.66
DQI	1.442	0.2432	0.907	0.4544	4.169	0.0183	23.52

FC – first count, E – emergence, SL – shoot length, RL – root length, SD – stem diameter, NL – number of leaves, SDM – shoot dry mass, RDM – root dry mass, TDM – total dry mass, SSR – shoot/root ratio, DQI – Dickson quality index.



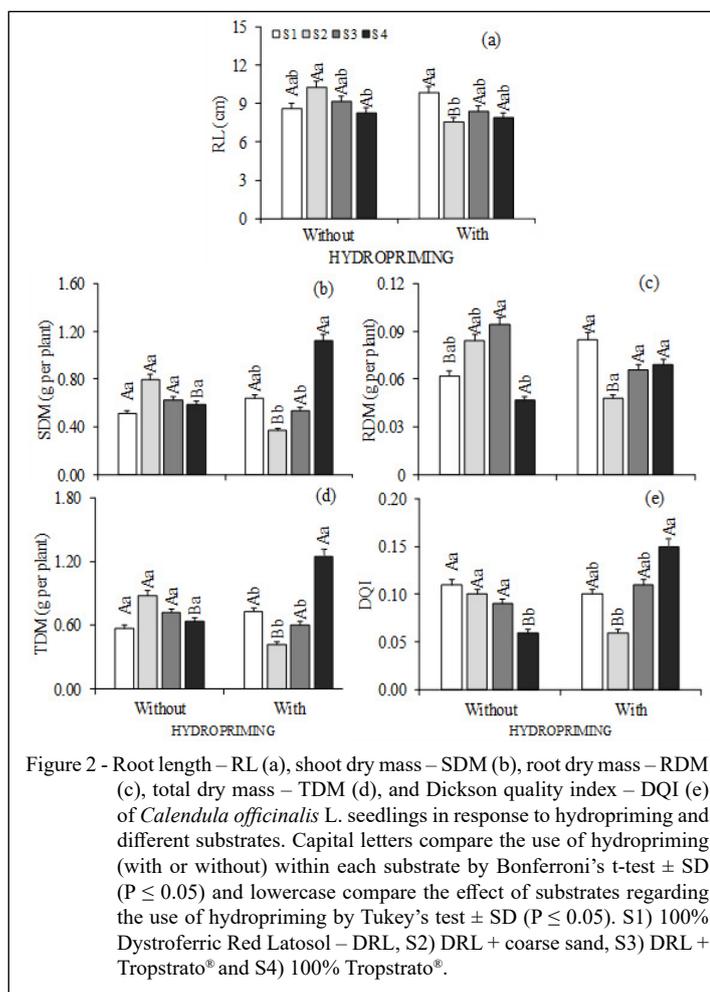
seeds (6.1%), that is, exposure to water for 24 hours may have been too long, and also due to the fact that the seeds of this species have low water content (LOPES et al., 2022), which caused release of electrolytes and damage to membranes (LEMMENS et al., 2019). Similarly, *Dalbergia nigra* (Vell.) Fr. All. ex Benth. (ATAÍDE et al., 2016) and *Lactuca sativa* L. (BISOGNIN et al., 2016) seeds, when submitted to hydropriming, had lower expression of the physiological and germinative potential. For *C. officinalis* seeds it may be that the period of exposure to hydropriming should be shorter, which can improve the physiological quality, becoming a perspective for new tests.

As for the substrate, the lower first count and final emergence value is due to the fact that the commercial substrate has high porosity; and therefore, the contact surface with seeds and water holding capacity is reduced, which can impair root protrusion (SOUZA et al., 2018), unlike what was observed using substrate containing DRL and/or coarse sand. For germination to occur, seeds need to have adequate contact with the substrate so that they absorb water, and thus start the process of using reserves and synthesizing hormones

associated with this process (MAITAN et al., 2020; IBANHES NETO et al., 2021).

With regard to root length without hydropriming, the highest value (10.25 cm) occurred in *C. officinalis* seedlings produced in DRL + coarse sand, statistically differing from that observed in 100% Tropstrato®. This is because the addition of sand in the substrate formulation favored root expansion. Conversely, with hydropriming, the lowest value was observed in seedlings produced in DRL + coarse sand, differing from that observed in seedlings produced without hydropriming in the same substrate and from those produced with hydropriming and DRL (Figure 2a). Hydropriming contributed for *C. officinalis* seeds by favoring root expansion even in DRL, but did not promote a higher percentage of emergence and other characteristics of seedling production.

The highest SDM and TDM occurred in 100% Tropstrato® with hydropriming compared to the other substrates (Figure 2b and 2d). These results are due to the fact that the commercial substrate contains in its constitution higher concentration of readily available nutrients compared to DRL, especially phosphorus, potassium and calcium, which are fundamental in the nutrition and metabolism of plants,



which favored the production of photoassimilates; in addition, as hydropriming contributed to higher and more quick RL, *C. officinalis* seedlings had better nutrient use efficiency.

In addition, DRL showed high aluminum content ($1.00 \text{ cmol}_c \text{ dm}^{-3}$) and lower base saturation, characteristic of soils from the Cerrado region, which are more weathered and have low natural fertility (SILVERIO et al., 2021), which harmed the development of seedlings regarding shoots. Therefore, the addition of other materials in the formulation of substrates containing this soil class in their constitution is an important practice for plant growth. BARBOSA et al. (2010) reported that *C. officinalis* seedlings grown in substrate consisting of Cerrado soil + bovine manure (1:1 v/v) had higher shoot dry mass and root system values.

For RDM without hydropriming, the highest value (0.094 g) occurred in DRL + Tropstrato®,

statistically differing only from 100% Tropstrato®, which promoted the lowest value (Figure 2c). With hydropriming, seedlings produced in 100% DRL had higher RDM value (0.085 g) although not statistically differing from the other substrates under this condition. The Dickson's quality index varied according to the presence of hydropriming, especially when using 100% Tropstrato®, since the lowest value was observed in this substrate without hydropriming, while using the same substrate, the highest DQI (0.15) value was observed, but hydropriming (Figure 2e) is a fact associated with root protrusion speed and nutrient use efficiency in the metabolism and growth of seedlings.

In Pearson's linear correlation analysis, SL–SD, RL–RDM, SDM–TDM and SDM–DQI had high positive correlation ($r; > 0.70$), while NL with SDM and TDM showed negative correlation (Table 2), suggesting that the low amount of formed leaves (general mean: 6.4 leaves) may impair light

Table 2 - Pearson's linear correlation (r) of the evaluated characteristics in *Calendula officinalis* L. seedlings as a function of hydropriming and different substrates.

Characteristics	SL	RL	NL	SD	SDM	RDM	TDM	SRR	DQI
E	0.32	0.39	0.44	0.41	-0.56	0.22	-0.55	0.05	-0.31
SL		0.47	0.66	0.77	-0.42	0.01	-0.44	0.32	-0.51
RL			0.45	0.68	0.13	0.76	0.14	-0.01	0.09
NL				0.64	-0.71	0.22	-0.70	0.38	-0.49
SD					-0.27	0.47	-0.27	0.40	-0.37
SDM						0.37	1.00	-0.23	0.77
RDM							0.41	0.09	0.44
TDM								-0.22	0.79
SRR									0.15

E – emergence, SL – shoot length, RL – root length, NL – number of leaves, SD – stem diameter, SDM – shoot dry mass, RDM – root dry mass, TDM – total dry mass, SSR – shoot/root ratio, DQI – Dickson quality index ($P \leq 0.05$).

interception, energy production and CO₂ assimilation. These results demonstrated that there is a balance in the height/diameter ratio and between growth and accumulation of photoassimilates, that is, the growth is proportional in the different vegetative structures, and that there is little or no possibility of damping-off of seedlings. Furthermore, it was observed that much of biomass partitioning is allocated in shoots, which is mainly responsible for the good quality of *C. officinalis* seedlings.

DQI is a characteristic evaluated in order to verify the vigor pattern of a seedling, since it is an indicator that associates growth, represented by height and diameter, with biomass production between the different organs of the plant (SANTOS et al., 2023), and the higher its value, the greater its development quality, with greater probability of good post-transplantation development. However, the response of the species in the field is variable with several other factors, making further studies necessary.

It was observed that the principal component analysis (PCA) explained 78.95% of the experimental variance, considering the representativeness of each PC. The characteristics taken from PCA were FC and SRR, which showed low experimental representativeness. In PC 1, the characteristics in descending order were NL, SL and emergence, while in PC 2, characteristics were RDM, RL, DQI, SD, TDM and SDM (Table 3). In the cluster analysis (cophenetic correlation= 0.928), seedlings with the smallest distance (1.63), that is, with greatest similarity, were those produced in the 100% DRL and DRL + Tropstrato® with and without seed hydropriming, while the group of seedlings farther

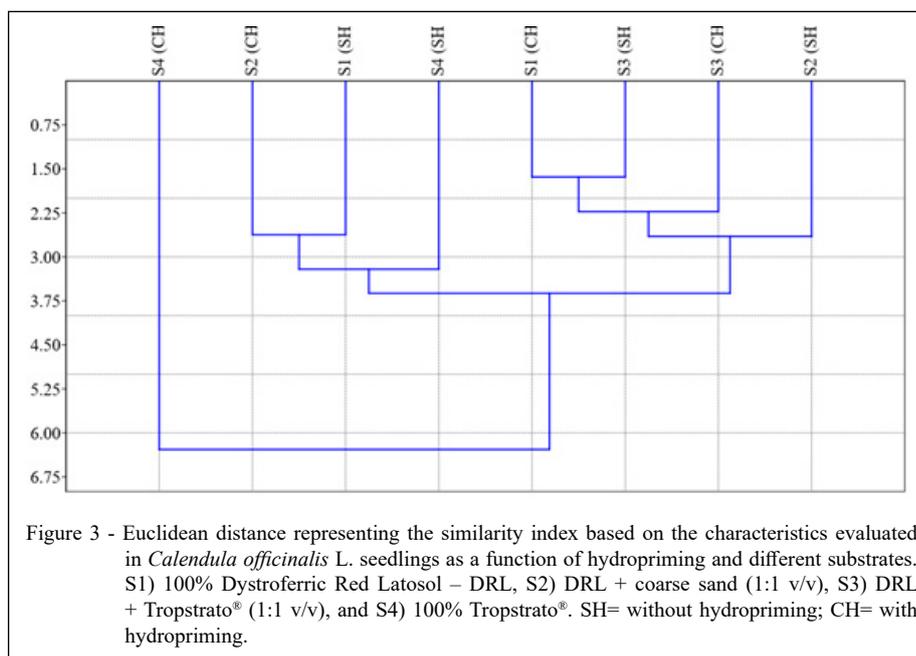
from the other seedlings were those produced in 100% Tropstrato® with seed hydropriming (Figure 3) due to the higher DQI.

However, it is noteworthy that the highest DQI occurred in the 100% Tropstrato® substrate, the same with the lowest emergence percentage. From the practical point of view; although, seedlings were more vigorous in this substrate, it would be necessary to use more seeds in sowing, making the activity costly due to costs with seeds, trays, commercial substrate and space, in addition to labor. In view

Table 3 - Factorial scores of the characteristics evaluated in *Calendula officinalis* L. seedlings as a function of hydropriming and different substrates through principal component analysis (PC).

Evaluated characteristics	PC 1	PC 2
	48.70%	30.25%
E	0.3027	0.0802
SL	0.3666	0.1371
RL	0.1631	0.5191
NL	0.4169	0.1033
SD	0.3405	0.3414
SDM	-0.3975	0.3015
RDM	-0.0577	0.5560
TDM	-0.3975	0.3023
DQI	-0.3414	0.3877

E – emergence, SL – shoot length, RL – root length, NL – number of leaves, SD – stem diameter, SDM – shoot dry mass, RDM – root dry mass, TDM – total dry mass, DQI – Dickson quality index.



of these results, we suggested an alternative option for the production of *C. officinalis* seedlings, which would be to use 100% DRL or DRL + Tropstrato®, with and without seed hydropriming, respectively, a fact observed in the shortest Euclidean distance, in which higher emergence percentages and intermediate DQI values were observed.

Further studies should associate seed hydropriming times and other materials in the formulation of substrates with the aim of increasing the options to produce *C. officinalis* seedlings, in addition to their subsequent development in the field, aiming to add information and strengthening the productive chain of medicinal and ornamental plants.

CONCLUSION

Substrates containing Dystroferic Red Latosol contributed to the emergence of *Calendula officinalis* L seedlings. The use of 100% Tropstrato® substrate favors biomass production and seedling quality. Dystroferic Red Latosol pure or combined with Tropstrato® substrate is recommended for the production and quality of *C. officinalis* seedlings. The hydropriming impaired first count and emergence, requiring new studies testing shorter exposure times to this technique.

ACKNOWLEDGEMENTS

The authors thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for granting the scholarships, and the Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul (FUNDECT) and CAPES (Finance code 001), for financial support.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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