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Irrigation management and capitulum order in the production and quality of *Eryngium foetidum* L. propagules

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ABSTRACT: There are few recommendations for management of production and quality of propagules of *Eryngium foetidum* L. Thus, the aim of this study was to evaluate irrigation management during plant flowering and the harvest of different orders of inflorescences on the production and quality of *E. foetidum* propagules. For that purpose, a completely randomized experimental design was used in a 5 × 4 factorial arrangement consisting of five capitulum orders (1st, 2nd, 3rd, 4th, and 5th orders) and four irrigation intervals (1, 2, 4, and 6 days). The following traits were evaluated: number of propagules, 1000-propagule weight, plant yield, first germination count, germination rate, hard propagules, relative frequency of germination, germination speed index, mean germination time, and mean time for seedling emergence in sand. After data collection, the means were compared using Tukey's test ($p < 0.05$). To obtain higher yield and physiological quality of *E. foetidum* propagules, they should be produced under irrigation at a 4-day interval, and harvest should occur from the 1st to the 5th orders of the capitula.

Index terms: Amazon chicory, germination, non-conventional food plants, vigor.

RESUMO: As recomendações para o manejo da produção e qualidade de unidades de propagação de *Eryngium foetidum* L. são escassas. Por isso, esse trabalho objetivou avaliar o manejo de irrigação durante o florescimento das plantas e a colheita de diferentes ordens de inflorescências, na produção e qualidade de unidades de propagação de *E. foetidum*. Para isso, utilizou-se o delineamento inteiramente casualizado, em esquema fatorial 5 x 4, cinco ordens de capítulos (1ª, 2ª, 3ª, 4ª, e 5ª ordem) e quatro manejos de irrigação (1, 2, 4 e 6 dias). Foram avaliadas as características: número e peso de mil unidades de propagação, e produção por planta, primeira contagem, germinação, unidades de propagação duras, frequência relativa de germinação, índice de velocidade e tempo médio de germinação e emergência das plântulas em areia. Após a obtenção dos dados, as médias foram comparadas pelo teste de Tukey ($p < 0{,}05$). Assim, para a obtenção de maior produção e qualidade fisiológica de unidades de propagação de *E. foetidum*, estas devem ser produzidas em condição de irrigação em intervalo de 4 dias e a colheita realizada da 1ª a 5ª ordem dos capítulos.

Termos para indexação: Chicória da Amazônia, plantas alimentícias não convencionais, germinação, vigor.

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INTRODUCTION

Eryngium foetidum L. (Apiaceae) is a tropical perennial vegetable classified as a non-conventional food plant (*Plantas Alimentícias Não Convencionais* - PANC). It is known as Amazon chicory, culantro, or sawtooth coriander (*chicória da Amazônia*, *chicória do Pará*, *coentrão*, and *coentro selvagem* in Brazil) (Gomes et al., 2013), and it stands out for its aromatic and medicinal properties (Mozumder et al., 2017).

It is used as a seasoning in food preparation (Gomes et al., 2020) and for the treatment of stomach and respiratory diseases, joint pain, and nervous system disorders (Rosero-Gómez et al., 2020) due to the presence of saponins, flavonoids, reducing sugars, and tannins in its leaves, stem, and roots (Mendoza et al., 2022). It is important to emphasize that compounds from Amazonian biodiversity extracted from plants have increasingly been used by industry (Silva et al., 2016; Machado and Kinupp, 2020), raising interest in plants grown in the Amazon region.

Regarding plant morphology during the reproduction phase, *E. foetidum* produces long stems with indeterminate growth and continuous flowering (Ekpong and Sukprakarn, 2006). Since it is not a widely domesticated species and shows genetic variability among genotypes (Gonçalves et al., 2022), individual plants can exhibit numerous stems. On the branches of each stem are inflorescences classified as capitula (flower heads), typical of plants of the Apiaceae family (Cardozo et al., 2021). Capitula up to the 10^{th} order can emerge on each stem, and the primary capitulum consists of a single inflorescence (Ekpong and Sukprakarn, 2006; Moraes et al., 2021).

Studies on *E. foetidum* fruit and seed production found a relationship between the order of the capitula and the quality of the propagules. Ekpong and Sukprakarn (2006) observed that the propagules of the 1st order capitula had greater weight, though they had a smaller quantity of seeds per capitulum compared to the other orders.

However, despite the availability of information on the position of propagules on the plant and their production and physiological quality, management practices during the reproductive phase of this vegetable in different geographic locations is hindered by the lack of information on how environmental factors affect development of the plant, its fruit, and its seeds. As it is a species native to tropical regions with high rainfall and relative humidity (Rodrigues et al., 2022), there are no studies addressing the consequences on fruit and seed production and quality of *E. foetidum* when exposed to environments with low water availability. According to Taiz et al. (2017), environmental factors outside the normal limits for a species generally lead to biochemical and physiological consequences in plants.

Therefore, to advance the domestication process and rational use of the economic potential of *E. foetidum*, it is necessary to develop techniques for commercial production of seeds and seedlings (Silva et al., 2016; Jeromini et al., 2018), especially considering the possibility of expanding the crop into areas limited by water deficit or with irregular rainfall distribution. Thus, the aim of this study was to evaluate the effect of irrigation management during plant flowering and the harvest of different orders of capitula on the production and the quality of *E. foetidum* propagules.

MATERIAL AND METHODS

It is important to note that *E. foetidum* has schizocarpic fruit (dry and indehiscent), consisting of subunits called mericarps or cypselae (Cardozo et al., 2021). Therefore, the term used throughout this study is “propagules”, since the material used is not the seed itself.

E. foetidum propagules were harvested in the Vegetable Crop area of the Universidade Federal Rural da Amazônia, Capanema-Pará Campus, and sent to the School of Agricultural and Veterinary Sciences of UNESP-Jaboticabal, where the experiment was set up and conducted in a greenhouse (T = 25 ± 2 °C and RH = 60% ± 3%) from August 2022 to April 2023.

Seedlings were produced in 128-cell polyethylene trays, with a cell volume of 22 mL. Trays were filled with a mixture of sieved soil, Bioplant© commercial substrate, coconut fiber, and earthworm humus at the volume ratio of 5:1:1:1/2, respectively. N-P-K (10-25-7) fertilizer was also incorporated.

Laboratory chemical analysis was conducted on the mixture used for producing seedlings in trays and later used for filling pots for transplanting. Chemical properties were as follows: pH (CaCl2) = 6.6; organic matter = 45 g.dm^{-3}; P = 220 mg.dm^{-3}; K^+ = 8.7 mmolc dm^{-3}; S = 26 mg.dm^{-3}; Ca^{2+} = 59 mmolc.dm^{-3}; Mg^{2+} = 22 mmolc.dm^{-3}; B = 0.60 mg.dm^{-3}; Cu = 3.3 mg.dm^{-3}; Fe = 20 mg.dm^{-3}; Mn = 4.9 mg.dm^{-3}; and Zn = 3.1 mg.dm^{-3}.

Three to four seeds were sown in each cell. Following emergence of seedlings and the first true leaf in at least one individual, the plants were thinned, leaving only one seedling per cell, the one with best development. The seedlings were kept in trays until emergence of the third true leaf, which occurred at around 82 days after sowing, when the seedlings were transplanted into pots.

The bottom of the 5-L capacity pots was covered with micro-perforated non-woven fabric (Perflex©) to allow water to drain without loss of the substrate through the holes at the bottom of the pot. The pots were filled with the same substrate as that used in the trays for seedlings production.

The plants were trained with wooden stakes and manually irrigated whenever necessary until the various irrigation treatments were applied.

Irrigation: following anthesis of the basal flowers of the 1st-order capitulum until the harvest of the propagules, the plants were irrigated daily and at intervals of 2, 4, and 6 days. These treatments resulted in moisture contents in the substrate of 57%, 45%, 27%, and 22%, respectively, determined by using the methodology proposed by Caputo and Caputo (2017) and measured at the time of harvest of the 5th-order capitulum after each irrigation interval. The amount of water used for irrigation was up to saturation of the substrate and draining of the excess through the hole in the pot.

Capitulum order: capitula from the 1st to the 5th order were harvested (Figure 1) when they had ripe fruit, characterized by a brownish-black color (Ekpong and Sukprakarn, 2006) and dehiscence when touched. Due to the unevenness of capitulum emergence and fruit maturation, fruit harvest was staggered. Capitula from six plants were harvested in each irrigation management treatment.

The capitula were placed to dry on paper on a laboratory bench at 22 ± 2 °C for one week. After that, the fruit was extracted by rubbing with the fingers and manually cleaned to remove unfertilized mericarps (cypselae) and plant fragments.

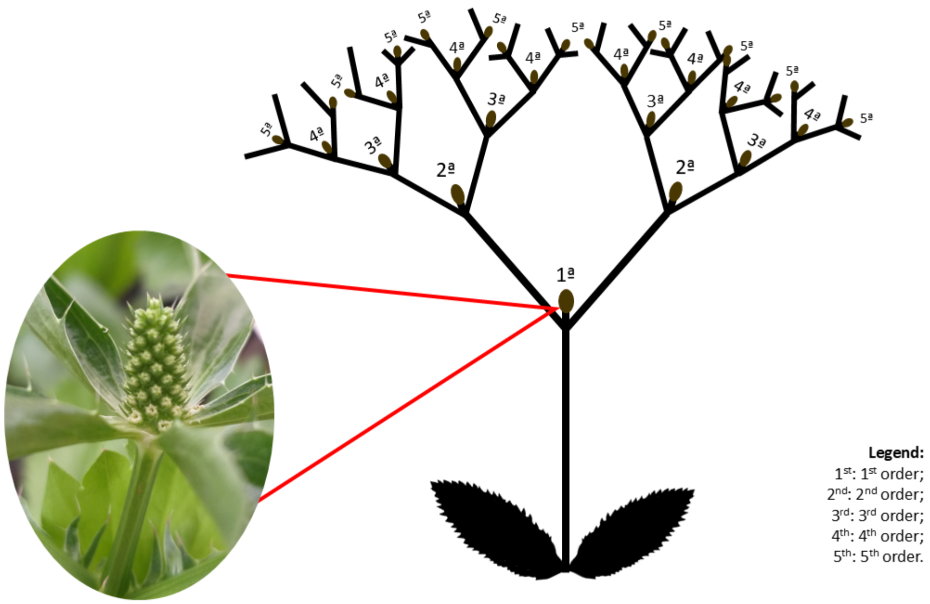

Figure 1. Diagram representing the orders of the capitula during the reproductive phase of *E. foetidum*.

The following tests were performed and determinations made:

1000-propagule weight (mg): based on the methodology adapted from Brasil (2009), and determined using four 100-propagule subsamples.

Number of propagules (number of cypselae.plant^{-1}) and *plant yield (mg.plant^{-1})*: after cleaning, the cypselae were manually counted and weighed on a precision analytical balance (0.001 g).

Germination rate (%): performed with four subsamples of 50 cypselae at a temperature of 25 °C and an 8-hour photoperiod (Brasil, 2009) for 38 days, as germination stabilized after that period (Affonso et al., 2018).

Hard propagules (%): at the end of the germination test, the hard cypselae were identified (Brasil, 2009).

First germination count: performed together with the germination test, calculating the percentage of normal seedlings on the 14th day after sowing, when the count reached 50% of the final germination, based on results reported by Tomaz et al. (2016).

Germination speed index (GSI) and *mean germination time (MGT):* performed together with the germination test and obtained by daily counting of the germinated propagules until the end of the test, using the methodologies proposed by Maguire (1962) and Labouriau (1983), respectively.

Relative frequency of germination (RF): performed together with the germination test and calculated using the formula proposed by Labouriau and Valadares (1976).

Seedling emergence in sand (%): performed with four subsamples of 25 cypselae sown in transparent plastic boxes (22.0 × 15.0 × 5.0 cm), following the methodology of Brasil (2009). The test was performed in a greenhouse (25 ± 2 °C and RH = 60% ± 3%) and the substrate was irrigated daily for 28 days, because emergence did not increase after that period.

Experimental design and statistical procedure: a completely randomized experimental design was used in a 5 × 4 factorial arrangement, with five capitulum orders (1st, 2nd, 3rd, 4th, and 5th orders) and four irrigation intervals (1, 2, 4, and 6 days), for a total of 20 treatments.

The data were first analyzed for meeting the assumptions of ANOVA. Residual normality was tested using the Shapiro-Wilk test, and homogeneity of variances was assessed using Bartlett's test. The probability density function and the QQ-plot were also used to help interpret these analyses. The data on propagule production per plant were transformed using the methodology of Box and Cox (1964), while the data on first germination count and 1000-propagule weight were transformed through $(x + 1)^{1/2}$.

Analysis of variance was used on the mean values and they were compared using Tukey's test at a 5% probability level. All the analyses were performed using the statistical computation software "RStudio" (version 2023.12.0+369) (R Core Team, 2023).

RESULTS AND DISCUSSION

Regarding 1000-propagule weight, no significant difference was observed between the capitulum orders when irrigation was applied daily or at 6-day intervals. In contrast, in the treatments with irrigation at intervals of 2 and 4 days, there was significant difference between the values obtained. Heavier cypselae were obtained from the 4th and 5th order capitula for the 2-day interval and from the 2nd and 3rd order capitula for the 4-day interval (Table 1).

The lowest 1000-propagule weight was from the cypselae harvested from the 1st to 3rd order when the plant was irrigated every 2 days, which may have been due to the initial stress brought about by water limitation. As of the 4th order, the plant adapted to the location and increased cypsela weight. However, when irrigated every 4 days, the plant began to enter senescence as of the 4th order, hindering storage of reserves in the propagules.

In regard to the effect of irrigation on cypsela weight in each capitulum order, there was significant difference

Table 1. Mean values of 1000-propagule weight and number of propagules per *E. foetidum* plant as a function of irrigation and order of capitula.

Traits evaluated	Irrigation interval (days)	Order of the capitula				
		1st	2nd	3rd	4th	5th
1000-propagule weight (mg)	1	521.6 aA	525.0 aA	532.5 aA	532.5 aA	532.5 aA
	2	480.0 bC	507.5 aB	485.0 bBC	550.0 aA	547.5 aA
	4	500.0 abB	515.5 aAB	532.5 aA	497.5 bB	459.4 bC
	6	490.0 bA	481.8 bA	480.0 bA	465.0 cA	476.9 bA
CV (%)		1.3				
Number of propagules per plant	1	86 aC	109 aC	145 aC	331 abB	464 bcA
	2	92 aC	123 aC	190 aC	380 aB	659 aA
	4	91 aC	146 aC	151 aC	394 aB	535 bA
	6	72 aC	94 aC	110 aC	241 bB	385 cA
CV (%)		31.4				

Mean values followed by the same lowercase letter in the column and uppercase letter in the row do not differ from each other according to Tukey's test at 5% probability.

among the results. In general, the heaviest cypselae were produced by plants irrigated daily (1 day), and the lightest were produced under 6-day irrigation intervals (Table 1).

Possibly, the lower water content in the substrate resulting from longer irrigation intervals (6 days) interfered in the seed development phases, particularly the cell division and elongation phase and the dry matter accumulation phase. According to Miranda et al. (2017), the cell division and elongation phase determines seed size and, consequently, reserve storage capacity. Likewise, in the dry matter accumulation phase, water availability to the plant is essential, otherwise lighter weight seeds are produced (Carvalho and Nakagawa, 2012).

For the number of propagules produced per plant under each irrigation condition, significant difference was observed in the values. A larger number of cypselae was obtained the later the capitulum order emerged in the plant cycle, regardless of the irrigation interval (Table 1). These results are consistent with those obtained by Ekpong and Sukprakarn (2006) and Moraes et al. (2021) in studies on *E. foetidum*, who reported an increase in the number of capitula due to the number of orders, and consequently, an increase in the number of cypselae.

There was significant difference in the number of propagules produced per plant only in the 4th and 5th orders, regardless of the irrigation applied. In the treatment with irrigation at 6-day intervals, the lowest numbers of propagules were observed in both orders, with 241 and 385 propagules, respectively (Table 1). This result may have been caused by plant senescence, because the plants exhibited dry or yellowish-colored leaves due to low water availability. There was therefore reduction in the photosynthesis rate and in photoassimilate production, interfering with the formation of propagules. Scalon et al. (2011) report that water deficit generally leads to reduction in the photosynthesis rate. The authors performed a study on mutambo (*Guazuma ulmifolia* Lam.) seedlings and observed leaf loss in the treatment with lower water availability.

Regarding propagule production per plant, there was a significant effect of the irrigation and capitulum order factors, independently. Senescence in the plants under irrigation at 6-day intervals not only reduced the number of propagules, but also reduced cypsela production per plant (Table 1 and Figure 2A). As for the effect of the capitulum order on propagule production per plant, production increased as the orders advanced (Figure 2B). This result is mainly due to an increase in the number of propagules per plant as the number of capitula increased from the 1st to the 5th order, with the 1st order producing a single inflorescence, the smallest propagule production per plant (Table 1 and Figure 2B).

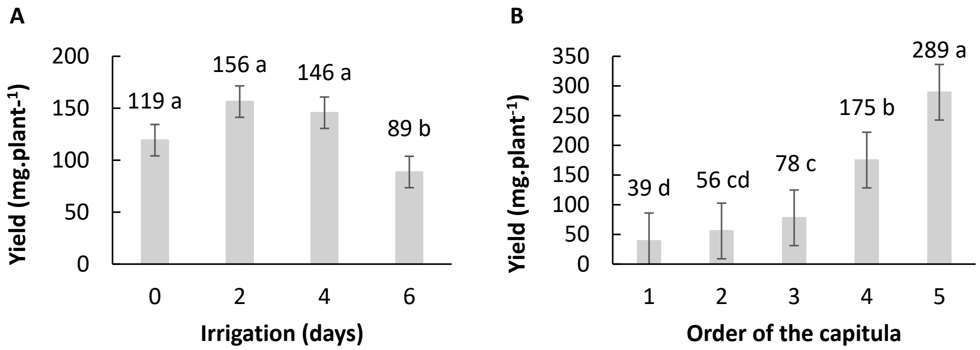

Figure 2. Production of *E. foetidum* propagules as a function of irrigation (A) and order of capitula (B). Mean values presented from original data.

Generally, first germination count in each order showed that the germination rates of the propagules produced by the plants under daily irrigation (1 day) were statistically lower compared to the other irrigation treatments (Table 2).

Applying irrigation at 2-, 4-, and 6-day intervals likely interfered with plant metabolism and development. Due to the water limitation caused by these treatments, the plants may have developed a shorter vegetative period and larger number of propagules that reached physiological maturity as a species survival strategy (Singh et al., 2022), favoring a higher germination rate in the first germination count (Table 2).

Observation of the effect of the capitulum order in plants under each irrigation condition on first germination count showed a significant difference among the treatments, with the highest values observed in the 1st order in the treatments with irrigation at 2- and 6-day intervals, and in the 1st and 2nd orders in the treatment with irrigation at 4-day intervals (Table 2).

Possibly, the lower values observed from the 3rd to the 5th order of first germination count under the 4-day interval irrigation condition is due to the fact of the increased competition for resources as the plant develops. Various inflorescences are produced on each branching of the stem during the reproductive period, which increases the number of sinks. According to Walker et al. (2021), older reproductive organs inhibit the growth of newer ones, and regular harvest is necessary for continued development of fruit and seeds.

Regarding germination rate of the cypselae obtained from the 1st order, a significant difference was seen only in the results obtained from the daily (1 day) irrigation treatment compared to the other intervals (Table 1). With daily irrigation, germination reduced by more than 40% compared to the other treatments. Beginning with the 2nd order of the capitula, irrigation at 4-day intervals provided for the highest germination rates, ranging from 75% to 82%. Reduction in the germination rate in the cypselae obtained in the 1st order produced under the daily irrigation condition may have been due to uneven flowering. Maintaining irrigation up to the time of harvest may have led to fruit and seeds that did not achieve physiological maturity (Mera et al., 2011), thereby reducing seed physiological quality. Miranda et al. (2017) observed that in carrots (*Daucus carota* L.), immature seeds exhibited a low germination rate, due to lower accumulation of dry matter.

Furthermore, observing the effect of capitulum order under each irrigation treatment showed that under daily irrigation and irrigation at 6-day intervals, the results of germination rates fluctuated depending on the order. Irrigation at 2-day intervals led to a higher germination rate only in the cypselae from the 1st order, at 84%. And under irrigation at 4-day intervals, no significant difference was found among the umbel orders. However, higher germination rates were found for the cypselae obtained from the 1st to the 5th orders (ranging from 75% to 88%) (Table 2).

Irrigating plants at 4-day intervals may have led to uniform flowering and maturation of the propagules within each order, resulting in higher germination rates from the 1st to the 5th order. Due to the water limitation brought about by this treatment, this result may reflect a species survival strategy. In the event of some limitation in environmental factors, the plant directs its resources to seed development, with the aim of promoting greater physiological quality to maintain the species in the area (Taiz et al., 2017).

Table 2. Mean values of first germination count (FGC), germination rate (G), hard propagules (H), germination speed index (GSI), and mean germination time (MGT) of *E. foetidum* propagules as a function of irrigation and order of capitula.

Traits evaluated	Irrigation (days)	Order of the capitula				
		1st	2nd	3rd	4th	5th
FGC (%)	1	2 cB	0 cBC	0 bBC	0 cC	7 bA
	2	25 bA	15 bB	18 aAB	21 aAB	15 aB
	4	38 aA	34 aA	19 aB	3 bB	16 aB
	6	33 abA	10 bC	23 aAB	17 abBC	18 aBC
CV (%)		15.05				
G (%)	1	46 bAB	28 cC	38 bBC	31 cBC	59 bcA
	2	84 aA	54 bB	50 bB	62 bB	52 cB
	4	88 aA	81 aA	75 aA	82 aA	80 aA
	6	90 aA	36 cC	77 aAB	64 bB	74 abAB
CV (%)		13.6				
H (%)	1	16 aB	31 aA	25 abAB	14 bB	23 aAB
	2	4 aB	26 aA	35 aA	29 aA	27 aA
	4	5 aA	10 bA	14 bA	5 bA	6 bA
	6	6 aB	32 aA	16 bB	16 bB	10 bB
CV (%)		39.9				
GSI	1	1.29 bAB	0.63 dC	0.85 cBC	0.80 bBC	1.78 bA
	2	2.81 aA	1.84 bB	1.81 bB	2.20 aB	1.74 bB
	4	3.32 aA	3.06 aAB	2.59 aBC	2.53 aBC	2.48 aC
	6	3.23 aA	1.21 cC	2.62 aB	2.19 aB	2.53 aB
CV (%)		14.1				
MGT (days)	1	20 cB	22 cD	21 bCD	20 cBC	17 bA
	2	16 bA	15 bA	14 aA	15 aA	16 abA
	4	14 aA	14 aA	15 aA	17 bB	17 bB
	6	15 bcA	15 bA	16 aA	15 aA	15 aA
CV (%)		5.0				

Mean values followed by the same lowercase letter in the column and uppercase letter in the row do not differ from each other according to Tukey's test at 5% probability.

Regarding the percentage of hard propagules at each irrigation interval, fluctuation was observed in the results for daily irrigation (1 day), varying according to the capitulum order. Irrigation at 2-day intervals led to a lower percentage of hard propagules (4%) only in the 1st order. Meanwhile, irrigation at 4-day intervals did not result in significant differences among capitulum orders. However, low percentages of hard propagules were found in all the orders (5% to 14%), due to the higher germination rate at this irrigation interval (Table 2).

Regarding the effect of applying irrigation in each capitulum order on the percentage of hard propagules, a significant difference was found in the values obtained only from the 2nd order on. Higher percentages of hard propagules were observed in daily irrigation and in the 2-day interval treatment in the 3rd, 4th, and 5th orders, reaching a maximum of 31% and 35%, respectively (Table 2).

This result may have occurred due to the natural behavior of the plant. It is a vegetable crop native to tropical regions, with high rainfall and humidity conditions (Rodrigues et al., 2022). Dormancy is its adaptation and survival strategy to these climate conditions, through control of water uptake (Carvalho and Nakagawa, 2012). This prevents germination from occurring before reaching the optimal period or under conditions unfavorable to growth and survival (Vivian et al., 2008), particularly in the periods of highest rainfall.

In contrast, plant senescence brought about by the conditions of irrigation at 4- and 6-day intervals, with lower water availability in the substrate, affected the plant survival mechanism. In other words, due to the limitation of environmental resources, there was smaller production of hard propagules as a way of ensuring continuity of the species (Table 2).

Observing the effect of the capitulum orders in each irrigation treatment on the germination speed index showed that daily irrigation led to fluctuation in the results, varying according to capitulum order. However, under irrigation conditions at 2- and 6-day intervals, higher germination speed was found only at the 1st capitulum order (Table 2).

This variation in the results under daily irrigation may be due to the fact of *E. foetidum* being a non-domesticated species, resulting in high variability in seed germination performance in response to environmental factors, such as water deficit. Fortes et al. (2008) state that responses to tests for evaluation of physiological quality can vary within the same species, especially those performed on seeds of non-domesticated plants.

Regarding the effects of irrigation in each capitulum order, the propagules produced under daily irrigation (1 day) exhibited lower germination speed indices in the 1st, 2nd, 3rd, and 4th orders, and therefore, the longest germination times (Table 2). These results may be due to the larger number of immature seeds at the time of harvest. Perennial plants seek to ensure ideal storage of resources that enables them to survive, and thus tend to adjust their metabolic and developmental programming (Taiz et al., 2017), prolonging the maturation period. According to Bennet et al. (2012), under optimal conditions, perennial plants allocate more limited resources to their reproductive parts, mainly due to their prolonged life cycle, and it is more advantageous to retain photosynthetic capacity while there are favorable conditions.

Daily irrigation (1 day) not only led to a longer mean germination time, but also brought about uneven germination of the propagules obtained from the 1st to 4th orders up to the 38th day after sowing, as can be observed in the relative frequency graphs (Figure 3). That differs from the results obtained under the 2-, 4-, and 6-day interval irrigation treatments for the propagules from the 1st to the 4th orders of the capitula, where germination was more concentrated up to around the 18th day after sowing.

Therefore, these results can help reduce the time and costs associated with producing *E. foetidum* seedlings and decrease losses of propagules in the field. For the longer the germination time, the greater the susceptibility to attack from pests and diseases.

Regarding seedling emergence in sand, no significant interaction was found among the factors; only the capitulum order had an effect. Therefore, the propagules obtained from the 1st and 2nd orders showed the highest emergence percentages, at 80% and 76%, respectively (Figure 4). Lamichaney and Sooganna (2016) reported that the position of the seed in the plant affects seed physiological quality. In *Pimpinella anisum* L. [Apiaceae], the authors found better quality of seeds from the 1st and 2nd order umbels than the quality of seeds from the 3rd and 4th orders.

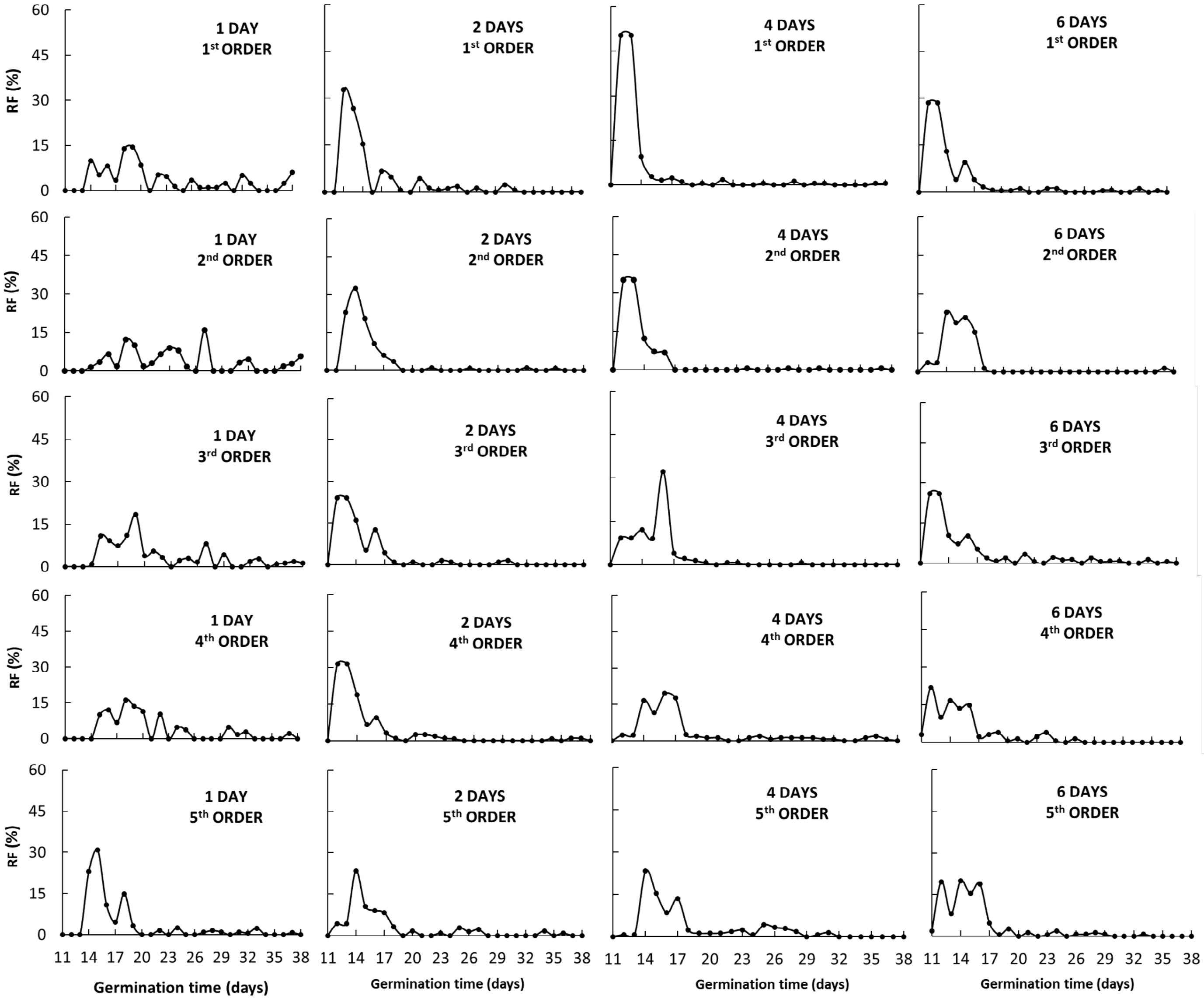

Figure 3. Relative frequency of germination (RF) of the *E. foetidum* propagules as a function of daily irrigation (1 days) and irrigation at 2-, 4-, and 6-day intervals and of the 1st, 2nd, 3rd, 4th, and 5th orders of the capitula.

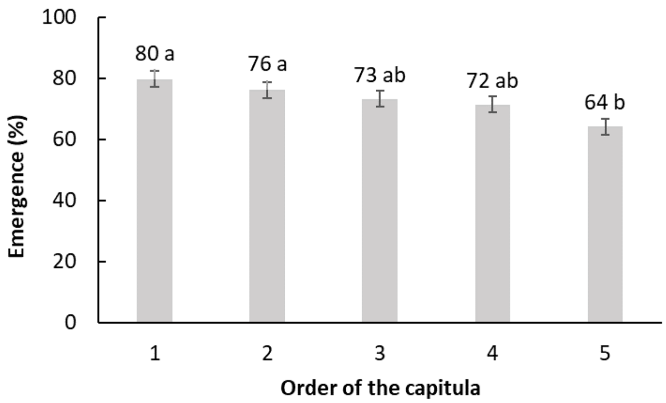

Figure 4. Plantlet emergence of *E. foetidum* in sand as a function of the order of the capitula.

CONCLUSIONS

To achieve greater production and physiological quality of *E. foetidum* propagules, they should be produced under the condition of irrigation at 4-day intervals; and harvest should occur from the 1st to the 5th orders of capitula, with a staggered harvest.

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AUTHORS' CONTRIBUTION

Isabelle Caroline Bailosa do Rosário contributed with: Conceptualization, Methodology, Investigation, Formal analysis, Data Curation, Visualization, Writing - original draft, review and editing; Laura Monteiro Pedrosa contributed with: Methodology, Writing - review & editing; Giovanna de Castro contributed with: Methodology; Rafaelle Fazzi Gomes contributed with: Writing - review & editing; Cibele Chalita Martins contributed with: Conceptualization, Methodology, Supervision, Resources, Writing - review & editing.

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