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Articles

Seedling emergence and survival of three Fabaceae species in response to different substrates and containers

Emergencia y supervivencia de tres especies de Fabaceae en respuesta a diferentes sustratos y contenedores

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ABSTRACT

In forest nurseries, germination and seedling growth are influenced by the choice of planting materials, which must be suited to the plant's developmental stages. Substrate composition and container type influence structural support, nutrients, and water availability. This study investigated the effect of different substrate mixtures and container types on the emergence and survival of *Neltuma laevigata*, *Havardia pallens* and *Ebenopsis ebano.* The substrates were composed of peat moss, perlite, vermiculite, and soil in different proportions. The containers tested were polyethylene bags (400 ml), polypropylene containers (170 ml), and polystyrene containers (163 ml). Emergence percentage, survival rate, time to 75% seedling emergence, and the emergence speed index were evaluated. While the emergence speed index remained consistent across treatments for all species, emergence and survival were significantly reduced in the soil-only substrate. On the other hand, polyethylene bags showed favorable effects when paired with a suitable substrate. These findings suggest that seedling production for each species should be tailored to specific substrate-container combinations to optimize emergence and survival.

Keywords: Nursery; Thornscrub; Germination; Reforestation

RESUMEN

La germinación y crecimiento de plántulas en vivero están ligados a los materiales utilizados, los cuales deben adaptarse a las etapas del desarrollo vegetal. El sustrato y el contenedor influyen en la disponibilidad de soporte, nutrientes y recursos hídricos. Este estudio exploró el efecto de diferentes combinaciones de sustratos y contenedores sobre la emergencia y supervivencia de *Neltuma laevigata*, *Havardia pallens* y *Ebenopsis ebano*. Los sustratos incluyeron peat moss, perlita, vermiculita y suelo en distintas proporciones; los contenedores fueron bolsa de polietileno (400 ml), contenedor de polipropileno (170 ml) y poliestireno (163 ml). Se evaluaron el porcentaje de emergencia y supervivencia, el tiempo para la emergencia del 75% de las plántulas y el índice de velocidad de emergencia, este último no varió entre tratamientos para ninguna especie. En general, la emergencia y supervivencia se redujeron en el sustrato compuesto únicamente por suelo. Por otro lado, la bolsa de polietileno puede producir efectos favorables cuando está en combinación con un sustrato adecuado. Se concluye que la producción de plántulas de cada especie debe adaptarse a combinaciones específicas para optimizar la emergencia y supervivencia.

Palabras clave: Vivero; Matorral espinoso tamaulipeco; Germinación; Reforestación

1 INTRODUCTION

Reforestation, defined as plant recovery in degraded areas, is a crucial tool for ecological restoration that not only supports biodiversity conservation, but helps mitigate climate change and enhances food security (Di Sacco *et al.,* 2021). A key component of reforestation is the production of high-quality plants, a trait that directly influences their successful establishment and survival in the field (Haase and Davis, 2017).

Plant survival is closely linked to plant quality, which depends largely on the materials used in their production (Mexal *et al.,* 2008). Characteristics such as the substrate, container type, irrigation regime, and light availability must be tailored to species-specific needs at different growth stages. The substrate is particularly important, not only because it provides physical support and nutrients to seedlings, but also by determining water retention, porosity, bulk density, and root development. These characteristics directly affect seedling emergence and survival in the nursery, and ultimately plantation success (Mariotti *et al.,* 2023). Different substrate mixtures have been tested in plant production, with results varying depending on the species and growing conditions. Mixtures that include peat moss, compost, or bark with inorganic components such as perlite or vermiculite are frequently used to improve porosity and drainage (Wilkinson *et al.,* 2014). In Mexico, soil-based substrates are common, but pose risks such as pathogen transmission, increased seedling mortality, and environmental degradation due to soil extraction (Ogunsiji *et al.,* 2020).

Container type also plays a critical role in seedling development by influencing the volume, temperature, and water retention of the substrate. For example, polyethylene bags are widely used in Mexico due to their low cost, but their design often promotes root spiraling, which reduces field performance after planting (Haase *et al.,* 2021). Container volume is directly correlated with seedling biomass (Lima *et al.,* 2019; Freitas *et al.,* 2021; Pachajoa *et al.,* 2023). Furthermore, substrate temperature and water retention are influenced by the interaction between the container's material and the volume of the substrate used. A lower substrate volume and darker or less porous container materials can increase substrate temperature and reduce water availability (Nambuthiri *et al.,* 2015a). Given that seeds require sufficient water absorption during imbibition, and excessive substrate temperatures can reduce water availability and damage the roots, the material and color of the container, which influence heat retention, can significantly affect seed germination, seedling emergence, and survival in the nursery. These factors are especially critical in arid and warm climates, where substrate temperature and water availability are major constraints in seedling production (Nambuthiri *et al.,* 2015b).

The increasing extent of degraded lands in the arid and semiarid regions of northern Mexico and the southern United States has made reforestation a critical strategy for biodiversity restoration and climate change mitigation. Species from the Fabaceae family are particularly abundant in these areas and provide timber, forage, and food, play a key role in nitrogen fixation, and serve as regeneration nuclei by facilitating the establishment of other plants in degraded areas (Valdes-Alameda *et al.,* 2024).

To improve their propagation practices for these species, it is essential to understand how substrate composition and container type affect early seedling development. This study aims to provide key information to optimize production practices for three common leguminous species in Mexico. To that end, seedling emergence and survival were evaluated for *Neltuma laevigata* (Humb. & Bonpl. ex Willd.) Britton & Rose (formerly *Prosopis laevigata*), *Havardia pallens* (Benth.) Britton & Rose, and *Ebenopsis ebano* (Berland.) Barneby & J.W. Grimes under different combinations of container types and substrate mixtures.

2 MATERIALS AND METHODS

The study was conducted in a traditional forest nursery covered with 70% Raschel mesh, located in Linares, Nuevo León, Mexico (coordinates 24º 4′ 42″ N, 99º 32′ 29″ W), at an elevation of 390 meters above sea level. The local climate is classified as dry to semi-dry, with average annual rainfall and temperature of 650 mm and 22.4°C, respectively.

Three native leguminous species (*Neltuma laevigata*, *Havardia pallens*, and *Ebenopsis ebano*) were selected based on their reported high germination potential (Jurado *et al.,* 2000). Seedlings were grown in three different containers: 1) a polypropylene container with 54 cavities (170 ml each); 2) a Styrofoam container with 77 cavities (163 ml each); and 3) black polyethylene bags (400 ml volume). To assess the influence of substrate composition, three mixtures were formulated using varying proportions of soil, peat moss, perlite, and vermiculite. The soil was collected from the study area and classified as vertisol with a fine clay-loam texture (Yáñez Díaz *et al.,* 2018). The study followed a factorial experimental design with nine treatments, each representing a different combination of container type and substrate mixture (Table 1). Each treatment consisted of five replicates of 20 seeds per species (100 seeds per species per treatment), totaling 900 seeds per species.

Table 1 – Treatments assessed across the three species investigated

Treatment	Container	Substrate
T1	Black polyethylene bag	Substrate 1: 50% soil, 20% perlite, 20% peat-moss and 10% vermiculite
T2	Black polyethylene bag	Substrate 2: 70% peat-moss, 20% perlite and 10% vermiculite
T3	Black polyethylene bag	Substrate 3: 100% soil
T4	Styrofoam container	Substrate 1: 50% soil, 20% perlite, 20% peat-moss and 10% vermiculite
T5	Styrofoam container	Substrate 2: 70% peat-moss, 20% perlite and 10% vermiculite
T6	Styrofoam container	Substrate 3: 100% soil
T7	Polypropylene container (plastic)	Substrate 1: 50% soil, 20% perlite, 20% peat-moss and 10% vermiculite
T8	Polypropylene container (plastic)	Substrate 2: 70% peat-moss, 20% perlite and 10% vermiculite
T9	Polypropylene container (plastic)	Substrate 3: 100% soil

Source: Authors (2024)

Seeds were collected between January and April 2023 in Linares, Nuevo León. Prior to sowing, the seeds were cleaned to remove those that were visibly immature or insect-damaged. The remaining seeds were soaked in water for 24 hours as a pre-germination treatment. All containers were disinfected using a 10% chlorine solution (10 parts water to 1 part chlorine). After sowing, a balanced fertilizer (15N - 9P - 12K) was applied twice, the first application three weeks after sowing, and the second two months later. Pest management measures were initiated five days after the second fertilizer application, by applying the fungicide Captan (20 g/liter). Irrigation was carried out daily, with 30 milliliters of water administered per cavity. Seedling emergence was monitored daily over a period of 83 days.

2.1 Statistical analysis

Seedling performance across the different treatments was evaluated using three main metrics. The emergence percentage (EP) was selected as the most commonly used measure for sample comparisons, and calculated according to Equation (1):

$$EP = \frac{SE}{TS} * 100 \tag{1}$$

where: EP = emergence percentage; SE = number of emerged seedlings; TS = Total seeds sown.

The time to 75% seedling emergence (T75) was used to compare emergence dynamics among species, since it is a recognized metric for distinguishing germination speed (Lezama and Morfin, 1992). T75 was determined by linear interpolation between observation days and cumulative percentage.

The emergence speed index (ESI), a measure of seedling vigor, was calculated using Equation (2):

$$ESI = \sum \left(\frac{ni}{t}\right) \tag{2}$$

where: ESI = emergence speed index; n_i = number of emerged seedlings on day *i;* t = time (in days) from sowing to the emergence of the last seedling.

Additionally, the seedling survival percentage (SP) was used to evaluate seedling establishment success and resilience in Equation (3).

$$SP = \frac{PV}{SE} * 100 \tag{3}$$

where: SP = survival percentage; PV = number of surviving seedlings at the end of the experiment; SE = number of emerged seedlings.

A factorial analysis of variance (ANOVA) was performed separately for each species to identify the effects of substrate mixture and container type, each tested at three levels. When statistically significant differences between means were detected, Tukey's post-hoc test was used for multiple comparisons. All analyses were performed using R Studio (version 4.4.1).

3 RESULTS AND DISCUSSIONS

The ESI did not vary significantly across treatments for any of the three species, with no significant effects for substrate composition (*N. laevigata*: F = 3.17, P = 0.06; *H. pallens*: F = 2.93, *P* = 0.07; *E. ebano*: F = 2.93, *P* = 0.07) container type (*N. laevigata*: F = 0.455, *P* = 0.63; *H. pallens*: F = 0.48, *P* = 0.62; *E. ebano*: F = 0.48, *P* = 0.62), or their interaction (*N. laevigata*: F = 0.36, *P* = 0.83; *H. pallens*: F = 2.46, *P* = 0.06; *E. ebano*: F = 1.10, *P* = 0.37). These findings suggest that seed samples exhibited similar emergence rates and uniformity across treatments. This early emergence behavior may reflect inherent seed vigor, defined as the physiological potential for rapid and uniform germination and seedling development even under sub-optimal conditions (Marcos-Filho, 2015). While ESI alone may not capture all aspects of seed vigor, the lack of significant differences suggests that the seeds used in this study exhibited comparable physiological quality. Therefore, observed variations in emergence and survival are likely due to the effects of container and substrate.

By contrast, EP was significantly influenced by the substrate mixture in all three species (*N. laevigata*: F = 11.63, *P* < 0.01; *H. pallens*: F = 10.36, *P* < 0.01; *E. ebano*: F = 3.92, *P* = 0.03). The highest EP values were recorded for substrate mixtures 1 and 2, while mixture 3 negatively affected seedling emergence. Similar results have been reported in previous studies on *Neltuma* and *E. ebano*, which found higher emergence in substrates that exclude soil (Vilela and Ravetta, 2001; Luera and Gabler, 2022). The presence of soil in nursery substrates may increase the risk of disease transmission affecting seeds and seedlings during early development (Ogunsiji *et al.,* 2020). Moreover, the soil used in this study, characterized by high clay content (Yáñez Díaz *et al.,* 2018), typically results in high bulk density and limited aeration (Hart *et al.,* 1980). Under these conditions, the use of substrate mixture 3, composed entirely of local soil, could hinder germination and emergence. This effect has been observed in other species, where less porous substrates restrict gas exchange and limit initial root development, thus affecting both germination and emergence (Benvenuti, 2003; Hallett *et al.,* 2014).

By contrast, emergence percentages for *H. pallens* and *E. ebano* were relatively low compared to germination percentages reported in previous studies (Luera and Gabler, 2022; Carranza *et al.,* 2024). However, these findings align with research indicating less than 50% emergence in substrates containing 50-100% soil, even when intensive pre-germination treatments are used (Luera and Gabler, 2022). This highlights a gap between germination and emergence for these species, suggesting either early seedling mortality or insufficient substrate conditions to support emergence. The similar response to different substrates across the three species reinforces the conclusion that substrate characteristics play a critical role in determining seedling emergence.

Container type also influenced EP, but the effect was statistically significant only for *E. ebano* (F = 4.58, P = 0.01), with no significant effects observed for *N. laevigata* (F = 1.58, P = 0.22) or *H. pallens* (F = 2.12, P = 0.13). Among the container types tested, the polypropylene container (170 ml) yielded the highest EP for *E. ebano*. No previous studies were found that directly compared the effects of container type on emergence in these species. Interestingly, the results obtained here contrast with previous findings across a range of species, whereby larger container volumes are typically associated with increased root development, stem diameter, and biomass (Dominguez-Lerena *et al.,* 2006; Lima *et al.,* 2019; Freitas *et al.,* 2021; Pachajoa *et al.,* 2023).

For *E. ebano*, previous studies have shown that heat is a favorable factor that facilitates germination (Carranza *et al.,* 2024). In the present study, the polypropylene container, due to its dark color and material, likely contributed to elevated substrate temperatures (Sánchez-Aguilar *et al.,* 2016). Moreover, its relatively small volume may further expose seeds to these increased temperatures. This suggests that the observed contrast between germination and development may be explained by differing requirements across developmental stages: while larger container volumes support seedling growth, during the germination phases, factors such as substrate temperature and water-holding capacity, which are more directly affected by container size and material, play a critical role. If this is the case, seedling production strategies should be tailored to match the distinct needs of each stage in seedling development. Therefore, future studies should investigate the relationship between container volume and substrate temperature and how these variables influence critical stages such as germination and emergence. A better understanding of these interactions could help optimize production practices and improve both germination rates and subsequent seedling performance in the field.

A significant interaction between substrate and container was observed only for *N. laevigata* in terms of emergence percentage (*N. laevigata*: F = 8.10, $P < 0.01$), with no interaction effects detected for *H. pallens* (F = 1.53, $P = 0.22$) or *E. ebano* (F = 1.77, $P = 0.16$). This indicates that the emergence of *N. laevigata* is not determined by substrate or container type alone, but rather by interaction between the two. For instance, both treatments 7 and 4 used substrate mixture 1, but exhibited the highest (96%) and lowest (67%) EP values, respectively, highlighting the role of container type (Table 2). Similarly, the performance of the polyethylene bag varied with the substrate, resulting in 97% emergence when paired with substrate 2, but only 67% with substrate 3 (Table 2). These findings underscore the importance of jointly considering substrate and container selection when aiming to optimize *N. laevigata* emergence.

The time to 75% seedling emergence (T75) varied by species and treatment. *N. laevigata* emerged rapidly, with no significant influence from substrate mixture (F = 0.10, $P = 0.89$). By contrast, emergence times for *E. ebano* and *H. pallens* were strongly affected by substrate composition, with faster emergence occurring in substrates 1 and 2 (*H. pallens*: F = 101.62, $P < 0.01$; *E. ebano*: F = 952.71, $P < 0.01$). These trends align with earlier EP findings, where substrate 3 consistently hindered performance.

N. laevigata emerged predominantly within the first 2 to 3 days, an early pattern consistent with prior observations for this and other leguminous species (Dorneles *et al.,* 2013; Rodríguez-Laguna *et al.,* 2018). This trait may reflect an adaptative response to the limited water availability typical of arid and semi-arid regions.

Table 2 – Emergence and survival results of *Neltuma laevigata* seedlings under different substrates and containers

T	EP (%)	T75 (days)	ESI	SP (%)
1	79 (±3.82) a	2.00 (±0.82) a	0.95 (±0.35) a	74.64 (±7.12) a
2	97 (±6.00) b	2.50 (±1.29) a	1.17 (±0.22) a	93.86 (±2.16) b
3	67 (±10.51) a	2.75 (±1.71) a	0.82 (±0.24) a	89.57 (±5.37) bc
4	67 (±8.86) a	1.75 (±0.96) a	0.80 (± 0.35) a	88.14 (±4.21) bc
5	83 (±7.57) a	2.00 (±0.82) a	1.00 (± 0.31) a	77.68 (±12.89) ac
6	78 (±9.52) a	1.75 (±0.50) a	0.95 (±0.35) a	83.52 (±10.44) abc
7	96 (±5.65) b	2.25 (±1.89) a	1.16 (±0.37) a	84.47 (±6.92) ac
8	82 (±12.43) ab	2.25 (±1.89) a	1.00 (±0.37) a	87.82 (±1.83) c
9	67 (±8.86) a	2.00 (±1.41) a	0.81 (±0.98) a	83.70 (±4.45) ac

Source: Authors (2024)

In where: T= treatment, EP= emergence percentage, T75 = time (in days) to 75% seedling emergence, ESI=emergence speed index, SP = survival percentage. Values in parentheses represent standard deviation. Different letters indicate significant differences according to Tukey’s test.

Conversely, although *H. pallens* and *E. ebano* have been described as having intermediate to high germination rates, with 50% germination typically occurring between days 3 and 6 (Flores and Jurado, 1998), in the present study 75% emergence was only reached after 12 days (Table 3). This difference may be due to the use of distinct pre-germinative treatments. Hard seed coats, a common feature among legumes, usually require scarification or other treatments to accelerate water uptake and improve germination (Statwick, 2016). The less aggressive pre-treatment used here may have been insufficient to fully break physical dormancy, partially explaining the slower emergence in *H. pallens* and *E. ebano*. Additionally, substrate composition appears to influence T75, since the compaction, low porosity, and limited aeration of clay soils may not only delay emergence by restricting water availability and gas exchange, but reduce the number of emerged seedlings.

Neither container type nor the interaction between container and substrate significantly affected T75 in *N. laevigata* (F = 0.56, P = 0.57; F = 0.15, P = 0.96, respectively). This result contrasts with the emergence percentage for the same species, which was significantly influenced by the interaction of these two factors. Conversely, both container type and its interaction with substrate significantly affected T75 in *H. pallens* and *E. ebano*. For *H. pallens*, emergence was slower in black polyethylene bags and faster in polypropylene trays (F = 51.40, $P < 0.01$). The slower emergence in the former, especially when combined with substrate 3 ($\bar{x}$ = 38 days, F = 41.65, $P < 0.01$), may be due to their reduced permeability, which can result in anoxic conditions or high temperatures (Nambuthiri *et al.,* 2015b). By contrast, faster emergence was observed when polypropylene trays were paired with substrate 2 ($\bar{x}$ = 12 days; Table 3). Although, polyethylene bags filled with soil are widely used in the region due to their low cost, this combination has been reported to yield lower-quality plants and higher long-term economic and environmental costs (Haase *et al.,* 2021).

E. ebano seedlings showed slower emergence in Styrofoam containers and faster emergence in polypropylene containers (F = 640.13, $P < 0.01$). However, the container type x substrate interaction revealed a more complex pattern. While the combination of polypropylene and substrate 3 delayed emergence ($\bar{x}$ = 82 days; Table 4), the same container type with substrate 1 led to rapid emergence ($\bar{x}$ = 20 days; F = 419.87, $P >$ 0.01; Table 4). These findings suggest that polypropylene containers promote faster emergence in both *E. ebano* and *H. pallens* when paired with suitable substrates.

Table 3 – Emergence and survival results of *Havardia pallens* seedlings under different substrates and containers

T	EP (%)	T75 (days)	ESI	SP (%)
1	40 (±8.64) abc	14.75 (±1.50) cd	0.49 (± 0.16) a	56.77 (±5.17) a
2	39(±10.51) abc	16.50 (±2.38) bcd	0.47 (±0.25) a	57.61 (±8.87) a
3	23 (±10.00) c	38.50 (±2.38) a	0.27 (±0.11) a	29.26 (±28.46) a
4	43 (±8.86) ab	17.75 (±2.75) bc	0.51 (±0.29) a	53.04 (±3.96) a
5	40(±10.32) abc	17.00 (±1.82) bc	0.49 (± 0.18) a	58.05 (±5.91) a
6	40(±10.32) bc	18.75 (±2.50) bc	0.40 (±0.09) a	45.06 (±12.48) a
7	39 (±5.03) b	13.25 (±1.70) ce	0.47 (± 0.19) a	66.76 (±10.84) a
8	53 (±6.00) a	12.50 (±1.29) e	0.62 (±0.18) a	66.07 (±6.90) a
9	31 (±8.24) bc	19.25 (±1.25) bc	0.36 (± 0.20) a	54.37 (±22.58) a

Source: Authors (2024)

In where: T= treatment, EP= emergence percentage, T75 = time (in days) to 75% seedling emergence, ESI=emergence speed index, SP = survival percentage. Values in parentheses represent standard deviation. Different letters indicate significant differences according to Tukey's test.

Table 4 – Emergence and survival results of *Ebenopsis ebano* seedlings under different substrates and containers

T	EP (%)	T75 (days)	ESI	SP (%)
1	23 (± 8.86) b	47.25 (±2.21) c	0.30 (±0.22) a	83.33 (±33.33) a
2	19 (± 9.45) b	73.75 (±2.62) b	0.22 (±0.22) a	95.00 (±10) a
3	18 (± 5.16) b	72.00 (±2.16) b	0.23 (±0.12) a	100 (±0) a
4	22 (± 10.06) b	70.50 (±1.29) b	0.26 (± 0.15) a	100 (±0) a
5	15 (± 10.51) b	69.25 (±1.70) b	0.22 (±0.08) a	100 (±0) a
6	20 (± 14.96) b	72.50 (±1.29) b	0.26 (±0.21) a	100 (±0) a
7	45 (± 8.86) a	20.00 (±0.81) e	0.54 (±0.16) a	100 (±0) a
8	29 (± 11.94) ab	39.00 (±0.81) d	0.36 (±0.22) a	100 (±0) a
9	18 (± 11.54) b	82.50 (±1.00) a	0.21 (±0.13) a	100 (±0) a

Source: Authors (2024)

In where: T= treatment, EP= emergence percentage, T75 = time (in days) to 75% seedling emergence, ESI=emergence speed index, SP = survival percentage. Values in parentheses represent standard deviation. Different letters indicate significant differences according to Tukey's test.

No significant differences in survival were observed for *N. laevigata* across substrates (F = 1.08, P = 0.35) or container types (F = 0.55, P = 0.58). However, a significant substrate x container interaction was detected (F = 4.83, P < 0.01), suggesting that specific combinations influence seedling survival. Notably, the combination of substrate 1 with polyethylene bags (treatment 1) exhibited the lowest survival (74%), whereas pairing the same container with substrate 2 (treatment 2), increased survival to 93% (Table 2). The larger volume of polyethylene bags (400 ml) may have allowed more space for root development. This is consistent with findings for other forest species, where greater container volume is associated with improved seedling quality, growth and survival (Dominguez-Lerena *et al.,* 2006; Escobar-Alonso and Rodríguez, 2019). These results suggest that the negative effects typically linked to polyethylene bags can be mitigated by selecting appropriate substrates.

In *H. pallens*, survival was significantly higher in substrates 1 and 2 (F = 5.73, P < 0.01), indicating a positive effect of these mixtures, while substrate 3 had a negative impact, consistent with patterns observed for other variables. In treatments 3, 6, and 9, where substrate 3 (100% soil) was used, survival rates were lower across all containers

(Table 3). Negative effects are likely due to compaction and pathogen risks associated with soil. Additionally, survival was higher in polypropylene containers (F = 3.36, *P* = 0.04), as observed in treatments 7, 8, and 9 (Table 3). This contrasts with *N. Laevigata* and *E. Ebano*, which showed no significant differences in survival across container types. No significant interaction effect was found between container and substrate (F = 0.67, *P* = 0.61).

For *E. ebano*, survival did not differ significantly across substrates (F = 0.75, *P* = 0.49), container types (F = 1.55, *P* = 0.23), or their interaction (F = 0.72, *P* = 0.58). It should be noted that *E. ebano* seeds were the largest among the species studied, a factor often associated with better seedling survival in early stages (Moles and Westoby, 2004), since seedlings rely more on stored reserves than environmental conditions. Furthermore, the species' delayed emergence may have minimized its exposure to treatment-related variability. A longer-term study is recommended to more accurately assess its survival, given its slower use of substrate resources.

The results highlight the importance of considering both container and substrate characteristics to optimize seedling production in nurseries. Factors such as volume and material, along with substrate composition, significantly affect emergence and survival. Moreover, the interaction between these elements is crucial in some cases. Since no single combination was optimal for all species, nursery practices should be tailored to the specific developmental needs of each species to enhance early-stage performance and long-term establishment.

4 CONCLUSIONS

Substrate 3 (100% soil) negatively affected all evaluated parameters, particularly when combined with polyethylene bags.

For *N. laevigata*, the highest emergence and survival occurred in treatment 2, which used polyethylene bags with substrate 2 (70% peat moss, 20% perlite, and 10% vermiculite).

In *H. pallens*, optimal emergence percentage, emergence time, and survival results were achieved with substrates 1 (50% soil, 20% perlite, 20% peat moss, and 10% vermiculite) and 2. Seedlings in polypropylene containers showed higher survival regardless of substrate. A negative effect was observed in treatment 3 (polyethylene + substrate 3), especially in terms of emergence time and survival.

E. ebano exhibited faster and higher emergence in substrate 1 and polypropylene containers, evaluated independently. Treatment 7, where these two factors were combined, produced the fastest emergence. However, survival did not vary significantly across treatments.

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