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Biodegradable containers affect the morphological and nutritional aspects of *Eucalyptus urophylla* seedlings

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ABSTRACT: Forest seedlings production generally uses polypropylene plastic tubes due to their advantages, as root protection and easy operation. However, these tubes have limitations, as small substrate accommodates capacity, there is a possibility of root deformation, and there is a composition of pollutant and non–renewable material. It is necessary to find environmentally correct alternatives. This study aimed to evaluate the morphological and nutritional aspects of *Eucalyptus urophylla* seedlings produced in biodegradable containers, compared to plastic polypropylene tubes. For this, seedlings were produced in different containers/treatments (plastic tube, pig and poultry litter, and coconut fiber) in a greenhouse for 75 days. The analysis included height, collar diameter, length, and number of principal root ramification, leaves, stem and roots dry matter, number of leaves, leaf area, chlorophyll *a* and *b*, and N, P, K, Ca, and Mg concentration in leaves. Experiment design was conducted completely randomized with 24 replications, with each plant a sampling unit. Dickson quality index (DQI) and relationships between dry matter fractions and height and diameter were calculated. *E. urophylla* seedlings production in biodegradable containers, especially coconut fiber, promoted greater growth compared to plastic tubes, especially larger leaves and stem dry mass, and shoot and roots dry mass ratio. This favored root development and leaf area, increasing nutrient absorption and photosynthetic efficiency. Despite seedlings in plastic tubes having greater chlorophyll, N, and Mg concentrations, the biodegradable containers provided adequate P, K, and Ca concentrations, which are essential for development. A positive correlation between collar diameter, dry mass, and leaf area with DQI confirms that biodegradable containers are an effective alternative for producing *Eucalyptus* spp. seedlings.

Keywords: agro–industrial waste, animal litter, coconut fiber, nursery eucalypt, plastic tubes.

INTRODUCTION

Planted forests cover 131 million hectares worldwide, representing 7 % of the global forest area (FAO, 2020). However, commercial forest plantations only supply <40 % of worldwide demand for wood, elucidating the growth potential of the forestry sector (Paquette and Messier, 2010; Kulmann et al., 2022a). *Eucalyptus* genus is the most important and representative because it is fast–growing, adequate wood properties and is highly adaptable to different edaphoclimatic conditions (Flores et al., 2016), covering around 20 million hectares worldwide, distributed in more than 100 countries (Booth, 2013; Myburg et al., 2014; Elli et al., 2019). Brazil is a major world producer of *Eucalyptus* spp., with around 9.94 million hectares planted (IBÁ, 2023), and reaching an average productivity of 38.9 m^3 ha^{-1} yr^{-1}, which is considered the largest of the genus in the entire world (Binkley et al., 2017; IBÁ, 2021).

A fundamental factor for the success of high productivity of *Eucalyptus* spp. planted forests is the production of high–quality seedlings (Prates et al., 2012; Ehlers and Arruda, 2014; Silva et al., 2022), because this directly affects survival and initial development of plants, impacting on plantation uniformity (Ehlers and Arruda, 2014; Wolschick et al., 2016; Silva et al., 2022). Heterogeneity of forest plantations harms the homogeneous distribution of natural resources, such as solar radiation capture, access to water, and nutrients available in soil (Aspinwall et al., 2011). Studies show that heterogeneous plantations can reduce wood volume productivity between 5 and 20 % in *Eucalyptus* spp. plantations (Stape et al., 2010).

The rapid growth of the forestry sector in Brazil (Abraf, 2010; IBÁ, 2023) caused forestry companies to choose seedlings production in reusable containers, as polypropylene plastic containers (Lopes et al., 2014), which is the material most used in forest nurseries currently (Freitas et al., 2017). The advantage of polypropylene containers is their striated internal structure, which protects and directs root growth, minimizing the entanglement commonly observed in plastic bag seedling production system, providing a longer planting period, with greater survival rates and initial growth (Flores et al., 2011; Menegatti et al., 2017). However, plastic containers accommodate a low substrate volume and have a small rigid structure and dimensions, which commonly cause root deformations, such as bending and strangulation, restricting the seedlings development in the field (Flores et al., 2011; Freitas et al., 2013). Moreover, polypropylene plastic containers are produced from propylene (C_3H_6), unsaturated hydrocarbon, by processing liquefied petroleum gas (LPG) or petroleum refining by–product, i.e., a non–renewable fossil raw material (Phung et al., 2021). Thus, polypropylene containers become pollutant materials at lifespan end, requiring >100 years to degrade (Flores et al., 2011). Therefore, it is necessary to propose other materials for container manufacturing, such as biodegradable materials from plants, animals, and/or agro–industrial waste (e.g., poultry and pig litter, and coconut fiber). Biodegradable containers can be transported to field and discarded, without noxious impact to environment, as well as serving as a nutrients source for seedlings, providing the penetration roots in the porous walls, guaranteeing the root system integrity, and reducing remain time spent in the nursery, increasing production capacity (Flores et al., 2011; Menegatti et al., 2017).

Several studies have reported positive responses to production forest seedlings using plant and/or animal waste as a nutrient source, as a total or partial substitute for mineral fertilizers (Flores et al., 2011; Camara et al., 2020; Mendonça et al., 2021; Griebeler et al., 2023). Partial addition of poultry litter to commercial substrate increased by 166 % the height of *E. dunni* (Menegatti et al., 2017) and doubled the height of *Mimosa setosa* seedlings (Faria et al., 2016). In addition, the pig wastewater compost use associated with commercial substrate use contributed to the emergence, quality, and growth of *E. benthamii* (Da Ros et al., 2018). In another study, the organic compost from agro–industrial waste resulted in an alternative potential to produce *E. grandis* seedlings (Silva et al., 2014). Conversely, organic composts can be contaminated by pathogens, invasive plant seeds, and chemical residues, damaging the development of seedlings (Liu et al., 2022). Studies report that sterilization, such as heat, chemical and solarization treatment, is crucial for the seedlings production with organic residues due to the reduction or elimination of the contaminants action (Isaka et al., 2021; Luo et al., 2022). Positive responses to organic waste use in partial or total composition of substrates are due to an increase and fractionation of nutrient availability and associated microorganisms growth, directly affecting the production and seedlings quality (Faria et al., 2016). However, the use of waste from plants and/or animals as a raw material source of containers for seedling production is incipient in the literature.

Biodegradable containers have become a trend in the quest to produce quality forest seedlings (Flores et al., 2011). Seedling quality is usually determined by morphological and/or physiological measurements. Physiological measurements are generally expensive, with the germination rate index being the most used parameter. Thus, morphological measurements, such as height, collar diameter, height and collar diameter ratio, shoot and root dry matter, and Dickson quality index (DQI), are more commonly used worldwide to determine quality forest seedlings, due to the measurement facility (Menegatti et al., 2017).

Several studies on the use of plant and/or animal waste as substitute for mineral fertilizers and/or commercial substrate have reported positive responses in growth, morphological, nutritional, and quality seedling production in *Eucalyptus* spp. (Silva et al., 2014; Menegatti et al., 2017; Da Ros et al., 2018; Camara et al., 2020) and native Brazilian species (Faria et al., 2016; Mendonça et al., 2021; Griebeler et al., 2023). However, few studies have investigated the direct replacement of the container for *Eucalyptus* spp. seedlings production and their responses in growth, morphological, nutritional, and seedling quality parameters.

We hypothesized that substituting plastic tubes for biodegradable containers produced from organic waste can favor root growth, increasing nutrient access and absorption, incrementing foliar nutrition, contributing to growth in height and collar diameter, and shoot and root dry matter, resulting in enhanced quality *E. urophylla* seedlings. Thus, this study aimed to evaluate the morphological and nutritional aspects of *E. urophylla* seedlings produced in biodegradable containers, compared to plastic polypropylene tubes.

MATERIALS AND METHODS

Study site

The study was carried out from March to June 2019 in a greenhouse, located in the Soils Department of the Agronomy Institute of the Federal Rural University of Rio de Janeiro (UFRRJ), in Seropédica, Rio de Janeiro state, southeast Brazil (22° 45’ 32” S and 43° 41’ 50” W, 33 m altitude). According to Köppen classification system, the predominant climate region is tropical with a dry winter (Aw), characterized by rainy season in summer and mean temperature of coldest month above 18 °C (Alvares et al., 2013). Mean accumulated annual rainfall is 1,294 mm yr^{-1} (Oliveira Júnior et al., 2014) and mean temperature of 23.9 °C (Carvalho et al., 2011). During the experiment, the greenhouse was maintained at a mean air temperature of 30 ± 2 °C and relative humidity of 60 %.

Experimental design and treatments

Experiment design was conducted in a completely randomized design with 24 replications. Each container type was considered a treatment, and each plant a sampling unit. Evaluations were concentrated on the central plants, with a double row border. Treatments consisted of four different container types for seedling production: plastic polypropylene tubes; and biodegradable containers from poultry, pig, and coconut fiber (Supplementary

Material 1). Organic waste containers were produced and available by Toco Engenharia company (http://biotoco.com.br) and are commercially designated Biotoco®, with a registered patent in Brazil at Instituto Nacional da Propriedade Industrial (INPI) No. BR 102020013631–3.

Containers presented 10 ± 0.5 mm wall thickness and a standardized volume of 90 cm^3 (Supplementary Material 1). The characteristics of the containers enable the irrigation water to infiltrate and the roots to penetrate their lateral and inferior walls, favoring the natural development of the root system architecture (Supplementary Material 2). Previously to seedlings production, biodegradable containers samples were nutritionally analyzed for N, P, K, Ca, Mg, Al and Na content, according to Tedesco et al. (1995). More details of nutritional results can be found in Supplementary Material 3.

Seedling production

The species used was *Eucalyptus urophylla* S.T. Blake because it is a world most economically important tree, planted as pure species and hybrid combinations, presenting resistance to diseases, as canker (*Cryphonectria cubensis*) and blight (*Austropuccinia psidii*), drought tolerance, high wood density, and rooting rate (Silva et al., 2018). Seeds were sourced from seed production area, collected, processed, and donated by the seed sector of Instituto de Pesquisas e Estudos Florestais (IPEF – http://www.ipef.br).

Commercial substrate used to seedlings produce was Mecplant florestal, composed of bio–bio-stabilized pine bark and vermiculite, commonly used in forest seedlings production. Nutritional composition of substrate was: 1.8, 4.2, 6.3, 1.7, 3.1, and 8.6 g kg^{-1} for N, P, K, Ca, Mg, and Al, respectively. Commercial substrate was homogenized with mineral fertilizers and then placed in containers. Sowing was carried out directly in containers, using three seeds per container. Then, containers in trays were transferred to the greenhouse, where they received micro–sprinkler irrigation (8 mm day^{-1}). Thinning occurred after 30 days, and the most vigorous seedling was maintained.

Seedlings were fertilized in two periods: (i) base and (ii) top–dressing, according to recommendations proposed by Gonçalves et al. (2015). Base fertilization was carried out on quantities, sources, and nutritional concentration: 15, 33.3, 3.3, and 3 g of ammonium sulphate (20 % N and 24 % S), simple superphosphate (18, 16, and 10 % of P_2O_5, Ca, and S, respectively), potassium chloride (58 % K_2O), and FTE (9, 1.8, 0.8, 2.0, 3.5 and 0.1 % of Z, B, Cu, Mn, Fe and Mo, respectively), respectively. Top–dressing fertilization was carried out 30 days after thinning, on quantities, sources, and nutritional concentration: 10 g ammonium sulphate (20 % N and 24 % S) and 2.6 g potassium chloride (58 % K_2O), dissolved in 1 L of distilled water. The application was carried out via aqueous solution, using graduated syringes (10 mL per container). The frequency of top–dressing was 15 and 30 days for N and K, respectively.

Morphological evaluation

Growth measurements were carried out periodically in 15 days until the experimental period end at 75 days–old. Plant height (cm) was measured using a graduated ruler (Digimess, 600.004, Brazil) and stem diameter (mm) using a digital caliper (MTX, 316.119, Brazil), with a precision of 0.01 mm. Moreover, at the end of the experimental period, principal root length (cm) (between collar and root end) was assessed using a graduated ruler (Digimess, 600.004, Brazil).

Photosynthetic pigments and leaf area evaluation

To assess the photosynthetic pigments concentration, chlorophyll *a* and *b* were determined at 75 days–old using a digital chlorophyll meter (Falker, CFL 1030, Brazil), according to recommendations of Traini et al. (1983). After, the total number of per seedling was counted manually. Leaf area per plant was assessed using an automatic meter (Li–Cor, 3600, USA).

Dry mass and quality seedling evaluation

Plants were collected, cut near the substrate, and separated into leaves, stem, and roots at the end of the experiment (75 days–old). Root system was carefully washed under tap water until the substrate was completely removed. Shoot was separated into leaves and stem by completely removing leaves from each plant. Each fraction (leaves, stem, and roots) was placed in paper bags, duly identified, and dried in the oven with forced air circulation (65 ± 1 °C) until constant mass. After, fractions were weighed in a digital balance (Bel Engenharia, Precision balance L, Brazil) with a precision of 0.01 g, to determine dry mass. With these results, the dry mass ratio between shoot and root, leaves and roots, leaves and stem, and stem and roots were calculated. Moreover, the seedling production quality was assessed using DQI proposed by Dickson et al. (1960).

Nutritional composition evaluation

Immediately after drying, the leaves were milled in Willey with a 2 mm mesh size and reserved for chemical analysis of leaf tissue according to Tedesco et al. (1995). Part of the leaf samples was submitted to sulfuric digestion for N determination using a micro–Kjeldahl distiller (TE 0363, Tecnal, Brazil). Another part of the leaf samples was submitted to nitro–perchloric digestion to determine P, K, Ca, and Mg concentrations. Phosphorus was determined using colorimetric method (Murphy and Riley, 1962), by UV–visible spectrophotometer (Model SF325NM, Bel Engineering, Italy); K by flame spectrophotometry (Micronal B 462, Tecnal, Brazil); Ca and Mg by atomic absorption spectrophotometry (EAA; Varian SpectrAA–600, Australia).

Statistical analysis

All data obtained from morphological parameters (height, collar diameter, H:D, length and number principal root ramification), dry matter production (leaves, stem, roots and their ratios), seedling quality (DQI), nutritional (N, P, K, Ca, and Mg in leaves), and photosynthetic pigments (chlorophyll *a* and *b*) were submitted to analysis of variance (ANOVA). The normality of residuals and homogeneity of variance were tested using Shapiro–Wilk and Bartlett tests, respectively. The results were considered statistically significant when $p<0.05$. Means were compared using Tukey test ($p<0.05$). ANOVA and Tukey test were carried out using the "ExpDes.pt" R software package (Ferreira et al., 2022). To check for correlation effects between response variables and distribution of treatments, the data were submitted to Pearson correlation using "corrplot" (Wei et al., 2017) in R software.

RESULTS

Morphological response

Morphological growth of seedlings was affected significantly by container type (Figures 1a and 1b; Supplementary Material 4). During the study, a significant difference was observed at 30 days–old for height (Figure 1a), while the collar diameter at 30–75 days–old (Figure 1b). The coconut fiber container provided a 12 % increase in collar diameter values at 75 days–old seedlings compared to plastic tube production. Poultry and pig litter increased the collar diameter an average of 4 % at 75 days–old, compared to plastic tube. The greatest values for height and collar diameter ratio (H:D) were visualized in seedlings grown in plastic tubes (Table 1).

Container types significantly affected the morphological root growth of seedlings (Figures 2a and 2b). Plastic tube provided a 2 cm increase in principal root length (Figure 2a). In contrast, the pig litter promoted the greatest number of principal root ramification of seedlings compared to the plastic tube (Figure 2b). Biodegradable containers (pig litter, poultry litter, and coconut fiber) provided better conditions for root penetration and seedling container occupancy than plastic tube.

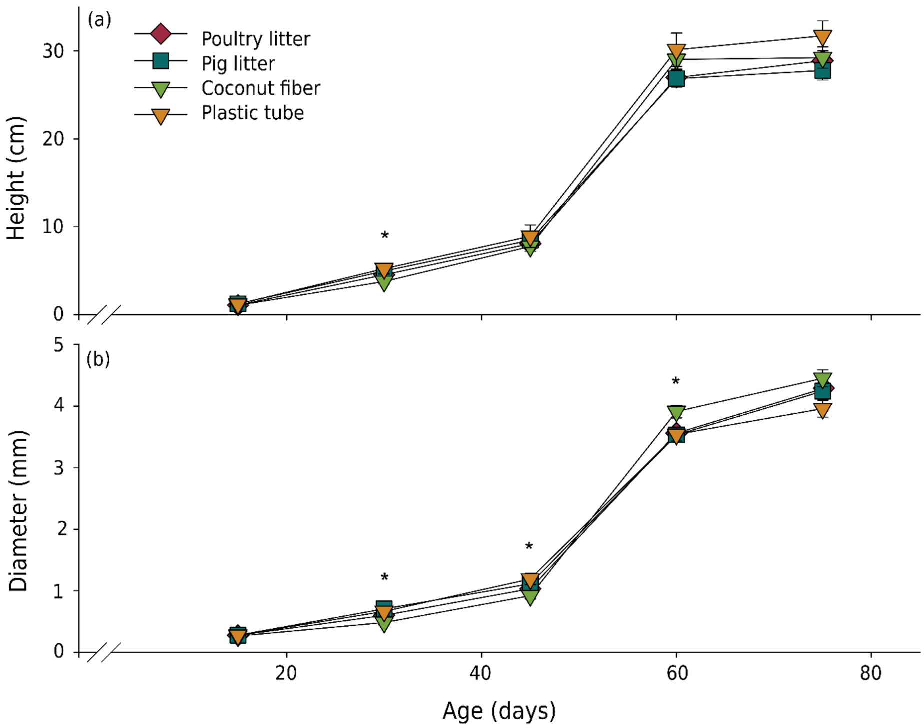

Figure 1. Height (a) and collar diameter (b), growth of *Eucalyptus urophylla* seedlings under different types of containers. The vertical bars indicate the standard deviation, and the asterisk indicates a significant difference between different types of containers by the LSD test (p<0.05).

Table 1. Relationships between growth variables of *Eucalyptus urophylla* seedlings, 75 days after germination, under different types of containers

Treatment	H:D	L:S DM	S:R DM	DQI
Poultry litter	6.75 ± 0.91 b[(1)]	3.52 ± 0.83 a	1.30 ± 0.50 a	0.37 ± 0.07 a
Pig litter	6.63 ± 1.35 b	3.06 ± 0.69 a	1.35 ± 0.27 a	0.39 ± 0.12 a
Coconut fiber	6.70 ± 1.57 b	3.42 ± 2.85 a	1.60 ± 0.53 a	0.44 ± 0.09 a
Plastic tube	8.07 ± 1.56 a	2.32 ± 0.57 a	1.16 ± 0.20 a	0.38 ± 0.11 a

[(1)] Values represente mean ± standard deviation (n = 24). Different lowercase letters indicate significant differences between fertilizations by Tukey test (p<0.05). H:D: height and collar diameter ratio; L:S DM: leaves and stem dry matter ratio; S:R DM: stem and roots dry matter ratio; DQI: Dickson quality index.

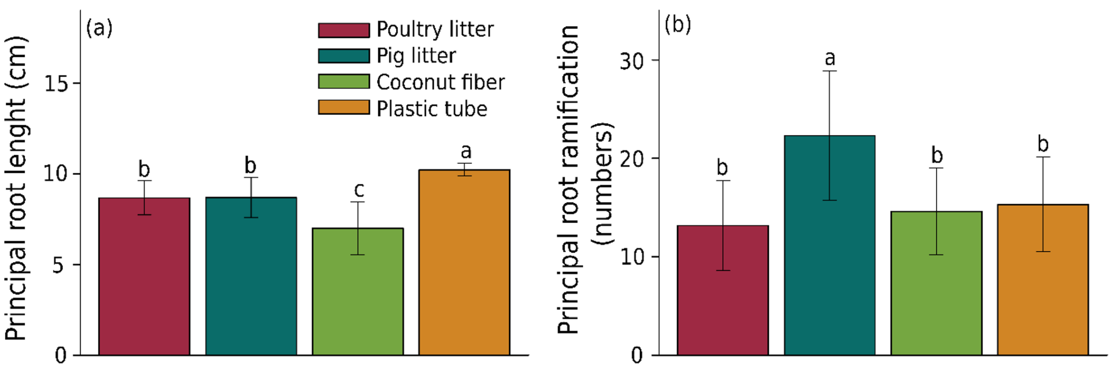

Figure 2. Principal root length (a) and principal root ramification (b) of *Eucalyptus urophylla* seedlings, 75 days after germination, under different types of containers (plastic tube (C.1), pig litter (C.2), poultry litter (C.3), and coconut fiber (C.4)). Different lowercase letters indicate significant differences between containers by Tukey test (p<0.05). Vertical bars represent the standard deviation.

Dry mass and quality of the seedlin

Container types significantly affected the leaves, stem, and shoot dry mass, and shoot:root, leaves:root dry mass ratio of seedlings (Figures 3a, 3b, 3c, 3e and 3f). Coconut fiber container increased 47, 24, and 40 % in leaves, stem, and shoot dry mass production, respectively, compared to the plastic tube (Figures 3a, 3b and 3c). Biodegradable containers (pig litter, poultry litter, and coconut fiber) promoted an increase of 52 and 64 % in shoot:root and leaves:root dry mass ratio of seedlings, respectively, compared to the plastic tube (Figures 3e and 3f). Container types did not significantly influence the roots dry mass production (Figure 3d) and leaves:stem and stem:roots dry matter ratio, and Dickson quality index (Table 1).

Photosynthetic pigments and leaf area response

Container types significantly affected the photosynthetic pigments and leaf area of seedlings (Figures 4a, 4b and 4d). Plastic tube provided the greatest chlorophyll *a* (27.8) and *b* (5.5) values, followed by the coconut fiber container (Figures 4a and 4b). Although non–significant (p>0.05), the number of leaves was 35 % larger in the coconut fiber container than in the plastic tube container (Figure 4c). The greatest leaf area values were found in seedlings grown in coconut fiber container, causing an increase of 74 cm^2 $plant^{-1}$ of seedlings compared to plastic tube (Figure 4d).

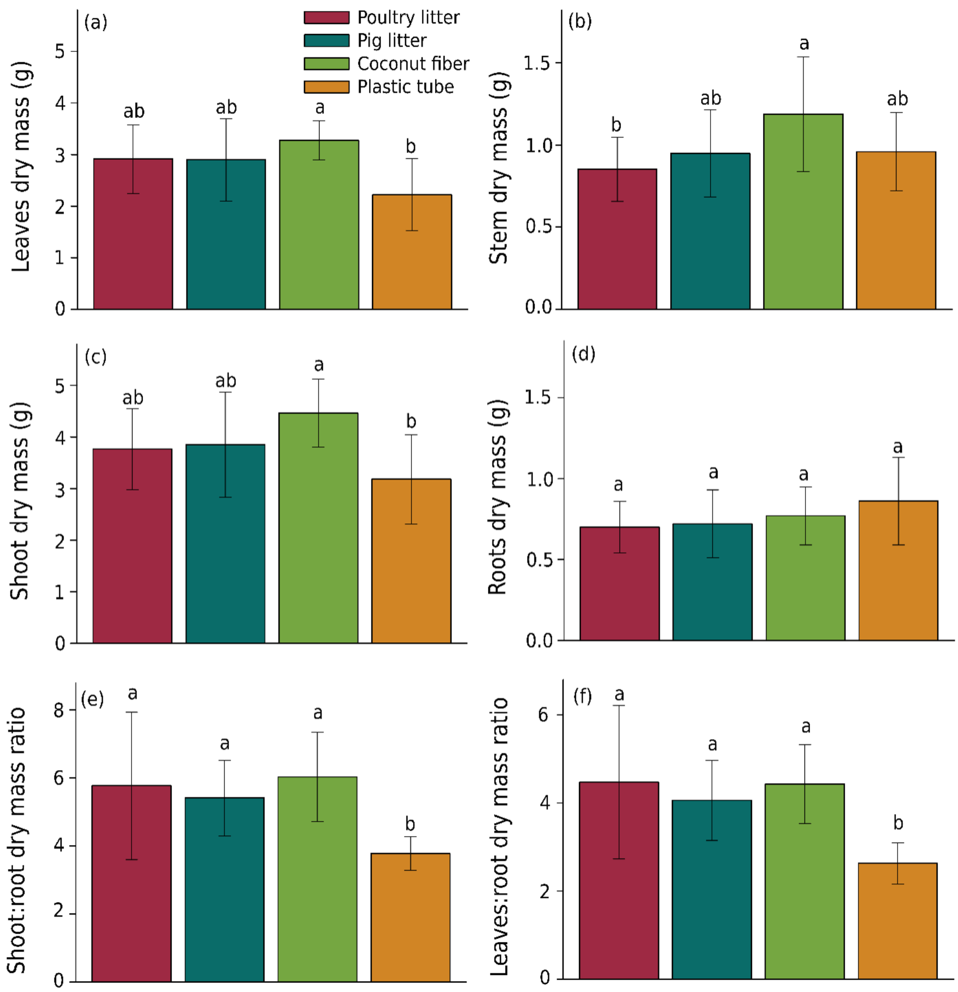

Figure 3. Leaves (a), stem (b), shoot (c), roots (d) dry mass, shoot: root (e), and leaves: root dry mass ratio (f) of *Eucalyptus urophylla* seedlings, 75 days after germination, under different types of containers. Different lowercase letters indicate significant differences between containers by Tukey test (p<0.05). Vertical bars represent the standard deviation.

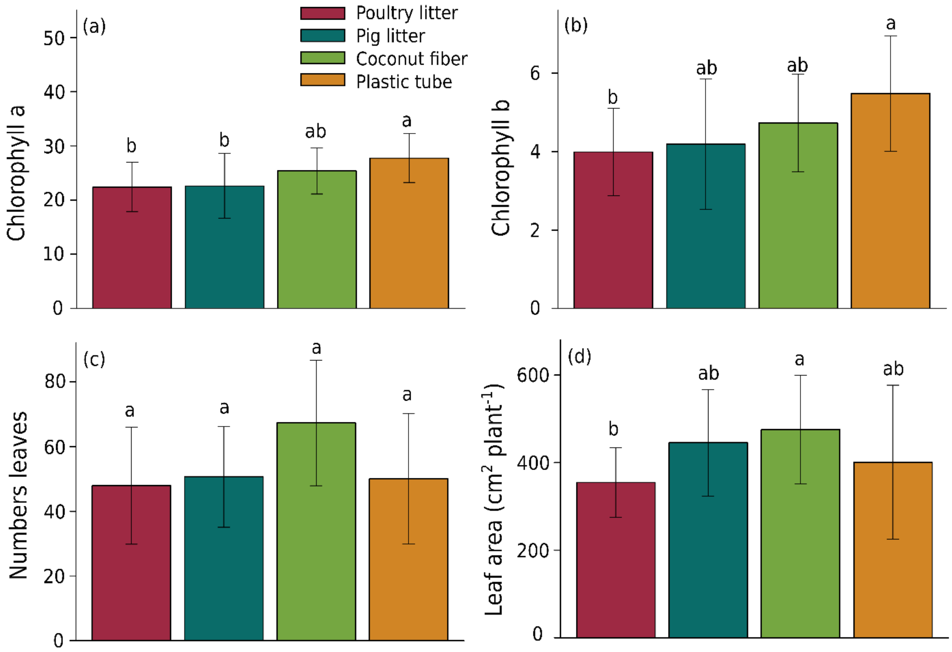

Figure 4. Chlorophyll *a* (a), chlorophyll *b* (b), number leaves (c), and leaf area (d) of *Eucalyptus urophylla* seedlings, 75 days after germination, under different types of containers. Different lowercase letters indicate significant differences between containers by Tukey test ($p<0.05$). Vertical bars represent the standard deviation.

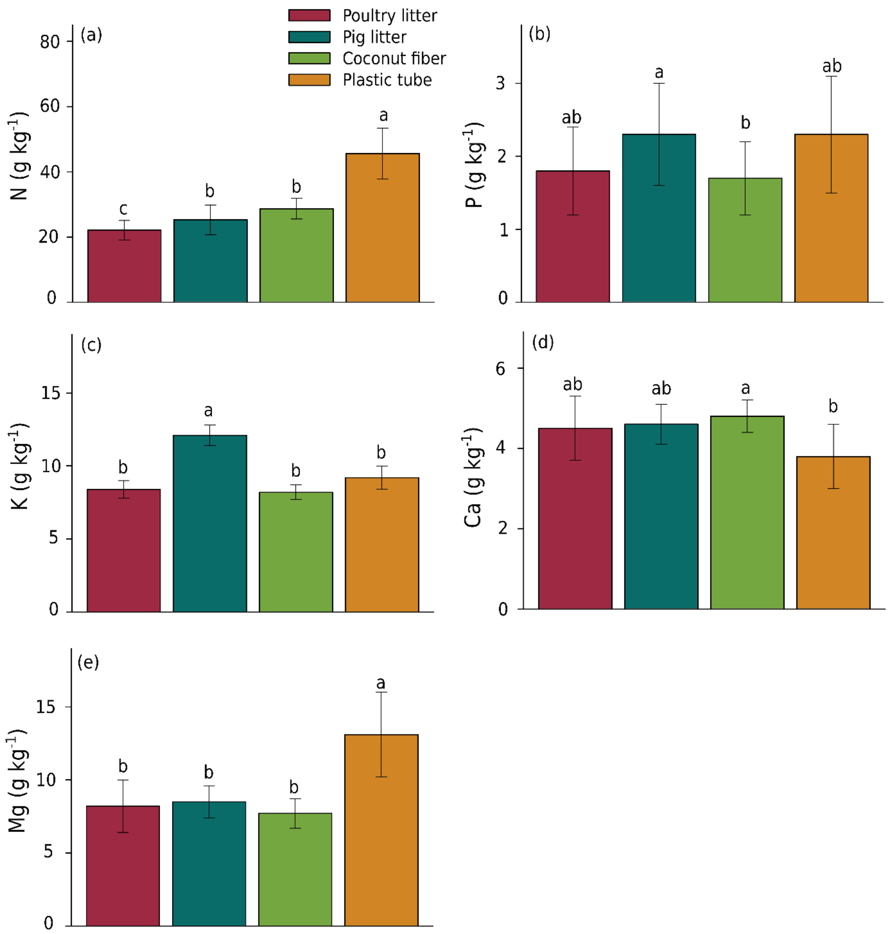

Figure 5. Nitrogen (a), phosphorus (b), potassium (c), calcium (d), and magnesium (e) concentration in leaves of *Eucalyptus urophylla* seedlings, 75 days after germination, under different types of containers. Different lowercase letters indicate significant differences between containers by the Tukey test ($p<0.05$). Vertical bars represent the standard deviation.

Nutritional composition response

Container types significantly influenced the nutrient concentration in the seedling leaves (Figures 5a, 5b, 5c, 5d, 5e and 5f). Plastic tube provided the greatest N and Mg concentrations (Figures 5a and 5e). However, seedlings produced in the pig litter container promoted the greatest P and K concentrations (Figures 5b and 5c). Coconut fiber container provided the largest Ca concentrations in leaves (Figure 5d).

Pearson correlation response

Pearson correlation showed that growth parameters, such as collar diameter, were positively correlated with DQI, leaves, shoots, and roots dry mass and leaf area, while height was positively correlated only with leaf area (Figure 6). Moreover, seedling production quality parameter (DQI) showed a positive correlation of 0.66, 0.7, 0.72, 0.81, 048, and 0.5 with collar diameter, leaves, shoot, and roots dry mass, number of leaves, and leaf area, respectively.

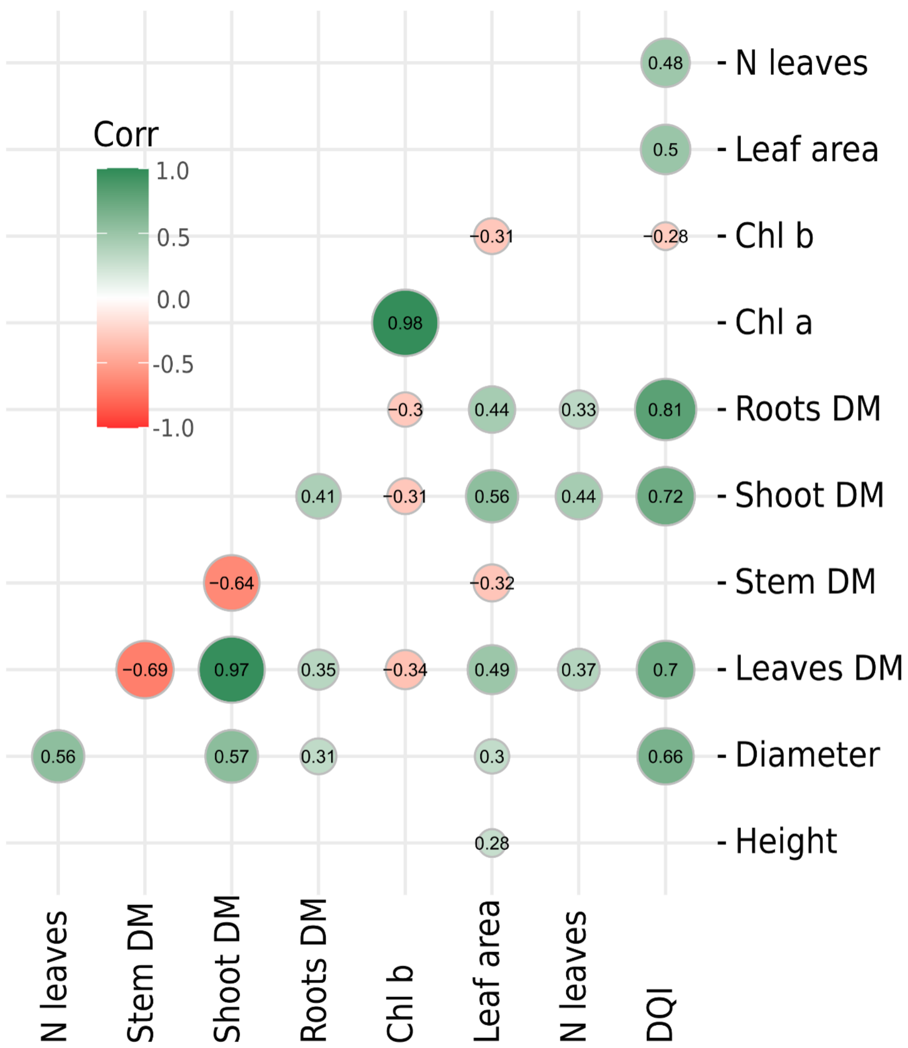

Figure 6. Pearson correlation between growth variables of *Eucalyptus urophylla* seedlings, 75 days after germination, under different types of containers. Apparent circles were considered significant (p<0.05). Non–significant correlations (p>0.05) have been hidden. Size of the circle represents the level of correlation between the variables. Greenish indicates a positive relationship, and reddish indicates a negative one.

DISCUSSION

Morphological response

Coconut fiber container provided the greatest morphological growth at 75 days–old compared to the plastic tube. This can be explained by root system growth, because the biodegradable containers (pig litter, poultry litter, and coconut fiber) provided larger conditions for seedlings to occupy the internal space and penetrate the container walls than plastic tube. This may have occurred as a plant mechanism to increase the contact area between roots and substrate. An increase in root system is favorable for plant growth, as the root is a reserve organ for carbohydrates, amino acids and other nutrients (Bachega et al., 2016). As result, plants become less dependent on nutrients derived from mineral fertilizers (Kulmann et al., 2020). Moreover, part of the nutrients accumulated in the root system can be redistributed to the growing organs, such as leaves, assisting *Eucalyptus* spp. seedlings to resist conditions of low nutrient availability in the substrate (Bachega et al., 2016; Kulmann et al., 2022a).

Height and collar diameter ratio (H:D) of biodegradable containers (6.63–6.75) was lower than that of the plastic tube container (8.07), which is within the appropriate range of H:D ratio (5.4–8.1) for *Eucalyptus* spp. seedlings (Carneiro, 1995). This shows that biodegradable containers can be an alternative to producing *Eucalyptus* spp. seedlings, because H:D ratio is a parameter used to estimate seedling growth, because when a lower ratio is observed, greater is the survival and development of seedlings in the field (Carneiro, 1995; Berghetti et al., 2016). It is important to emphasize that H:D ratio should be used in combination with other parameters to determine the seedling quality.

Dry mass and quality seedling response

The biodegradable containers (pig litter, poultry litter, and coconut fiber) promoted an increase in shoot dry matter production (leaves and stem), and also increased in 52 and 64 % in shoot:root and leaves:root dry mass ratio of seedlings, respectively, compared to plastic tube (Figure 3). According to Gomes and Paiva (2006), the shoot:root dry mass ratio is an efficient and secure indicator for assessing the seedling quality; the greater its value is, the greater is the seedlings survival capacity, evidenced by greater values promoted by biodegradable containers for *Eucalyptus* spp. seedlings production. Moreover, coconut fiber container increased the leaves, stem and shoot dry mass productions by 47, 24 and 40 %, respectively, compared to the plastic tube. The greater leaf dry matter production is considered an indication that plants had a larger leaf area, and as a result the sunlight interception is enhanced, increasing photochemical processes, C fixation and photoassimilates content (Marschner, 2012; Lee et al., 2016), contributing to increase in total dry matter, as observed in this study. It is also important to note that the greater leaves dry matter production may increase transpiration, stimulating greater nutrient absorption by roots, potentiating the ions transport in plants (Lee et al., 2016; Aguilar et al., 2024). Coconut fiber container promoted the greatest stem dry mass production, this fraction is considered a nutrient reserve in forest species due to large nutrients accumulation (Viera et al., 2011; Salvador et al., 2016). Thus, the increase in stem dry mass production can be a plant strategy to reserve nutrients for maintenance, especially in organic form (Oliveira et al., 2017), enabling the redistribution of nutrients in subsequent growth period, a phenomenon reported in forest species (Berghetti et al., 2021; Kulmann et al., 2021). Thus, the remobilization and efficiency of plant nutrient use is increased, especially when planted in soils of low natural fertility (Kulmann et al., 2022a). Seedlings produced in biodegradable containers showed greater leaf area and nutrient reserves in their woodier tissues, with the same DQI (Table 1) as the seedlings produced in plastic tubes, indicating that they could be an alternative for producing more interesting *Eucalyptus* spp. seedlings for transplanting to the field.

Photosynthetic pigments and leaf area response

Plastic tube provided the greatest chlorophyll *a* and *b* concentrations, followed by coconut fiber container (Figure 4). This is possibly due to increased N and Mg concentration in leaves, contributing to the formation of greater quantities of photosynthetically active pigments (Teixeira Filho et al., 2011), which increases the absorption, energy transfer and proteins responsible for photosynthesis (Camarero and Carrer, 2017; Tanaka et al., 2018). This occurs because photosynthetic pigments are responsible for absorbing light in different spectrum regions in photosynthesis initial stages. Photochemical phase is completed if sufficient pigments are available to interact with photosynthetic radiation (Taiz et al., 2017). The reduction of chlorophyll *a* and *b* detected in poultry and pig litter containers can be related to seedlings production with lower nutrient absorption efficiency (Fernandes et al., 2016). This is because the decrease in photosynthetic pigments can be related to a decrease of: (i) concentration of nitrogenous base (adenine) in ATP formation; and (ii) phosphorylation, reducing the plant energy production (ATP and NADPH) and thus reducing the sugars synthesis in C fixation reactions (Taiz et al., 2017).

Seedlings produced on coconut fiber showed the greatest number of leaves and leaf area. Seedlings with a larger leaf area absorb more solar radiation and produce more photosynthates. Therefore, they have a greater transfer of signaling molecules to the root system, favoring the translocation of photosynthates from shoot to roots, contributing to increase the root morphological parameters (Figure 2) and greater nutrient absorption (Lee et al., 2016; Xuan et al., 2017; Kulmann et al., 2022a). Increased root system development can favor the root growth in porous walls of biodegradable containers (Figure 3), enabling access to nutrients contained in container composition, which is a rich source of macronutrients, as N, P, Ca and Mg (Supplementary Material 3).

Nutritional composition response

Plastic tube provided the greatest N and Mg concentrations, 45.6 and 13.1 g kg^{-1}, respectively (Figures 5a and 5e). However, the observed leaf N concentrations exceeded the optimal ranges of 25–38 g kg^{-1} (Dell et al., 1995) and 25–35 g kg^{-1} (Higashi et al., 2015). Additionally, Mg concentrations surpassed the reported upper threshold of 3–3.5 g kg^{-1} (Higashi et al., 2015) for plastic tube production. Nitrogen excess can result *E. urophylla* seedlings: (i) excessive vegetative growth, thus plants more fragile susceptible to pests and diseases; and (ii) decreased lignification, resulting in in low–density wood and more susceptible to physical damage; and nutritional imbalance, inducing deficiencies of other nutrients, especially P and K (Graciano et al., 2006; Kulmann et al., 2022a). This may also show that the N and Mg fertilization recommendation for the *Eucalyptus* genus seedling production, proposed by Gonçalves et al. (2015), may be generalized, evidencing its segmentation for each species, such as *E. uropylla*. In addition, biodegradable containers may have adsorbed N and Mg in their structure, making it possible to supply them to seedlings subsequently (Supplementary Material 3).

Conversely, biodegradable containers, especially pig litter and coconut fiber, promoted the greatest P, K, and Ca concentrations. The increase in P concentrations in leaves can have occurred due to increased P availability, potentiating the physiological demand, particularly of more developed plants, because P is considered to plant energy currency (Lambers et al., 2011; Marschner, 2012), promoting greater leaf area production, as observed in this study (Figure 4d). Thus, part of P is used to produce ATP and NADPH in the plant, contributing to an increase in shoot biomass production (Kulmann et al., 2022b), as verified in this study (Figure 3c). The larger P concentrations in leaves of produced plants in the biodegradable containers can favor the initial development of *Eucalyptus* spp. in the field, because this nutrient is key in fine root formation and nutrient absorption (Kulmann et al., 2022a; Aguilar et al., 2024). The greater K concentration in biodegradable containers can improve the rustification of *E. urophylla* seedlings, because K acts in osmotic regulation and thus directly affects transpiration and water absorption

(Gonçalves, 1995; Santos et al., 2021). Moreover, the greater Ca concentration values in coconut fiber container can contribute to cell wall stability, membrane integrity, and cell division of seedlings (Rocha et al., 2019). Due to gains in nutritional aspects, the *Eucalyptus* spp. seedlings produced in biodegradable containers may have characteristics that are more resistant to field planting.

Pearson correlation response

Growth parameter collar diameter showed a positive correlation with DQI, leaves, shoot, and roots dry mass and leaf area, while height correlated positively only with leaf area, according to Pearson's correlation (Figure 6). These results show that the production of high–quality *E. urophylla* seedlings can be measured by evaluating morphological parameters, as leaves, shoot, and roots dry mass and leaf area, and that these parameters contribute to greater plant height development, especially as they reflect increases in photosynthetic processes, with higher photosynthetic pigment concentration values. This also indicates that seedlings with a larger leaf area may be more physiologically efficient, especially by increasing the amount of energy directed to the photochemical stage of photosynthesis (Berghetti et al., 2021; Kulmann et al., 2023; Aguilar et al., 2024). As a result, plants tend to increase their efficiency in converting light energy into chemical energy and, consequently, have greater C assimilation and, therefore, shoot growth (Kulmann et al., 2023; Aguilar et al., 2024), as observed in this study. Moreover, studies that evaluated alternative substrates in *E. dunnii* and *E. benthamii* seedlings production have also found positive relationships between root system growth and seedling quality (Kratz et al., 2013; Mieth et al., 2019). Thus, the evaluation of shoot, especially leaves and root system appearance, can be good indicators of high–quality seedling production.

Organic waste containers can make it possible to produce seedlings in less time, due to root system protection and without the need to remove them for planting. This reduction in production time will make it possible to increase the nursery production capacity without altering the existing infrastructure. There are other potential gains: (i) no need to return the container to nursery (return transport costs); (ii) no need to wash and disinfect containers; (iii) no need to remove the seedling from the container before planting; (iv) less loss of seedlings due to damage to the root system by container removing; and (v) reduced fertilizers during seedling production. It is important to emphasize that this container type, produced from organic waste, has the advantage of plastic substituting and, at the same time, being part of a more sustainable action and in the circular economy context.

CONCLUSION

Production of *Eucalyptus urophylla* seedlings in biodegradable containers, especially coconut fiber, promoted greater growth compared to seedling production in plastic tubes. Seedlings produced in coconut fiber containers showed greater leaf and stem dry mass and a larger shoot:roots dry mass ratio. This pattern may be a response to greater root development and leaf area, which increase nutrient absorption and photosynthetic efficiency.

Seedlings produced in plastic tubes have greater concentrations of chlorophyll and leaf nutrients (N and Mg). In addition, biodegradable containers adequately satisfied the demand for P, K and Ca, essential for seedling development. The positive correlation between collar diameter, dry mass, and leaf area with the DQI confirms that biodegradable containers are a viable alternative to plastic tube containers for producing high-quality *Eucalyptus* spp. seedlings, thereby reducing environmental pollution and optimizing mineral fertilizer use.

SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://www.rbcsjournal.org/wp-content/uploads/articles_xml/1806-9657-rbcs-49-e0240216/1806-9657-rbcs-49-e0240216-suppl01.pdf

DATA AVAILABILITY

The data will be provided upon request.

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