

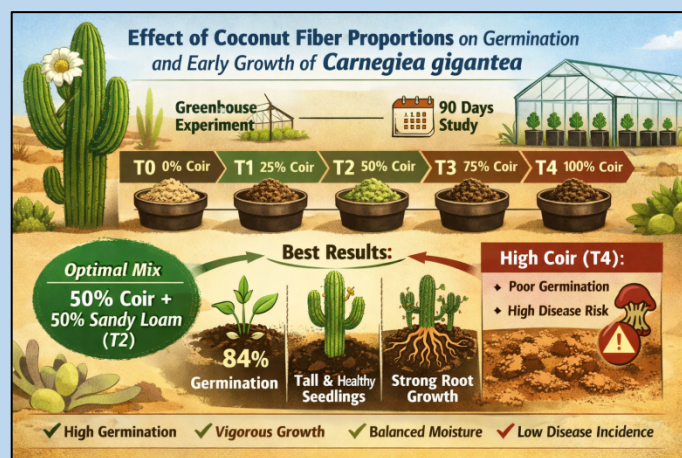
## Effect of Coconut Fiber Proportions in Substrate on Germination and Early Growth of *Carnegieia gigantea*

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**Abstract:** *Carnegieia gigantea* (saguaro cactus) is an indigenous species of the Sonoran Desert that grows at a very slow rate. As it has a vital role in the ecosystem and there is growing interest in cultivating *C. gigantea* as an ornamental environmental plant, optimization of germination and establishment of *C. gigantea* seedlings is very important. The substrate used during the early growth of *C. gigantea* seedlings is very important to provide adequate amounts of water, aeration and root development. The experiment documented here compares the effects of two different coir ratios added to the substrate on seed germination rates and early growth of *C. gigantea* seedlings. Five treatments including T0 (0% coir), T1 (25% coir), T2 (50% coir), T3 (75% coir) and T4 (100% coir), were subjected to seed germination testing on standard sandy loam soil. Seeds were grown under controlled conditions in a greenhouse. Monitoring of the germination percentage, seedling height, root length, and infection levels was done over a period of 90 days. Results indicated that T2 (50% coir) had the best overall performance; T2 had the highest average germination rate (84%) and average seedling height as well as the most root development. T1 (25% coir) also performed much better than the control. T4 (100% coir) performed poorly on account of lack of drainage, excess moisture retention and highest level of fungal infections leading to poor germination (40%) and weak seedlings. It can be concluded that a 50:50 mixture of coconut coir and sandy-loam substrate provides the best balance of moisture retention and aeration needed for successful propagation of *C. gigantea*. When used in the right amounts, coconut fiber is a sustainable and effective substrate material for growing desert cactus.



**Keywords:** seedling development, substrate optimization, water retention, germination rate, sustainable horticulture

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## Introduction

*Carnegiea gigantea* (Engelm.) Britton & Rose, known as the saguaro cactus, is a prominent and ecologically crucial cactus found in North America. It is native to the Sonoran Desert of the southwestern United States and northwestern Mexico and is a keystone species in its environment that benefits many desert-dwelling species. Its height provides nesting cavities for birds, such as the Gila woodpecker (*Melanerpes uropygialis*) and the elf owl (*Micrathene whitneyi*), and its fruits are a seasonal food source for mammals, reptiles, and insects [1, 2]. *C. gigantea* has ecological value as a native species that provides habitat and food for many species, but it is also commercially significant for Indigenous peoples and increasingly cultivated for ornamental gardens and for conservation.

The cultivation of *C. gigantea* is, despite its importance, limited by various biological and environmental factors. The natural seedling germination process is low because of temperature fluctuations, low and inconsistent precipitation, and high predation rates [3]. This species also demonstrates slow germination and long juvenile life history when grown in controlled greenhouse environments; therefore, optimal growing conditions are essential to enhance the early survival and growth of these plants. Substrate composition is considered to be one of the most significant variables impacting successful cactus propagation because it is directly tied to respective success rates on seed germination, seedling establishment, root system development, and/or disease tolerance [4,5].

The typical media used to grow desert plant species will generally mimic the typical dry soil profile of the native environment including a sandy and/or sand-loam texture where there is typically good drainage but less than the average amount of organic matter present. Even though these media may resemble the adult habitat of *C. gigantea*, they generally do not retain enough moisture or continue to hold sufficient moisture when seedlings are small, as seedlings are more prone to drying out and experiencing stress [6]. Organic amendments have been recommended as an approach to increase water-holding capacity, along with aeration and nutrient availability, resulting in better early plant growth.

Of the many categories of organic amendments, coconut fiber (coir) looks like an alternate amendment that is viable, due to its excellent physical and chemical properties. Coir is a waste material of the coconut industry obtained from the mesocarp of *Cocos nucifera*. Coir is renewable, biodegradable, and it has become a more common horticultural substrate as an environmentally friendly alternative to peat moss [7,8]. Coconut coir has high porosity, a moderate water-holding capacity, low bulk density, and a relatively stable structure that resists decomposition. Coconut coir also has low electrical conductivity and relatively neutral pH levels after processing, making it appropriate for many plant species [9].

Multiple studies have shown that substrates with coir improve germination, seedling biomass, and root architecture of several horticultural crops and ornamentals (e.g., tomatoes, chrysanthemums, and ferns) [10,11]. Such improvements have been attributed to increased water availability, improved oxygen movement in the root zone, and a decreased likelihood of compaction. However, the benefits depend on the ratio of coir in the substrate and interaction with the mineral components of the substrate. High ratios of coir can lead to excessive retention of water, anaerobic conditions, and greater incidence of fungal disease in plants from arid environments [12].

Although there is a growing interest in sustainable substrates, there is relatively little research on the use of coir to promote germination and early development of xerophytic

species like *Carnegiea gigantea*. *C. gigantea* represents a deser

t-adapted plant with physiological and morphological adaptations that regulate water use efficiency. Its seedlings probably lack sufficient cuticular development or root depth for drought stress resistance. Consequently, a substrate that provides an optimal balance of moisture supply and drainage and, additionally, adequate aeration is crucial to enable seed germination and establishment.

Despite its many advantages, coconut fiber (coir) also presents certain limitations that must be considered when used as a substrate component [13]. One of the primary concerns is its high water-holding capacity, which, when used in excessive proportions, can lead to poor aeration and oxygen deficiency in the root zone, particularly for xerophytic species adapted to well-drained soils [14]. Additionally, coir may contain residual salts, especially sodium and potassium, if not properly processed, which can negatively affect seed germination and early seedling growth. Nutrient imbalances have also been reported, as coir is relatively low in essential nutrients such as nitrogen and may immobilize calcium and magnesium [15]. Furthermore, under conditions of high moisture, coir-based substrates can promote the development of pathogenic fungi such as *Pythium* and *Phytophthora* [16]. These limitations highlight the importance of optimizing coir proportions and combining it with mineral components to achieve a suitable balance between water retention and aeration [17].

This study investigated how different amounts (0%, 25%, 50%, 75%, and 100% coconut fiber by volume) of coconut fibers in a sandy loam substrate affected the ability of *Cereus giganteus* to germinate and grow in a greenhouse. The main goal of our experiment was to determine which mix of organic and mineral components had the highest potential for producing healthy seedlings. We believe this study provides valuable information about how to propagate cacti and can help both conservation efforts and nurseries propagate this economically and culturally valuable cactus species utilizing renewable and sustainable resources.

## Materials and Methods

### Study Site and Conditions

The research was conducted in a controlled greenhouse facility in Pescia (Tuscany, Italy; latitude 43.54°N, longitude 10.42°E) at the Landscaping Plants and Nursery Research Unit of the Italian Council for Agricultural Research and Economics, under semi-arid climatic conditions. The greenhouse maintained average daytime temperatures of  $28 \pm 2$  °C and nighttime temperatures of  $18 \pm 2$  °C. Relative humidity fluctuated between 45–60% and was not actively controlled, in order to reflect typical commercial nursery conditions; however, we acknowledge that this range may influence seedling establishment and interact with substrate water-holding capacity. To standardize light conditions and ensure consistent photosynthetic input, supplemental illumination was provided using full-spectrum LED lamps delivering an average photosynthetic photon flux density (PPFD) of approximately 200–250  $\mu\text{mol m}^{-2} \text{s}^{-1}$  at canopy level, under a 14-hour photoperiod. Natural ventilation was used to promote air circulation, and irrigation was applied manually.

### Seed Source and Preparation

Mature seeds of *Carnegiea gigantea* were acquired from a recognized botanic facility (the Svalbard Global Seed Vault) that had assured their viability and that they were naturally pollinated. The seeds were surface sterilized with sodium hypochlorite at 10%, for five minutes, and then thoroughly washed three times with sterile distilled water to create uniformity in germination and limit possible microbial inoculation during germination.

### Substrate Composition and Preparation

Substrates were created with different coconut fiber (coir) and sand loam ratios by using coconut fiber as a growing medium for the final wood growth on five different types of substrates. The coir used was produced commercially, washed, and aged 3 months to remove the salts and phenols from coir. The coir had an average pH value of 5.8 - 6.5, average electrical conductivity (EC) of 0.4 - 0.8 dS m<sup>-1</sup>, and carbon to nitrogen (C:N) ration of about 70:1 - 100:1. These values are in line with those of horticultural-grade coir. The sandy loam was sieved with a 2 mm sieve and heated to 80°C for 4 hours in order to sterilize it. The sandy loam was also characterized by having a pH value of 7.2, low organic matter (0.8% by weight) and approximately 70:20:10 proportions of sand:silt:clay. Prior to the use of any components of these five substrates they were all mixed well together to create a uniform product. The five treatments were as follows:

- T0 – 100% sandy-loam soil (control)
- T1 – 25% coconut fiber + 75% sandy-loam
- T2 – 50% coconut fiber + 50% sandy-loam
- T3 – 75% coconut fiber + 25% sandy-loam
- T4 – 100% coconut fiber

All mixtures were thoroughly homogenized before filling into plastic germination trays (32 cells per tray). Each cell had a volume of 70 mL and was pre-wetted to field capacity 24 hours before sowing.

#### Experimental Design

The experiment was arranged in a completely randomized design (CRD) with five treatments and three replicates per treatment, where each replicate consisted of one tray (experimental unit). Each tray initially contained 50 seeds distributed across 32 cells (approximately 1–2 seeds per cell), resulting in a total of 150 seeds per treatment (750 seeds overall) for germination assessment. After germination (7–10 days after sowing), seedlings were thinned to one plant per cell, retaining the most vigorous individual, resulting in 32 seedlings per tray and a total of 96 seedlings per treatment. Thus, while germination percentage was calculated based on the total number of seeds sown (n = 150 per treatment), all subsequent growth measurements (seedling height, root length, and vigor index at 60 and 90 DAS) were based on subsamples taken within each replicate tray. Specifically, ten seedlings per replicate were randomly selected for shoot measurements, and five seedlings per replicate were destructively sampled for root measurements at 90 DAS. Accordingly, the tray was considered the true experimental replicate (n = 3 per treatment) for statistical analysis.

#### Irrigation and Maintenance

Irrigation of all trays was performed uniformly using a fine mist spray to prevent any displacement of seeds. During the first 30 days, watering occurred twice a day, and watering continued once a day after day 30 to provide adequate moisture without causing waterlogging. No fertilizers were used throughout the entire experiment so that only the effects of the substrate would be evaluated. The presence of weeds and signs of fungal infection were observed daily. To minimize the number of seedlings dying from damping-off, a low concentration aqueous suspension of a biological control agent (*Trichoderma harzianum*) was applied uniformly to all treatments when the earliest symptoms were observed, and no rotting occurred. This provided for a consistent level of survival for all plants while preventing a significant difference in the number of interventions between treatments. Disease rates were recorded as a variable of experiment and stored for analysis.

#### Data Collection and Measurements

Data was collected at three-time intervals: 30, 60, and 90 days after sowing (DAS). The following parameters were measured:

- The percentage of germinated seeds at 30 DAS was calculated as the number of germinated seeds divided by the total number of seeds sown, multiplied by one hundred.
- At 60 and 90 DAS, ten seedlings were selected at random for measurement of height, from the base to the apical meristem, and all measurements were completed on all ten seedlings in each replicate.
- On DAS 90, root length was measured on five randomly selected seedlings per replicate. Although root length measurement would have been possible at both DAS 60 and 90, this measurement required destructive sampling, and thereby reducing the density of plants in the replicate and consequently affecting growth on subsequent observations. Hence, root length was measured only at DAS 90.
- Seedling Vigor Index (SVI): Calculated as germination percentage × mean seedling length (shoot + root).
- Substrate Moisture Content (%): Gravimetric method was used to assess water retention by weighing saturated and oven-dried (105°C for 24 h) substrate samples at 15-day intervals.
- Disease Incidence (%): Percentage of seedlings showing visible signs of fungal infection or damping-off symptoms.

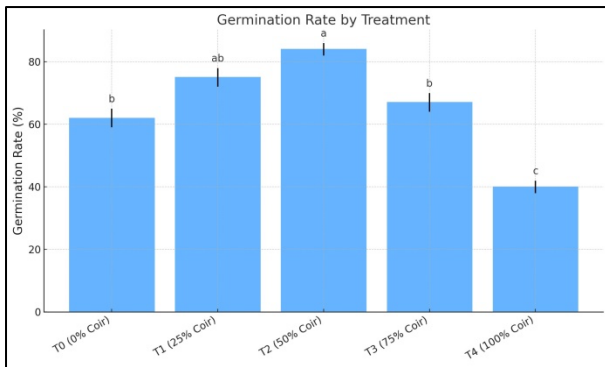
#### Statistical Analysis

The experiment was designed as a randomized complete block design. The data collected were analyzed for significant differences (P ≤ 0.05, 0.01 and 0.001) among treatments using a one-way ANOVA technique using the GLM univariate procedure. Once the analysis proportions were made, means were separated using the LSD multiple range test (P = 0.05). All the statistics and graphics were generated in Costat (version 6.451) and Excel (Office 2010).

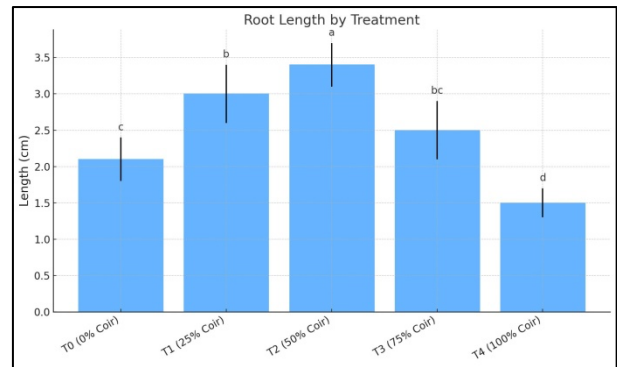
## Results and Discussion

#### Germination Rate

Germination response varied significantly among treatments (p < 0.05), where the highest germination response was in T2 (50% coir), which germinated 84% by 30 DAS (Figure 1), statistically higher than the 62% germination in the T0 control and significantly higher than T4 (100% coir), which had the lowest germination response at 40%. Intermediate levels of coir may improve the water retention and substrate porosity that are crucial during germination. Water availability influences enzyme activation, respiration, and cell elongation, important for radicle emergence [18,19]. On the other hand, excess water, such as in T4, can cause hypoxic levels to occur, thereby initiating decreases in aerobic respiration and inhibiting germination [20]. Coir amended substrates also are documented to have some advantages in the germination response of vegetables and ornamental species such as tomato (*Solanum lycopersicum*) and *Petunia hybrida* [21-23].



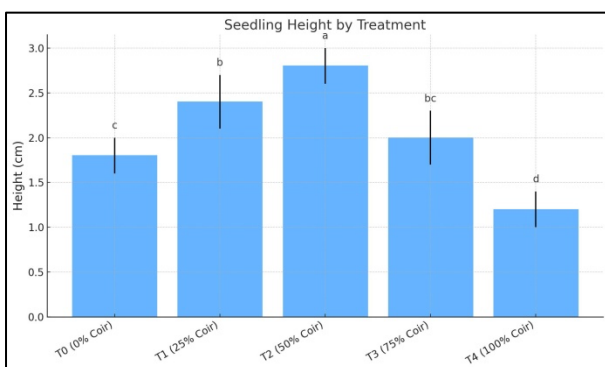
**Figure 1:** Germination rate (%) of *Carnegiea gigantea* seeds under different substrate treatments. Error bars represent standard error. Different letters indicate statistically significant differences ( $p < 0.05$ ).



**Figure 3:** Average root length (cm) of *Carnegiea gigantea* seedlings after 90 days under different substrate treatments. Error bars represent standard error. Different letters above bars indicate statistically significant differences ( $p < 0.05$ ).

### Seedling Height and Root Development

At 90 DAS, *Carnegiea gigantea* seedlings grown in T2 exhibited the highest seedling height and root length (2.8 cm and 3.4 cm, respectively), followed by T1 and the control (T0) (Figures 2 and 3). These results are consistent with the germination data and indicate enhanced early seedling development under moderate coir incorporation. Coconut coir likely improves substrate structure by increasing aeration while maintaining adequate moisture, thereby supporting cell division and elongation, particularly in root tissues [24]. Root elongation is especially important for desert-adapted species such as *C. gigantea*, as it facilitates early establishment and access to deeper moisture reserves [25] (Figure 4). Our results, align with previous studies, report a greater increase in shoot and root biomass in coir-based substrates in species such as lettuce (*Lactuca sativa*) and geranium (*Pelargonium × hortorum*) [26,27]. In contrast, seedlings grown in T4 showed limited root development, likely due to excessive moisture retention and reduced oxygen availability in the root zone, conditions known to impair root function and promote stress [28,29]. The same trends in seedling performance have been observed in succulent and desert shrub studies using high-coir substrates without mineral amendments [30]. Measurements taken at 60 DAS showed trends consistent with those observed at 90 DAS, with T2 (50% coir) exhibiting the highest seedling height, followed by T1 and T0, while T4 showed the lowest values (data not shown).



**Figure 2:** Average seedling height (cm) of *Carnegiea gigantea* after 90 days under different substrate treatments. Error bars represent standard error. Different letters above bars indicate statistically significant differences ( $p < 0.05$ ).



**Figure 4:** Comparison of root development under two substrate treatments: T2 (50% coir) shows denser, more fibrous root growth, while T0 (0% coir) exhibits thinner root distribution, highlighting the influence of coir content on root structure.

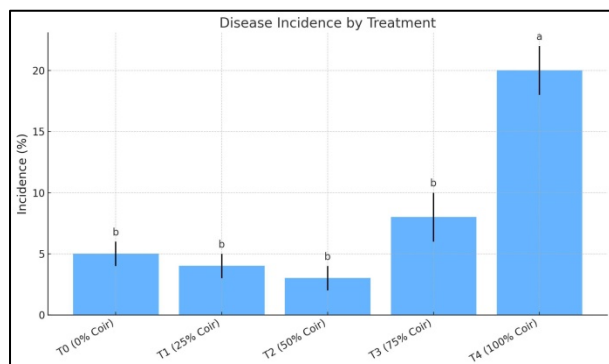
### Substrate Moisture Content

Moisture content measurements showed a progressive increase with increasing coir percentages ranging from 12% in T0 to 35% in T4. The acceptable range for *C. gigantea* performance was between 20–25% moisture. The T1 and T2 treatments met with this moisture condition. The high water-holding capacity (WHC) of coir is a result of the lignocellulosic structure with internal capillarity [31,32]. While moderate WHC is beneficial for plant establishment, high WHC of substrate or a saturated substrate can lower gas exchange and increase microbial risk [33,34]. This was evident in T3 and T4 as signs of over-watering and root decay were noted. Prasad (1997) [35] and Ansorena (1994) [36] both highlighted the importance of air porosity in conjunction with WHC, and this would be especially important for cacti which can easily succumb to root rot.

### Disease Incidence

Disease incidence varied among treatments, with T2 showing the lowest observed incidence (3%), followed by T1 and T0 (approximately 4–5%), while T3 exhibited a moderate incidence (8%) and T4 the highest level (20%) (Figure 5). Statistical analysis indicated that treatments T0–T2 did not differ significantly from one another ( $p < 0.05$ ), whereas T4 showed a significantly higher disease incidence compared to all other treatments. The relatively low incidence observed in T0–T2 suggests that moderate coir incorporation does not promote pathogen development. In contrast, higher coir content, particularly in T3 and T4, was associated with increased

moisture retention and reduced aeration, conditions that favor the proliferation of pathogenic fungi such as *Pythium*, *Phytophthora*, and *Fusarium* [37-39]. These findings are consistent with previous studies highlighting the importance of substrate drainage and aeration in controlling disease development [40-42].



**Figure 5:** Disease incidence (%) observed in *Carnegiea gigantea* seedlings under different substrate treatments. Error bars represent standard error. Different letters above bars indicate statistically significant differences ( $p < 0.05$ ).

### Seedling Vigor Index and Overall Performance

The Seedling Vigor Index (SVI), which combines germination and growth data into a single metric, was highest in T2 and T1 and both treatments were greater than T0, indicating that the organic amendment was supportive of early-stage plant establishment. SVI is a dependable metric of transplant success and is often used within forestry and horticulture to evaluate nursery success [43,44]. In addition to taller and deeper roots, the seedlings in T2 also had noticeably greener tissue and thicker stems, suggesting improved nutrient uptake and resistance to stress, in agreement with Reuveni et al. (2002) [45].

### Practical Implications and Horticultural Relevance

From a practical perspective, the use of coconut coir at 50% by volume represents a viable and sustainable option for the propagation of *Carnegiea gigantea*. Coir is a renewable alternative to peat and can help reduce some of the environmental impacts associated with peat extraction [46]. However, its overall environmental footprint depends on factors such as processing (e.g., washing, buffering, and drying) and transportation distances from production sites to end users. Therefore, comprehensive life cycle assessments are needed to fully evaluate its sustainability relative to other growing media. Despite these considerations, when used in appropriate proportions, coir remains an effective substrate component for improving germination and early seedling development. The study's results corroborate other studies that emphasized propagation studies on palms, succulents, and native desert species [47-50]. Future studies might be considering coir in combination with perlite, sand, or biochar, as suggested by Topa et al. (2019) [51], to address drainage and nutrient dynamics issues.

### Limitations and Recommendations for Future Research

Although greenhouses offer control and repeatability, they may not encapsulate the complexity found within an outdoor environment. Plant survival and growth continuity beyond the seedling stage should be researched through field trials using arid weather conditions [48]. Investigating the leaching of nutrients, microbial conditioning, and coir lesions on plant physiology will improve the use of this substrate in propagating desert plants.

### Conclusions

This study demonstrated that the 50% coir + 50% sandy-loam substrate (T2) achieved the highest germination rate (84%), as well as the greatest seedling height (2.8 cm) and root length (3.4 cm), and the lowest observed disease incidence (3%). However, disease incidence in treatments T0–T3 did not differ significantly from one another, indicating that moderate coir incorporation did not increase disease risk compared to the control. These findings suggest that T2 provides an optimal balance between moisture retention and aeration, promoting early seedling development without increasing susceptibility to pathogens. On the contrary, as coir rates increased beyond 50%-75%, moisture retention exceeded desirable levels causing moderate to severe fungal infections and poor vigor performance of seedlings. Coir seemed effective, but only if used properly. The results indicate that the best substrate formulation to cultivate *C. gigantea* in a greenhouse environment is a 50:50 mix of coir and mineral soil. This substrate improves early growth performance and supports sustainable horticultural practices by utilizing a renewable substrate as an alternative to peat. To conclude, moderate incorporation of coconut fiber in growing substrates promotes germination success, seedling vitality, and reduced disease incidence through improved substrate conditions in *Carnegiea gigantea*. Future research should examine long-term effects of coir-based substrates in field conditions and evaluate performance in conjunction with other soil amendments. This research will lead to more sustainable and efficient propagation methods for desert-adapted and conservation priority plant species.

### DISCLOSURE STATEMENT

- **Ethics approval and consent to participate:** Not applicable
- **Consent for publication:** Not applicable
- **Availability of data and materials:** The raw data required to reproduce these findings are available in the body and illustrations of this manuscript.
- **Author's contribution:** The authors confirm their contribution to the paper as follows: study conception and design: D.P.; data analysis and validation: D.P. & A.J.; draft manuscript preparation: D.P.. Editing and writing: A.J.. All authors reviewed the results and approved the final version of the manuscript.

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