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# Production of *Adenium obesum* (Forssk.) Roem. & Schult seedlings in different substrates

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## ABSTRACT

Low-cost substrates can potentially reduce production costs for ornamental plants such as *Adenium obesum*, popularly known as desert rose. The emergence and initial growth of desert rose in different substrates was evaluated. A randomized block design was used, with seven treatments and five replications. The treatments were sandy soil 100%; clay soil 100%; native vegetation soil 100%; sandy soil 50% + sawdust 50%; sandy soil 50% + fish farm sludge 50%; sandy soil 50% + crushed tobacco 50%; sandy soil 50% + pine bark with charcoal 50%. The fish farm sludge provided the highest emergence rate of 65%. At the end of the 180 DAS period, the SSF substrate had the highest average height, at 14.08 cm. The SSF and SPBC substrates had the highest average caudex diameter, at 32.42 mm. The SAN, NV, SSF, and SPBC substrates had the highest number of leaves parameter averages, with 41.80. Tobacco at a concentration of 50% hinders the emergence of desert rose seedlings. The substrate with a mixture of sandy soil and fish sludge provided the best development for the desert rose plants over the 180 days of evaluation.

**Keywords:** Initial plant growth, Seedling emergence, Desert rose.

## Produção de mudas de *Adenium obesum* (Forssk.) Roem. & Schult em diferentes substratos

## RESUMO

Substratos de baixo custo tem potencial para diminuir os custos de produção de plantas ornamentais, como a *Adenium obesum.* Avaliou-se a emergência e crescimento inicial de *Adenium obesum* em diferentes substratos. Foi utilizado o delineamento de blocos casualizados, com sete tratamentos e cinco repetições. Os tratamentos foram solo arenoso 100%; solo argiloso 100%; solo de vegetação nativa 100%; solo arenoso 50% + serragem 50%; solo arenoso 50% + lodo de piscicultura 50%; solo arenoso 50% + tabaco triturado 50%; solo arenoso 50% + casca de pinus com carvão 50%. O lodo de piscicultura proporcionou maior índice de emergência, de 65%. Ao final do período de 180 DAS o substrato ALP apresentou a maior média de altura, com 14,08 cm. Os substratos ALP e ACPC apresentaram a maior média para o diâmetro do caudex, com 32,42 mm. Os substratos ARE, VN, ALP e ACPC apresentaram maiores médias para o parâmetro número de folhas, com 41,80. A presença do tabaco na concentração de 50% inviabiliza a emergência de plântulas de rosas-do-deserto. O substrato com mistura de solo arenoso e lodo de piscicultura proporcionou melhor desenvolvimento das plantas de rosa do deserto ao longo dos 180 dias de avaliação.

**Palavras-chave**: Crescimento inicial de planta, Emergência de plântulas, Rosa do deserto.

## 1. Introduction

The *Adenium obesum*, popularly known as desert rose, is a herbaceous, shrubby, caudiciform succulent plant belonging to the Apocynaceae family (Varella et al., 2015). The species has only been considered economically profitable for a decade, so there is still a lack of information on how to manage its development properly. This plant has attracted attention for its exuberant flowering and also for the presence of a structure called a sculpted and swollen caudex (Colombo et al., 2018), which is an adaptation of the species for water storage, as its place of origin is very hot and water scarce (Varella et al., 2015), with special emphasis on savannas, open forests, dry and coastal forests with sandy or rocky soils.

Various technologies have been developed in the flower industry to increase productivity and reduce production costs. The development of ornamental plants requires a substrate that meets the demands of the crop. It is therefore important to conduct studies to develop cost effective substrates for desert rose production (Monteiro Neto et al., 2019), such as fish-farm sludge, pine bark, and tobacco.

Fish-farm sludge used as a substrate provides a range of nutrients, as it is a rich source of organic matter (OM), nitrogen (N), phosphorus (P), and potassium (K) (Eymontt et al., 2017). According to Collins (2017), with this wealth of nutrients, fish-farm sludge improves agricultural soils, increasing yield and minimizing the use of chemical fertilizers, reducing the negative effects on the environment. According to Senar (2019), besides having a rich concentration of nutrients, fish-farm sludge is a habitat for insects, animals, bacteria, and microorganisms, indicating the material excellent quality.

Another example of a low-cost substrate, pine bark, provides better drainage with low water absorption (Martin et al., 2006), a similar characteristic to charcoal, which also provides better drainage in the substrate (Chagas et al., 2005). Tobacco is another alternative material to be assessed as a substrate due to its high organic matter levels. Miranda et al. (2007) mentioned that substrates with high levels of poorly processed organic matter can be used as fertilizer for plant production by preparing compost.

In this context, tobacco has been assessed as a compost for substrate production, which contains high levels of organic matter. Miranda et al. (2007) mentioned that substrates with high levels of poorly processed organic matter can be used as fertilizer for plant production by preparing compost. Corroborating this, Paredes Junior et al. (2015) state that native vegetation soil enhances crop production as it stores large amounts of plant residues, which cooperate with the greater supply of energy, nutrients, and organic matter to the soil, causing an increase in the activity of microorganisms.

On the other hand, clay soils show high rates of compaction. In this context, Lucena et al. (2004) cited a low germination rate and initial growth of the *Senna siamea Lam* species, reporting that the substrate created an impermeable crust on the surface, making it difficult for the plants to emerge and grow. According to Fowler et al. (1998), this substrate makes the emergence and growth of the *Tabebuia cassinoides* species less vigorous and slower. This study aims to analyze the emergence rates and evaluate the morpho-biometric parameters of the initial growth of the desert rose in different low-cost substrates, which is justified because it is an ornamental plant with high added value.

## 2. Material and Methods

The study was conducted at the Mato Grosso do Sul State University (UEMS) in the Mundo Novo - MS, in a protected environment surrounded by 50% mesh and with a translucent cover, which provides ideal conditions for the development of the species evaluated. According to the international Köppen classification, the climate in the region is Cfa-type, humid subtropical, and mesothermal, with rainfall ranging from 1,500 to 1,700 mm per year and an average temperature of 23 °C (Sanesul, 2020). According to Semade (2015), the average temperature of the coldest and driest months is 14 °C to 15 °C, with frosts recorded.

The study used a randomized block design (RBD) consisting of seven treatments (Table 1) and five replications. Each experimental unit comprised one pot with four seeds, totaling 35 plots and 140 seeds. The seeds were pre-soaked in water for three hours to break the seeds dormancy. The seeds were then placed under the soil and covered with a thin layer of sand.

The soils used in the experiment were collected in Guaíra - PR (clay soil) and Mundo Novo - MS (sandy and sandy soils with native semi deciduous forest vegetation). The fish-farm sludge was collected in Maripá - PR (at the bottom of an excavated pond used for tilapia production). The Federal Revenue of Brazil (FRS) - 1st Tax Region, located in Mundo Novo - MS, seized and donated the tobacco. The sawdust came from untreated wood from various tree species, and the pine bark (70%) and charcoal (30%) were purchased commercially. Substrate samples were collected at the beginning of the experiment for chemical and particle size characterization in a specialized laboratory (Table 2).

**Table1.** Experimental conditions of treatments permormed.

| Treatments | Experimental Condition | Abbreviation |
|---|---|---|
| 1 | Sandy soil 100% | SAN |
| 2 | Clay soil 100% | CLA |
| 3 | Sandy Soil with Native Vegetation 100% | NV |
| 4 | Sandy Soil 50% + Sawdust 50% | SS |
| 5 | Sandy Soil 50% + Fish Farming Sludge 50% | SSF |
| 6 | Sandy Soil 50% + Crushed Tobacco 50% | ST |
| 7 | Sandy Soil 50% + Pine Bark and Coal 50% | SPBC |

**Table 2.** Chemical and particle size properties of the substrates used in the experiment.

| Variables Responses | | Samples | | | | | |
|---|---|---|---|---|---|---|---|
| | | SAN | CLA | NV | S | FS | T |
| Sand | g $kg^{-1}$ | 845 | 101 | 617 | - | - | - |
| Silt | g $kg^{-1}$ | 39 | 276 | 265 | - | - | - |
| Clay | g $kg^{-1}$ | 116 | 623 | 118 | - | - | - |
| pH | $CaCl_2$ | 4.76 | 5.10 | 5.62 | - | 5.95 | 4.98 |
| OM | g $dm^{-3}$ | 14.78 | 20.59 | 16.23 | 955.80 | 38.96 | 574.95 |
| P | mg $dm^{-3}$ | 6.85 | 21.80 | 9.70 | 12.33 | 418.26 | 5.10 |
| K | $cmol_c dm^{-3}$ | 0.12 | 0.60 | 0.40 | 1.52 | 0.43 | 49.15 |
| Ca | $cmol_c dm^{-3}$ | 1.52 | 6.30 | 4.32 | 5.36 | 10.93 | 22.69 |
| Mg | $cmol_c dm^{-3}$ | 0.84 | 1.30 | 1.00 | 3.32 | 4.72 | 6.73 |
| Al | $cmol_c dm^{-3}$ | 0.07 | 0.00 | 0.03 | - | 0.01 | - |
| H+Al | $cmol_c dm^{-3}$ | 2.68 | 3.20 | 2.70 | - | 3.40 | - |
| SB | $cmol_c dm^{-3}$ | 2.47 | 8.20 | 5.53 | - | 16.07 | 78.57 |
| CEC | $cmol_c dm^{-3}$ | 5.16 | 11.40 | 8.05 | - | 19.48 | - |
| V | % | 47.98 | 71.92 | 68.69 | - | 82.52 | - |

Granulometry: pipette method. Chemical characterization - Calcium chloride (pH); Mehlich (P and K); KCl 1N (Ca, Mg, and Al); Calcium acetate pH 7.0 (H + Al); Potassium dichromate oxidation (OM). SAN: sandy soil. CLA: clay soil. NV: native vegetation soil. S: sawdust. FS: fish-farm sludge. T: tobacco.

After sowing, the number of emerged seedlings in each treatment was daily counted to assess the following parameters:

a) emergence obtained by counting the emergence of the seedlings.

b) emergence speed index (ESI), obtained using the Maguire equation (1962):

$$ESI = \frac{N1}{D1} + \frac{N2}{D2} + \cdots + \frac{Nn}{Dn} \quad \text{Equation (1)}$$

N1, N2...Nn: corresponds to the number of normal seedlings that emerged.

D1, D2...Dn: corresponds to the number of days after sowing.

The emergence speed index (ESI) is a parameter used to quantify the number of seedlings that emerge per day in a given substrate, so the higher the number, the better the quality of the substrate, providing better conditions for seed emergence (Maguire, 1962).

c) mean emergence time (MET) estimated according to the equation proposed by Labouriau (1983):

$$MET = \frac{\sum Ni * Ti}{\sum Ni} \quad \text{Equation (2)}$$

In which:

Ni: number of seedlings emerged per day.

Ti: evaluation time (days).

The MET variable indicates the average time for "n" quantity of seeds to emerge. The value is expressed in days; the lower the number of days taken for emergence, the better the treatment.

d) emergence percentage (E), as expressed by Equation 3:

$$E = \left(\frac{N}{A}\right) * 100 \quad \text{Equation (3)}$$

N: number of emerged seedlings.

A: number of seeds sown.

The data from the emergence percentage was subjected to Scott-Knott analysis (p=0.05) using the R Core Team software (2019). The ExpDes.pt package (Ferreira et al., 2018) was used. To ensure the normality of the data and independence of variance, the values of emergence percentage were transformed into $\sqrt{X+1}$.

In the initial growth of the plants for the experiment, with one plant per pot, using the most centralized seedling as the selection criterion, morpho-biometric parameters were evaluated: plant height, caudex diameter, and number of leaves every 45 days, with four evaluations, up to 180 days after sowing (DAS). Plant height was assessed using a ruler, diameter was assessed using an electronic caliper, and leaves were counted visually. Statistical analysis was conducted using the Scott-Knot test (p=0.05) using the R Core Team software (2019). All tests were conducted using the ExpDes.pt package (Ferreira et al., 2018).

## 3. Results and Discussion

The SSF substrate (50% fish-farm sludge + 50% sandy soil) resulted in the best performance in terms of emergence percentage, with the highest number of seedlings emerging in the shortest time. On the other hand, the ST substrate (Soil 50% + Crushed Tobacco 50%) had the lowest performance, with no seedlings emerging in any of the five replications (Figure 1), which can be explained by the allelopathic effects caused by nicotine, phenolic acids, coumarins, flavonoids, and terpenoids characteristic of tobacco.

Fish-farm sludge is rich in nutrients (Table 1) and has a high water retention capacity, which is essential for seedling emergence and may justify the results obtained in this study (Silva et al., 2018). The SSF substrate had a percentage of 65% of emerged seedlings. The SPBC and NV substrates had 50% of the emerged seedlings. The SS and CLA substrates had 35% emerged seedlings, the SAN substrate had 30% emerged seedlings, and the ST substrate had no seedlings.

Considering the absence of emerged seedlings for the ST substrate (tobacco) and the study conducted by Fenilli et al. (2010), in which the use of powdered tobacco in proportions of 30% and 40% affected the growth of Tucaneira (*Citharexylum Myrianthum*) seedlings and in conditions of 10% and 20% there was no difference, it was evident that the search for lower proportions of tobacco could favor the emergence and growth of the desert rose. According to Magalhães and Durães (2002), desert rose seeds absorb water under favorable conditions after sowing, triggering germination and emergence in an average of four to five days. However, germination can take around a week longer in less favorable conditions, predisposing them to attack by pathogens, which was found for the tobacco-based substrate.

Considering all the treatments, 52 plants emerged. Given that 140 seeds were sown, overall, there was a low percentage of emergence (37.14%). According to Oliveira et al. (2020), for the process of germination and emergence of seedlings, it is necessary to have the relationship of sensitivity of the amount of water needed by the species for germination/emergence and initial growth, and this species is not tolerant of waterlogged substrates, thus being considered the best substrate, which provides better drainage. According to Anacleto and Bueno (2021), there are substrates that, when subjected to daily watering with low water drainage, can cause the caulicles of newly emerged plants to rot. For CLA substrate (100% clay soil), daily watering caused "waterlogging" which caused two seedlings to rot.

The total number of seeds that emerged per substrate during the ten days showed that SSF substrate had the highest number of emerged seeds, 13 seeds, accounting for 65% of the total, while ST had no emerged seedlings, meaning that this substrate was not suited to the species needs for seed germination. The lack of germination in the ST substrate can be explained by Peron et al. (2014), who state that tobacco (*Nicotiana tabacum* L.) can interfere with seed emergence and seedling development, as it is characterized by the allelopathic effects caused by nicotine, phenolic acids, coumarins, flavonoids, and terpenoids.

It was found that this treatment, AT, resulted in the appearance of fly larvae due to the intrinsic characteristics of tobacco, leading to an attractive and favorable environment for the deposit of parasites. Notably, this treatment received 50% of its substrate composition with tobacco. Lower percentages of the substrate may produce more satisfactory results. Therefore, novel studies could be conducted, testing lower percentages of substrate composition with tobacco.

According to ESI (Figure 2), SSF was the substrate with the highest average emergence. It can also be seen that no seedlings emerged from the ST substrate. According to Silva et al. (2008), the shorter the time for emergence, the better, as the seedling is not so susceptible to adverse conditions. These conditions include reduced humidity and infestation by microorganisms that can deteriorate the seeds over days in the soil/substrate.

Concerning mean emergence time (MET), the NV substrate had the shortest MET, around four days, while the SPBC substrate had the longest MET, around six days (Figure 3). The process of seedling emergence is due to an increase in the concentration of water/moisture, together with elements that help to store water. However, a substrate that stores a large amount of moisture, with high porosity and low density, provides the highest seed germination rate, especially spherical seeds, as with desert rose, where contact with the substrate is enhanced (Santos, 2018).

The first evaluations of the morpho-biometric parameters (Table 3) showed that the plant height in the SAN, NV, SSF, and SPBC substrates had no statistically significant differences. The NV treatment had the highest average plant height, with 3.36 cm at 45 days after sowing (DAS). The CLA and SS substrates, on the other hand, provided the lowest average for this variable.

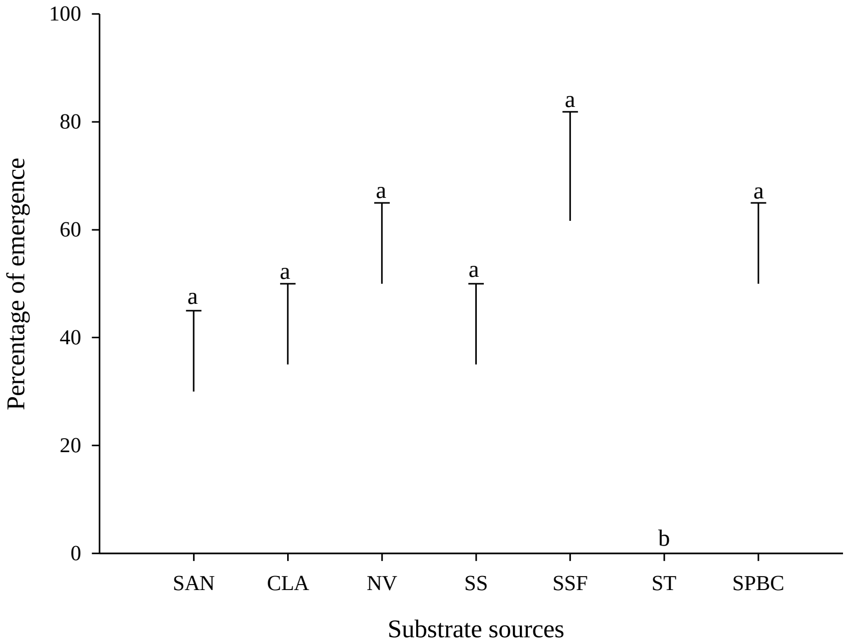


**Figure 1.** Confidence interval (95%), Scott Knott statistic, for the percentage of emergence of seedlings of desert rose. SAN: 100% sandy soil. CLA: 100% clay soil. NV: 100% native vegetation sandy soil. SS: sawdust 50% + sandy soil 50%. SSF: fish-farm sludge 50% + sandy soil 50%. ST: Sandy soil 50% + crushed tobacco 50%. SPBC: pine bark + charcoal 50% + sandy soil 50%. Vertical bars indicate standard deviation

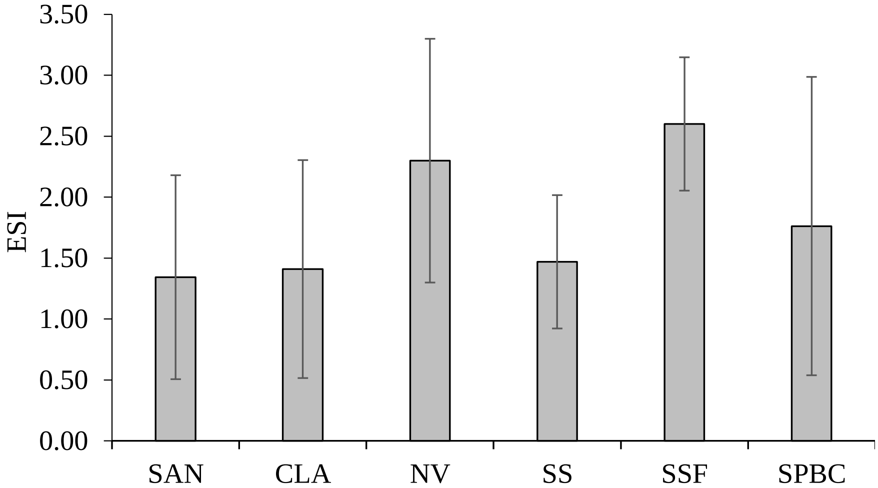


**Figure 2.** Emergence speed index (ESI). SAN: 100% sandy soil. CLA: 100% clay soil. NV: 100% native vegetation sandy soil. SS: sandy soil 50% + sawdust 50%. SSF: sandy soil 50% + fish farm sludge 50%. SPBC: sandy soil 50% + pine bark + charcoal 50%. Vertical bars indicate Standard Deviation.

The two substrates with the highest averages for the caudex diameter variable were SAN and SPBC, while the plants grown in the NV and SSF substrates showed intermediate results. At the same time, the lowest averages were found in CLA and SS. The number of leaves in the first 45 DAS had the highest average in the NV, SSF, and SPBC substrates. The lowest average for the number of leaves was found in the SS substrate. In the second evaluation of the morpho-biometric parameters, at 90 DAS, the plants grown on the SSF substrate were the tallest, with 8.84 cm, while the CLA substrate showed the lowest plant height at 2.52 cm. The SAN, SSF, and SPBC treatments had the highest averages for caudex diameter, with the SSF treatment having 18.67 mm, and the substrate with the lowest average was CLA, with 5.79 mm. The SAN, NV, SSF, and SPBC substrates had the highest and lowest values found in CLA and SS for the number of leaves.

In the third evaluation of the morpho-biometric parameters, at 135 DAS, it can be seen that, for plant height, the substrate with the highest average was SSF, with 13.68 cm, and the lowest was SS, with 2.38 cm. The substrates with the highest average for diameter were SAN, NV, SSF, and SPBC, and the lowest average for the SS substrate. For the number of leaves, the SAN, NV, SSF, and SPBC substrates had the highest averages, with SSF having the highest, 22.2, and SS having the lowest average, 5.00.

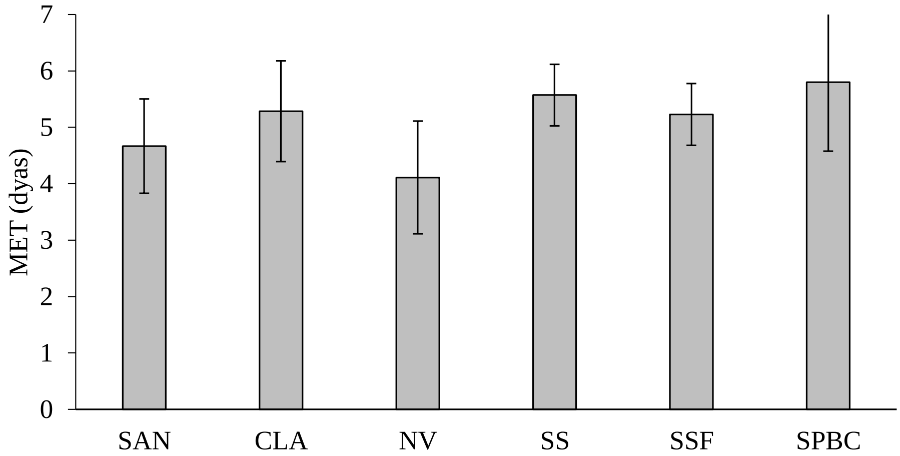


**Figure 3.** Mean emergence time (days) for the different substrates. SAN: 100% sandy soil. CLA: 100% clay soil. NV: 100% native vegetation sandy soil. SS: sandy soil 50% + sawdust 50%. SSF: sandy soil 50% + fish-farm sludge 50%. SPBC: sandy soil 50% + pine bark + charcoal 50%. MET: mean emergence time. Vertical bars indicate standard deviation.

**Table 3.** Morpho-biometric parameters of desert rose seedlings in different substrates at 45, 90, 135, and 180 days after sowing (DAS).

| Treat. | Height (cm) | Diameter (mm) | Number of leaves | Treat. | Height (cm) | Diameter (mm) | Number of leaves |
|---|---|---|---|---|---|---|---|
| | 45 DAS | | | | 90 DAS | | |
| SAN | 2.88 a | 8.32 a | 7.60 a | SAN | 6.26 b | 14.22 a | 16.00 a |
| CLA | 1.17 b | 3.62 b | 5.25 b | CLA | 2.52 c | 5.79 b | 6.00 b |
| NV | 3.36 a | 7.35 a | 9.00 a | NV | 5.82 b | 12.49 a | 14.60 a |
| SS | 1.32 b | 3.91 b | 3.80 b | SS | 2.64 c | 6.73 b | 6.00 b |
| SSF | 3.30 a | 7.13 a | 9.20 a | SSF | 8.84 a | 18.67 a | 15.00 a |
| SPBC | 3.16 a | 8.18 a | 9.60 a | SPBC | 6.58 b | 16.22 a | 17.20 a |
| CV% | 27.13 | 30.69 | 24.43 | CV% | 26.48 | 26.74 | 32.72 |
| Treat. | Height (cm) | Diameter (mm) | Number of leaves | Treat. | Height (cm) | Diameter (mm) | Number of leaves |
| | 135 DAS | | | | 180 DAS | | |
| SAN | 10.22 b | 23.18 a | 22.00 a | SAN | 11.06 a | 28.37 a | 30.00 a |
| CLA | 6.76 c | 15.32 b | 17.66 a | CLA | 6.96 b | 16.40 b | 24.66 a |
| NV | 10.10 b | 21.16 a | 22.20 a | NV | 11.76 a | 26.41 a | 31.00 a |
| SS | 2.38 d | 6.64 b | 5.00 b | SS | 1.62 c | 5.70 c | 4.60 b |
| SSF | 13.68 a | 27.61 a | 22.20 a | SSF | 14.08 a | 30.80 a | 41.80 a |
| SPBC | 8.46 b | 27.21 a | 22.00 a | SPBC | 10.18 a | 32.42 a | 34.60 a |
| CV% | 27.86 | 32.67 | 35.1 | CV% | 30.8 | 25.87 | 36.83 |

Means followed by the same letter in the column belong to the same group by the Scott-Knott test (5%). SAN: 100% sandy soil. CLA: 100% clay soil. NV: 100% native vegetation sandy soil. SS: sawdust 50% + sandy soil 50%. SSF: fish farm sludge 50% + sandy soil 50%. SPBC: pine bark + charcoal 50% + sandy soil 50%. CV: coefficient of variation. DAS: days after sowing. Trat.: treatment/substrate.

In the fourth evaluation of the morpho-biometric parameters, at 180 DAS, the SSF substrate had the highest plant height, 14.08 cm, and the SS substrate had the lowest, 1.62 cm. The SSF and SPBC substrates had the highest average for the caudex diameter, 32.42 mm, and the SS substrate had the lowest average (5.70 mm). The SAN, NV, SSF, and SPBC substrates had the highest averages for the number of leaves, with 41.80 leaves, and the SS substrate had the lowest average, with 4.60 leaves. As a result, the substrate that showed the best development for the morpho-biometric parameters assessed during the 180 DAS period was SSF. The substrate with the lowest development for morpho-biometric parameters was SS during the 180 DAS period.

Studying the number of leaves is important because they are the main site of photosynthesis, which is linked to plant growth. They are also used as a reserve store, a source of auxin and rooting cofactors, contributing to forming new tissues such as roots (Pereira et al., 1991). The SS substrate provided lower growth rates for height, diameter, and number of leaves. According to

Fontana et al. (2010), substrates with a high organic matter content interact more with clay soils, promoting greater stability of the organic carbon in the soil.

Stringheta (2005) states that the substrate containing sawdust, the material used as the basis for formulating the substrate for sowing desert rose, may contain lower pH, N, P, K, Ca, Mg, and S values compared to other substrates, but this does not make it unfeasible to use as some plants need low concentrations of nutrients. However, it can be inferred that the desert rose needs a high concentration of nutrients, the demand for which is met especially in the treatment with fish-farm sludge (Table 1).

In this context, Pes and Arenhardt (2015) corroborate when they state that the nutritional input offered by the substrates directly influences the growth and development of the plants, resulting in cell multiplication, elongation, and differentiation. Plants with a larger diameter have a better survival rate due to their ability to form and grow new roots, which indicates optimum seedling quality (Carneiro, 1995). According to Barroso Junior (2017), the substrate of pine bark (75%) and coconut dust (25%) provided greater growth in stem diameter. Similarly, the SPBC substrate provided the largest stem diameter in this study.

In the research by Santos et al. (2015), the height and diameter of desert rose seedlings, when submitted to the treatment with virgin forest soil (50%) + commercial substrate (50%) + fertilizer, showed better results when compared to the other substrates: 70% Sand + 30% Soil from virgin forest + Fertilizer, 90% Sand + 10% Commercial substrate + Fertilizer, 50% Sand + 25% Soil + 25% Commercial substrate + Fertilizer, 70% Sand + 30% Commercial substrate + Fertilizer, and 100% Soil from virgin forest + Fertilizer. The SSF substrate provided the best results for height and diameter in the present study.

According to the soil analysis in Table 1, the fish-farm sludge contains a high concentration of phosphorus (P), leading to the best development of the desert rose plants. This result is explained by Firoz (2009), who states that high phosphorus concentrations are good for root growth and nutrient absorption. Similarly, Nietsche (2021) corroborates this by stating that phosphorus can help speed up plant maturation and is essential for photosynthesis, respiration, and favoring the flowering period.

## 4. Conclusions

When mixed in equal proportions with the fish-farm sludge, the sandy soil provides more emerged seedlings in a shorter time. Tobacco at a concentration of 50% makes it impossible for desert rose seedlings to emerge. The substrate with a mixture of sandy soil and fish sludge provided the best development for the desert rose plants over the 180 days of evaluation.

## Authors' Contribution

Izabel Melz Fleck led the project, carried out analysis and wrote the manuscript. Jean Sérgio Rosset did the project correction and the manuscript, including the statical analysis. Leandro Fleck did the correction of the manuscript. Tiago Zoz assisted with statistical analyses. Jefferson Matheus Barros Ozório assisted with statistical analyses and correction of the manuscript.

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