CULTIVATION OF SEEDLINGS OF *Solanum melongena* L. FOR THEIR USE AS A BIOASSAY

CULTIVO DE MUDAS DE Solanum melongena L. *PARA SEU USO COMO BIOTESTE*

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ABSTRACT

The generation of waste and contaminants in the environment demands environmental monitoring through biotechnological tools. When employing plant bioassays, variables such as substrate type need to be standardized to ensure control over the results. In response to this necessity, this study aims to analyze the influence of different substrate compositions and field capacities on the development of pre-established *Solanum melongena* L., with the goal of assessing the use of this species as a bioassay for ecotoxicological assays. The evaluated substrates consisted of a mixture of sand and vermiculite compared to the commercial substrate Mococa. Irrigation conditions varied according to field capacity. After 14 days of acclimatization in controlled conditions, morphological and mass variables were assessed, including leaf area, stem base width, root and aboveground length, as well as fresh and dry mass of the root and aboveground parts. Results indicate significant interactions between substrate type and field capacity with vermiculite, favoring the development of stem base width and leaf area. In light of the obtained results, *S. melongena* shows potential as a test organism for evaluating phytotoxicity in various samples; however, the choice of substrate proves fundamental for plant quality.

KEYWORDS: Eggplant; growth parameters; phytotoxicity; plant bioassays; substrate; ecotoxicology.

RESUMO

A geração de resíduos e contaminantes no ambiente, apresenta como demanda o monitoramento ambiental através de ferramentas biotecnológicas. Quando utilizado biotestes vegetais, variáveis como o tipo de substrato precisam ser padronizados para que se tenha controle sobre os resultados. Diante desta necessidade, o objetivo com este estudo foi analisar a influência de diferentes composições de substratos e capacidades de campo no desenvolvimento de *Solanum Melongena* L. pré-estabelecidas, a fim de avaliar o uso dessa espécie como bioteste para ensaios ecotoxicológicos. Os substratos avaliados foram compostos por mistura de areia e vermiculia ao substrato comercial Mococa. As condições de rega variaram de acordo com a capacidade de campo. Após 14 dias, pós

aclimatação, em condições controladas, as variáveis morfológicas e de massa foram avaliadas, incluindo área foliar, largura da base do caule, comprimento de raiz e parte aérea, bem como massa fresca e massa seca da raiz e parte aérea. Os resultados indicam interações significativas entre o tipo de substrato e a capacidade de campo com a vermiculita, favorecendo o desenvolvimento da largura da base do caule e da área foliar. Diante dos resultados obtidos, *S. melongena* L. apresenta potencial como organismo teste para avaliação de fitotoxicidade de diversas amostras, contudo a escolha do substrato se mostra fundamental para a qualidade da planta.
PALAVRAS-CHAVE: Berinjela; biotestes vegetais; ecotoxicologia; fitotoxicidade; parâmetros de crescimento; substrato.

INTRODUCTION

With the advent of industrialization and the Agricultural Revolution, significant global concerns have arisen regarding environmental impacts and human health(1). Industrial activities have led to a series of detrimental consequences, including ecosystem pollution, deforestation, intensive exploitation of agricultural resources, climate change, soil erosion, and biodiversity loss(2). These activities result in the generation of waste that introduces contaminants into the environment, making the implementation of environmental monitoring measures essential(3).

Bioassays, in addition to serving as a methodology for environmental monitoring, represent a starting point for the development of new technologies in response to ecological changes. Biological assays demonstrate efficiency in monitoring environmental pollution(4). Systems that use plants as bioindicators are economical, sensitive to various contaminants, easy to analyze, exhibit good reliability and reproducibility, and show correlation with other animal and microbial bioassays(5–7).

The assessment of the toxicity of plant and environmental samples predominantly employs laboratory-scale bioassays(8). These assays address components of the initial development of seedlings, encompassing germination parameters (percentage of germination and various germination indices) and morphological parameters (root growth, aboveground part length, and characterization of abnormalities), representing direct exposure to the tested sample and providing a rapid response to stress—a widely recognized and established methodology(9,10).

For these bioassays, the substrate composition is crucial for the proper development of the bioindicator(11). Various factors affect seedling growth, with the substrate being responsible for providing nutrients, support, good soil drainage, and aeration[12]. Therefore, it is important to choose and understand the substrate composition to ensure the healthy development of the bioassay[13].

Among horticultural plants with the potential for use as bioindicators, *Solanum melongena* L. (eggplant) stands out due to its extensive cultivation in tropical and subtropical areas. Belonging to the Solanaceae family with over 1,200 perennial species, exhibiting shrubby or climbing characteristics, it includes some of the essential vegetables for human consumption and medicinal plants[14]. Additionally, the Solanum genus presents a great diversity of plants considered invasive, causing damage to commercial plantations[15–17].

The objective of this study was to analyze the influence of different substrate compositions and field capacities on the development of pre-established *Solanum melongena* L. with the aim of evaluating the use of this species as a bioindicator for ecotoxicological assays.

MATERIAL AND METHODS

Solanum melongena L. seedlings were commercially obtained in the municipality of Alfenas-MG, 31 days after sowing, and were kept for 10 days in a growth chamber under conditions of temperature at 25 °C ± 1 ºC, humidity ranging between 43% and 61%, and a photoperiod of 12/12 hours for acclimatization (Figure 1).

Figure 1. *Solanum melongena* L. plants under acclimatization conditions in the growth chamber.

After this period, the seedlings were transplanted into containers with a capacity of 500 cm3, providing ample space for root fixation, water and nutrient storage, thereby promoting plant growth and development. The containers were individually covered with black plastic to prevent direct light incidence on the roots.

Substrate Preparation: For the evaluation of different substrate types, the commercial substrate Mococa (composition: peat, pine bark, and charcoal) was used as a base. The substrates were prepared using the following mixtures: 10% sand with 90% Mococa for the first treatment; 70% Mococa supplemented with 30% vermiculite for the second treatment. Both treatments underwent drying in an oven at 60 °C for 4 days until they reached a constant weight.

*Field capacity:*The field capacity was determined for two different substrate compositions in 500 cm^3 containers, where distilled water was added until substrate saturation, indicated by water dripping through small holes made at the bottom of the container. Subsequently, the moist substrates were transferred to Petri dishes and dried in an oven at 60 °C until they reached a constant weight. The procedure was carried out in triplicate. By calculating the initial weight of saturated substrates in relation to the final weight of dried substrates, the amount of water in grams needed to achieve 100% field capacity was determined. This was 194.9 mL for 280 g of substrate plus vermiculite and 162.3 mL for 340 g of substrate plus sand.

For the field capacity tests, conditions of 70% field capacity and a critical soil water tension at -0.3 atm were determined. Therefore, for the treatment using vermiculite, 136.4 mL and 207 mL of distilled water were required to achieve 70% field capacity and -0.3 atm, respectively. For the treatment using sand in the composition, 113.62 mL and 168.9 mL were needed to achieve 70% field capacity and -0.3 atm, respectively.

Experimental procedure: The seedlings were transplanted 41 days after sowing into 500 cm^3 containers (Figure 2) containing the respective substrate types used for the experimental procedure. The plants were kept in the growth chamber for 14 days under conditions of temperature at 25 °C + 1 ºC, humidity ranging between 43% and 61%, and a photoperiod of 12/12 hours. During this period, they were irrigated with distilled water according to the field capacities employed.

Figure 2. Transplanted *Solanum melongena* L. seedlings in 500 cm^3 containers for the experimental procedure.

Evaluated parameters: After 14 days in the growth chamber, the developed seedlings were analyzed for the following parameters: leaf area (cm^2), base width (mm), root length (cm), aboveground part length (cm), fresh root mass (mg), fresh aboveground part mass (mg), dry root mass (mg), and dry aboveground part mass (mg).

Measurement methods: The measurements of base width and aboveground part length were determined using a digital caliper, while the parameters leaf area and root length were defined using ImageJ software. To determine fresh and dry masses, the seedlings were removed from the planting container, roots were washed under running water, and separated from the aboveground part. The fresh masses of roots and aboveground parts for each treatment were determined using an analytical balance and subsequently subjected to drying in an oven at 60 °C. After 6 days of drying, roots and aboveground parts were weighed again to determine the dry mass.

Statistical analysis and experimental design: The experimental design was completely randomized with a double 2x2 factorial, involving two types of substrate and two field conditions, with 6 repetitions for each factor. The data were subjected to analysis of variance ($p < 0.05$), and the means were compared using the Scott-Knott test[18].

RESULTS AND DISCUSSION

Throughout the experimental period, *S. melongena* L. seedlings were kept in a growth chamber with closely monitored temperature and humidity conditions (Table 1). The average temperature was maintained at 25 ºC, while the humidity level was stabilized at 56%.

Table 1. Variation in temperature and humidity conditions during the experimental conduct in the greenhouse.

Day	Temperature	Humidity
1st	24.6 °C	55%
4th	25.3 °C	61%
6th	25.9 °C	61%
8th	25.3 °C	60%
10th	24.8 °C	57%
14th	24.1 °C	43%

In the assessment of morphological parameters, the interaction between substrate types and field capacity factors was found to be significant for base width (Figure 3A), unlike the leaf area parameter (Figure 3B and C), where the interaction was not significant. However, the factors individually showed statistical differences. Combinations of substrates revealed that the addition of vermiculite benefited both base width and leaf area, as detailed in Figure 4.

When comparing the field capacity condition, using sand as a substrate showed no difference between treatments for base width. However, for vermiculite, the optimal condition was at a critical tension of -0.3 atm, a condition that also favored leaf area.

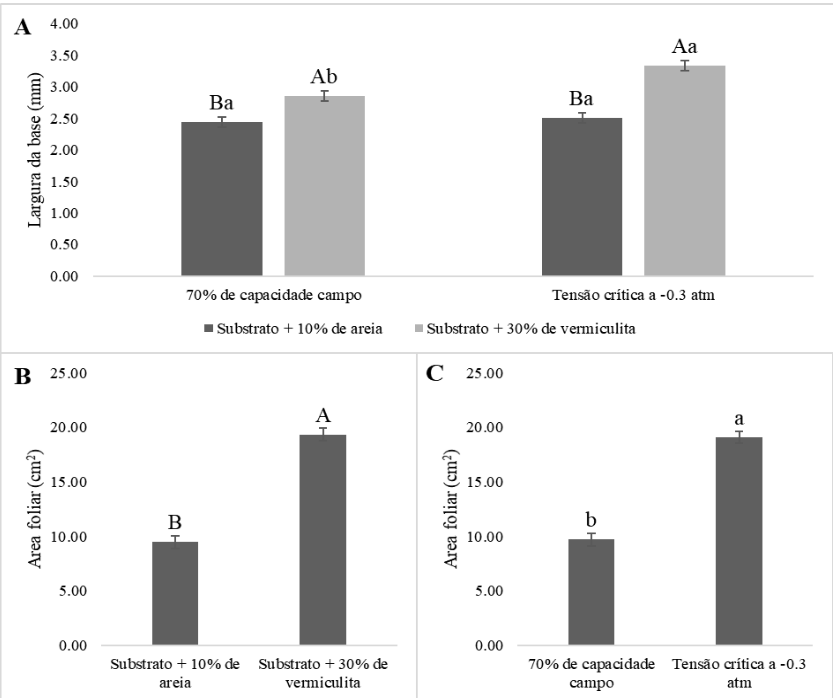

Figure 3. Assessment of leaf area and stem base of *Solanum melongena* L. plants in different substrates. Equal letters do not differ from each other by the Scott-knott test at a 5% significance level, where uppercase letters compare substrates, and lowercase letters compare field capacity.

Figure 4. *Solanum melongena* L. plants under acclimatization conditions in a growth chamber.

A The use of vermiculite in substrates has proven to be effective, particularly due to its absorption and moisture retention characteristics. This substance can retain up to five times its volume in water. Furthermore, vermiculite is recognized for positively contributing to the improvement of soil physical and chemical properties. The full application of vermiculite or its incorporation as a component in substrates results in a significant increase in their water retention capacity, promoting seedling development(19).

As observed for *Solanum melongena* L. leaves, the best result was achieved at a critical tension of -0.3 atm. This is explained by Schafer and Lerner(13), who discuss that for a substrate to be efficient, it is crucial to maintain water available at low tensions, fulfilling one of its vital functions: ensuring an adequate supply of water and air. In this context, the physical properties most frequently examined in substrates, which directly affect plant growth, mainly focus on the analysis of density curve and water retention capacity. These analyses aim to determine the optimal balance between water and air availability necessary for the healthy development of plants in a specific substrate.

Regarding root length (Figure 5C), an interaction between factors occurred. When comparing substrates, there was no difference when placed at 70% field capacity; however, at a critical tension of -0.3 atm, the vermiculite-containing substrate resulted in a longer root length. Comparing field capacity conditions, there was no difference for plants subjected to vermiculite-containing substrate, whereas for plants grown in sand, the best condition for root growth was at 70% field capacity.

Only the substrate factor was significant when analyzing the shoot length (Figure 5D). Plants grown in vermiculite had a greater shoot length compared to those grown in sand.

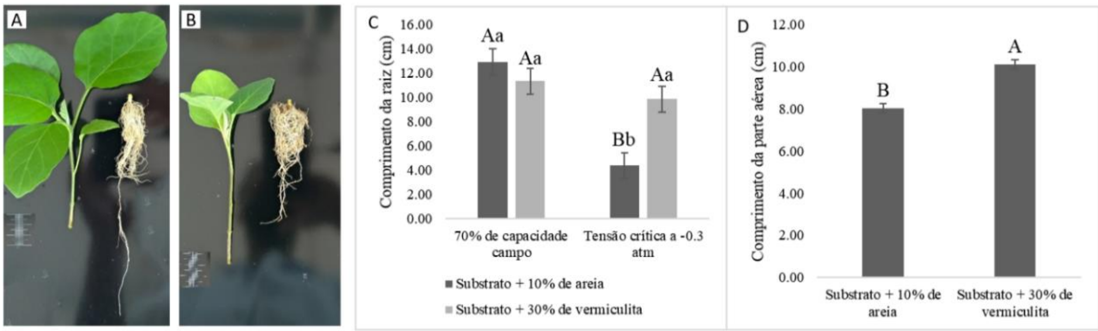

Figure 5. Morphological characteristics of *Solanum melongena* L. plants in different substrates. A) shoot and root in substrate +30% vermiculite; b) shoot and root in substrate +10% sand; c) root length; d) shoot length. Equal letters do not differ from each other by the scott-knott test at a 5% significance level, where uppercase letters compare substrates, and lowercase letters compare field capacity.

Changes in substrate composition, including the addition of vermiculite to the mix, can significantly influence the initial growth of plants, consequently altering the levels of pigments involved in photosynthesis. These changes affect the efficiency of light energy capture, impact the amount of assimilated substances produced by plants, and consequently increase the development of the shoot[(19)], as observed in *S. melongena* when cultivated in vermiculite.

The high adsorption capacity of vermiculite contributes to the gradual release of nutrients present in the substrate. This process significantly reduces leaching, improving the efficiency with which plants utilize available nutrients and consequently promoting an increase in root length, as this organ is the first to come into contact with the substrate and nutrients present[(20)].

When cultivated in sand, the roots of *S. melongena* showed better development at 70% field capacity. This is due to the porosity of the substrate in contact with this specific amount of water, resulting in an appropriate density that facilitates root development[(20)].

For all fresh and dry mass measurements (Figure 6), only the substrate factor was significant. Furthermore, for all analyzed parameters, both fresh and dry masses of shoot and root, the substrate that promoted the best plant development was the one using vermiculite in the composition. While the use of sand resulted in greater damage to shoot development, which is likely related to the low development of the root, leading to inefficiency in mass gain.

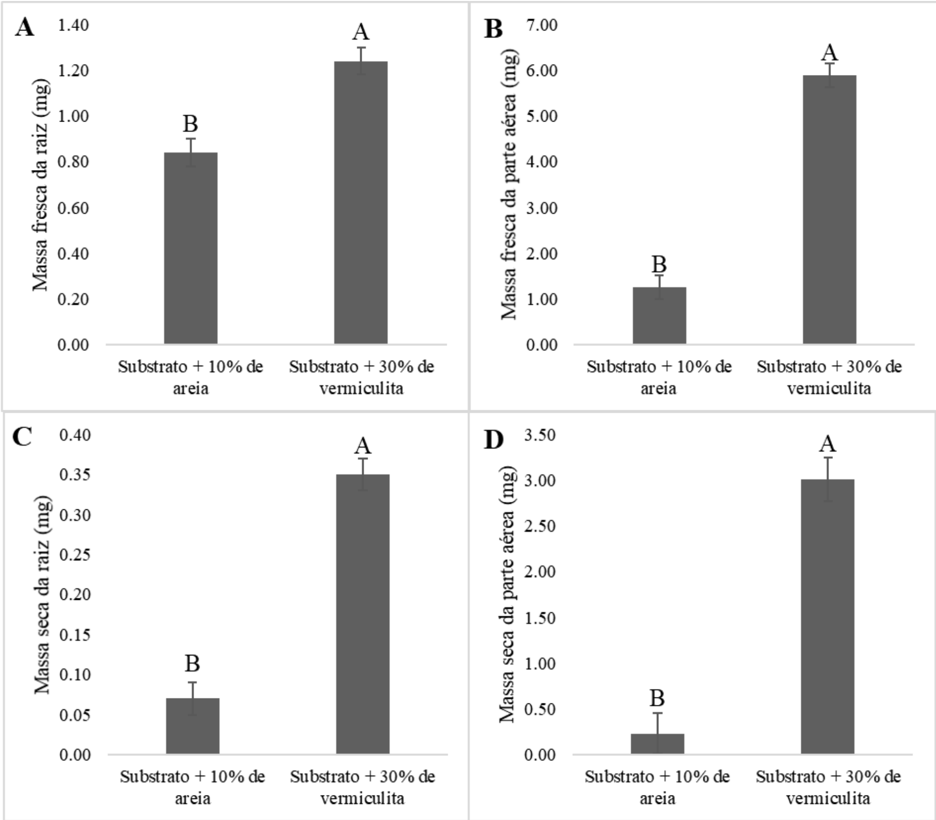

Figure 6. Evaluation of dry and fresh mass of *Solanum melongena* L. plants in different substrates. Equal letters do not differ from each other by the Scott-knott test at a 5% significance level.

The use of dense materials such as sand, either independently or in substrate compositions as in this case, has disadvantages due to its significant weight. This characteristic can complicate the management of plants in their containers, as well as interfere with the penetration and development of the plant's root system, negatively affecting water absorption and carbon allocation, consequently reducing both fresh and dry masses[19].

Considering the obtained results, the physical properties frequently analyzed in substrates, which have a direct impact on plant growth, are primarily focused on the analysis of density, reflected by the substrate composition, and water retention capacity. This approach aims to establish a balance between the availability of essential water and air for the optimal development of plants in a specific substrate[13].

CONCLUSION

The standardization of ideal parameters, including substrates and field conditions, is essential to ensure the accuracy and reliability of bioassays. The use of *Solanum melongena* L. as a bioassay is viable for phytotoxicity assessments. The results highlighted vermiculite as favorable for the development of stem base width and leaf area, emphasizing the importance of carefully selecting these parameters. It is recommended to cultivate seedlings in a substrate enriched with 30% vermiculite and field capacity with a critical tension of -0.3 atm. It is worth noting that for objectives focusing on root assessments, 70% field capacity can be used for *S. melongena* seedlings.

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