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SCIENTIFIC ARTICLE

Initial growth of zinnia seedlings in substrate with different proportions of biosolid

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Abstract

Zinnia (*Zinnia elegans* Jacq.) is a widely cultivated species and used as an ornamental plant in the world flower market. Although it has great potential for phytoremediation and compounds with anti-infective and antioxidant activities there are few studies that address the process of production of its seedlings especially using substrates from organic waste. This study aimed to evaluate the potential of biosolid as a substrate component in the initial growth of *Zinnia elegans* seedlings. The seedlings were produced in nursery conditions with 50% light control. The experimental design was entirely randomized. There were six treatments, four repetitions and five plants per plot. The treatments consisted of substrates resulting from the mixture of subsoil (SS) and biosolid (BIO) in different proportions: 100% SS (control); 80% SS + 20% BIO; 60% SS + 40% BIO; 40% SS + 60% BIO; 20% SS + 80% BIO; T6 100% BIO. The morphological and quality characteristics of the seedlings were evaluated. Polynomial regression analysis and Pearson correlation coefficient were performed. Quadratic regression fitting was observed for plant height, stem diameter, leaf area, total chlorophyll, and shoot and root dry mass, as well as total dry mass. The shoot height/stem diameter ratio was the characteristic that correlated least with the others, being observed a positive correlation only with plant height. The use of biosolid in the proportion of 60% in the substrate composition proved to be efficient for the initial growth of seedlings as it showed the best responses for most morphological and quality characteristics of the seedlings.
Keywords: organic compost, ornamental plant, quality seedlings, sewage sludge, *Zinnia elegans*.

Resumo

Crescimento inicial de mudas de zínia em substrato com diferentes proporções de biossólido

A zínia (*Zinnia elegans* Jacq.) é uma espécie amplamente cultivada e utilizada como planta ornamental no mercado floral mundial. Embora possua grande potencial para fitorremediação e compostos com atividades anti-infecciosas e antioxidante, poucos são os trabalhos que abordem sobre o processo de produção de suas mudas, principalmente utilizando substratos oriundos de resíduos orgânicos. Este estudo teve como objetivo avaliar o potencial do biossólido como componente de substrato no crescimento inicial de mudas de *Zinnia elegans*. As mudas foram produzidas em condições de viveiro com controle de luminosidade de 50%. O delineamento experimental foi inteiramente casualizado. Foram seis tratamentos, quatro repetições e cinco plantas por parcela. Os tratamentos foram constituídos por substratos resultantes da mistura de terra de subsolo (TS) e biossólido (BIO) em diferentes proporções: 100% TS (controle); 80% TS + 20% BIO; 60% TS + 40% BIO; 40% TS + 60% BIO; 20% TS + 80% BIO; T6 100% BIO. Foram avaliadas as características morfológicas e de qualidade das mudas. Realizou-se a análise de regressão polinomial e coeficiente de correlação de Pearson. Houve ajuste de regressão quadrática para altura da planta, diâmetro do coleto, área foliar, clorofila total, massa seca da parte aérea, raiz e total. A característica razão altura/diâmetro foi a que menos se correlacionou com as demais, sendo observada correlação positiva apenas com altura da planta. O uso do biossólido na proporção de 60% na composição de substrato se mostrou eficiente para o crescimento inicial de mudas, pois apresentou as melhores respostas para a maioria das características morfológicas e de qualidade das mudas.
Palavras-chave: composto orgânico, lodo de esgoto, mudas de qualidade, planta ornamental, *Zinnia elegans*.

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Introduction

Zinnia (*Zinnia elegans* Jacq.) is a species of the Asteraceae family used as an ornamental plant in the world flower market. It has stems with brightly colored and uniform flowers with a long shelf life. It tolerates dry and sunny environments, which makes it suitable for places where growing other types of flowers would be difficult (Zamiran et al., 2013; Ahmad et al., 2015; Pallavi et al., 2017). Although it has great potential for use medicine and phytoremediation, in Brazil there are few studies that address the process of seedling production using substrates from organic waste (Sousa et al., 2011; Pêgo et al., 2019; Barroso et al., 2020 Gomaa et al., 2020; Panda et al., 2020).

In this sense, the choice of the appropriate substrate is a key factor at the time of crop establishment, since its characteristics generally differ greatly from those of the soil (Soldateli et al., 2020), because the substrates in general have the function for water retention, support and aeration for the good development for the seedling, these must ensure the initial growth, and in some cases, it can provide the maintenance of the seedling development for a short to medium time (Kratz and Wendling, 2016).

Even though there are more recommended commercial substrates for the cultivation of certain species, the effect of different organic residues in the composition of substrates on the quality of the produced seedlings should still be evaluated, seeking to integrate the precepts of sustainability, reduce the time and costs of the production process, and provide options for nurserymen (Ferreira et al., 2018; Siqueira et al., 2019). In the composition of the substrate the organic part contributes for moisture retention, nutrients supply and its use in the production of seedlings has provided good results (Cordeiro et al., 2020; Oliveira et al., 2020).

Among the residues sewage sludge stands out, which when properly treated is called biosolid, is an alternative with economic and socio-environmental benefits, which contributes to sustainability, since it combines the need for an environmentally safe destination for these residues, which are solution for sewage treatment stations due to the high volume produced daily (Collivignarelli et al. 2020). The biosolid acquires characteristics that allows its use in agriculture. It is a material rich in organic matter and nutrients which can be used as a conditioner of the soil's physical, chemical, and biological properties. It also has a high capacity to partially or totally replace the use of substrates and fertilizers, meeting the nutritional demands of plants (Ferraz et al., 2016; Souza et al., 2019; Djandja et al., 2020).

Considering all these aspects, this study aimed to evaluate the potential of biosolid as a substrate component in the initial growth of *Zinnia elegans* seedlings.

Material and Methods

The experiment was carried out during the month of March 2020 in a greenhouse covered with a black screen (sombrite®) that allows 50% of shading, located in the state of São Paulo in the following coordinates: 21°15'2" S, 48°16'47" W, at 600 m altitude. The region climate is subtropical *Cwa* type (humid tropical with dry winter and rainy summer) with minimum, average and maximum temperatures of 19.8 ºC, 24.5 ºC and 32.5 ºC, respectively (Galzerano et al., 2012).

The experimental design was entirely randomized. There were six treatments, four repetitions and five plants per plot. The tested treatments consisted of substrates resulting from the mixture of subsoil (SS) and biosolid (BIO) in different proportions: 100% SS (control); 80% SS + 20% BIO; 60% SS + 40% BIO; 40% SS + 60% BIO; 20% SS + 80% BIO; T6 100%. To obtain the mixtures used, both the biosolid and the subsoil were sieved through a 3-mm steel mesh sieve and then homogenized in the proportions mentioned above.

The biosolid used for this experiment was obtained from Lageado Sewage Treatment Plant, located in Botucatu, São Paulo State, the residual material produced by the Treatment Station follows the supply standards for agricultural use. The biosolid meets the requirements according to Environmental National Council (Conama Resolution 375/2006) (Conama, 2006), being classified as belonging to type A and presents chemical analysis, including heavy metals, whose results were: organic carbon = 21.7% m m^{-1}; total cobalt = <0.001² % m m^{-1}; CTC = 500 mmol kg^{-1}; S = 3.3% m m^{-1}; Fe = 4.4 % m m^{-1}; P = 3.4 % P_2O_5 (m m^{-1}); Mg = 0.22 % m m^{-1}; N = 3.7 % m m^{-1}; pH (in $CaCl_2$) = 5.3; K = 0.12 % K_2O (m m^{-1}); C/N = 5.86 and the heavy metals: As = 3.9 mg kg^{-1}; Cd = 1.6 mg kg^{-1}; Pb = 24.6 mg kg^{-1}; Cr = 96.8 mg kg^{-1}; Hg = < 1.0 mg kg^{-1}; Ni = 28.5 mg kg^{-1}; Se = < 1.0 ² mg kg^{-1}; Ba = 296 mg kg^{-1} and Na = 678 mg kg^{-1}. The results were determined by the Laboratory of Fertilizers and Wastes of the Center for Research and Development of Soils and Environmental Resources belonging to the Agronomic Institute of Campinas (IAC).

The subsoil used was collected from an area at the Experimental Farm of FCAV/UNESP, at 20-40 cm depth. The soil of this area corresponds to a Dystrophic Red Latosol (Typic Haplorthox) with a clayey texture. The chemical characteristics were determined in the Laboratory of Soil Fertility (FertLab, FCAV/UNESP), with results: P resin = 1 mg dm^{-3}; K^+ = 07 $mmol_c$ dm^{-3}; CA^{2+} = 21 $mmol_c$ dm^{-3}; Mg^{2+} = 6 $mmol_c$ dm^{-3}; H + AL = 20 $mmol_c$ dm^{-3}; SB = 28 $mmol_c$ dm^{-3}; CEC = 48 $mmol_c$ dm^{-3}; BSI = 58%; OM = 9 g dm^{-3}; pH ($CaCl_2$) = 6.3 $mmol_c$ dm^{-3}.

Seeds of zinnia 'Red California Giants' used in this work were obtained from a commercial company, whose germination and purity information contained in the packaging were 86.0% and 99.0% respectively. Two seeds per cell were sown in expanded polystyrene trays of 98 cells with pyramidal bottom, with dimensions of 5.5 cm height, 3.5 cm top, 1.3 cm bottom and volumetric capacity of 30 mL per cell and were thinned after 5 days. The trays were suspended on steel benches 70 cm up from the ground in the greenhouse. Seedling irrigation was performed by automatic micro sprinklers, which remained on for one minute, at 1-hour intervals, with first irrigation at 6 am and the last at 6 pm.

After 21 days of seedling emergency, four plants/plot were evaluated for the following characteristics: shoot height (cm), obtained from the level of the substrate to the tip of the last leaf, and root length (cm) with the aid of a ruler graduated in centimeters; stem diameter (mm) determined at the level of the substrate, with the use of a digital pachymeter with precision of 0.01 mm (PRO DC-6, Western®); leaf number, verified by visual counting of the fully expanded leaves; leaf area (cm^2), using an electronic leaf area meter (Li-3100C, LI-COR®, Lincoln, Nebraska, USA); chlorophyll content, using a ChlorophiLOG device (CFL1030, FALKER®). Shoot dry mass (SDM), root dry mass (RDM) and total dry mass (TDM) were obtained after drying in an oven with forced air circulation at 70 ºC until reaching a constant weight and then weighed on a precision scale of 0.001g accuracy (AY220, SHIMADZU®); values were expressed in grams. The total dry mass was obtained by the sum of shoot and root dry mass.

From these measurements the following quality variables were determined: a) shoot height/stem diameter ratio (H/D); b) shoot dry mass / root dry mass ratio (SDM/RDM); and c) Dickson Quality Index (DQI), obtained by the formula DQI = [TDM/ (H/D + SDM/RDM)] according to Barroso et al. (2020) for the same species.

Data obtained were submitted to Shapiro-Wilk tests to analyse the normality of the residues and Bartlett's test for homogeneity of variance. Variance analysis (ANOVA) and polynomial regression analysis at 5% significance level ($p < 0.01$) were then performed, choosing the significant equations with the highest determination coefficient (R^2) (command lm). Pearson's correlation coefficient (r) ($p < 0.05$) (package coorplot) was also calculated among the characteristics. The statistical software used was R Studio® version 4.1.2 (R Core Team, 2021).

Results and Discussion

It was found that biosolid in the composition of the substrate had a significant effect ($p < 0.01$) in almost all morphological and quality characteristics evaluated of *Zinnia elegans* seedlings, not existing significance only for the shoot height/ stem diameter ratio (H/D). For the characteristics that presented quadratic behaviour it was possible to identify the maximum point for each of the tested biosolid proportions.

The shoot height showed a quadratic behaviour with means increasing until the 50.38% proportion of biosolid in composition of the substrate, followed by the reduction in the growth of the seedlings (Figure 1A). It is also observed that the treatments composed of biosolid obtained the highest means compared to the control treatment (100% subsoil). This result may be associated with the presence of nutrients in the biosolid, as shown in the chemical characterization performed, and therefore there were better nutrition and growth in height of the seedlings, as one of the main advantages of biosolid as a substrate is the better use of nutrients by the plants, since part of them are in organic form and are released gradually, supplying the nutritional needs of the seedlings adequately (Abreu et al., 2017). Thus, as there was no complementary fertilization for any of the treatments, the nutrients contained in the treatment composed only of subsoil were not adequate enough to help the growth of the seedlings, resulting in seedlings with undesirable characteristics.

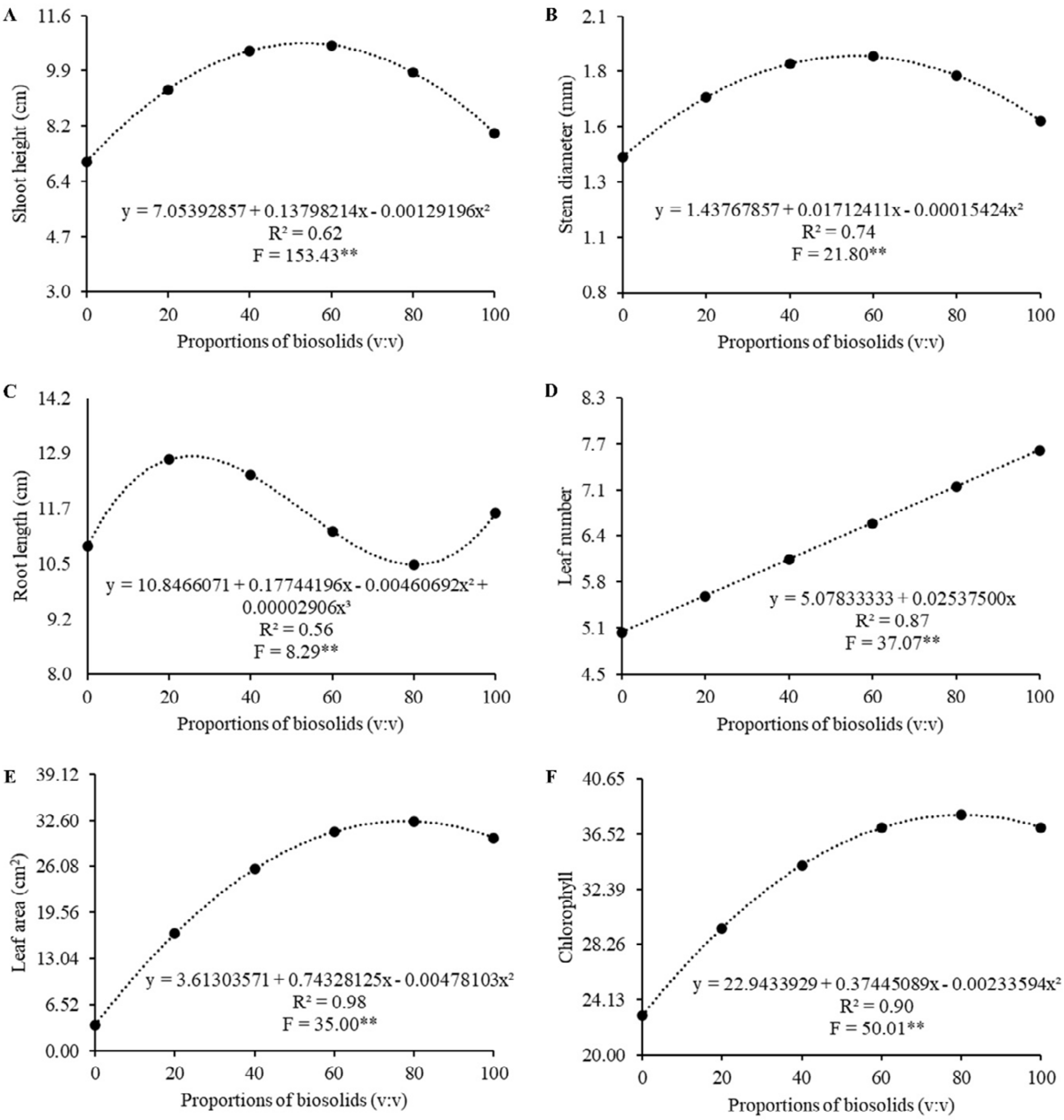

Figure 1. (A) Shoot height, (B) stem diameter, (C) root length, (D) leaf number, (E) leaf area and (F) total chlorophyll of *Zinnia elegans* Jacq. seedlings produced in substrates with different proportions of biosolid.
F-Test: ** Significant at 1% probability. Where: 0 = 100% subsoil; 20 = 20% biosolid + 80% subsoil; 40 = 40% biosolid + 60% subsoil; 60 = 60% biosolid + 40% subsoil; 80 = 80% biosolid + 20% subsoil; 100 = 100% biosolid.

For the ornamental species *Lantana camara* L., the authors Barone et al. (2018) also observed that sewage sludge, used pure or in mixtures with pupunha bark (*Bactris gasipae* Kunth) positively influenced the height growth of seedlings and attributed these findings to the high contents of nutrients and organic matter in the composition of these substrates. Additionally, Pêgo et al. (2019) when evaluating the quality of *Zinnia elegans* seedlings produced in alternative substrates obtained greater seedling height with the substrate obtained by composting agricultural waste and, Barroso et al. (2020) when producing seedlings of the same species in substrates based on carnauba bagasse observed an increase in height with the increase in the proportion of compost in the substrates. Thus, it is possible to observe that the presence of organic matter in the substrates has become indispensable for the growth in height of the seedlings, as reported by Cordeiro et al. (2020) and Oliveira et al. (2020) who stated that the organic portion is responsible for the supply of nutrients and presents the best results in the production of seedlings.

The stem diameter showed a quadratic behaviour with means increasing until the proportion of 56.56% of biosolid (Figure 1B). This is one of the most observed characteristics when evaluating the quality of seedlings in nurseries, as it can indicate the survivability and consequently the growth of seedlings in the field (Grossnickle and Macdonald, 2018).

The good development of the diameter stem, in addition to demonstrating a response to the growth of the seedling in relation to the treatments, is also related to a better distribution of phytohormones, carbohydrates, water supply and other nutrients to the aerial part, this being an essential variable for the continuous gain of dry mass, maintenance of metabolic and photosynthetic processes (Oliveira et al., 2013; Lima et al., 2018). Thus, it is possible to infer that the seedlings produced in substrates containing biosolid stood out compared to the control treatment (100% subsoil), indicating seedlings more suitable to be taken to the definitive cultivation site.

The root length trait fitted into the cubic regression model (Figure 1C). There was an increase of root length until the 20% biosolid proportion with a subsequent reduction until the treatment with 80% biosolid. It should be highlighted Zinnia is a fast-growing and short-cycle plant, therefore no need to use large volume containers, how should it be used for species that present slow growth or that will remain longer in the nursery (Cruz et al., 2016). Therefore, the tray used allows good root development. Sousa et al. (2011) and Pêgo et al. (2019) when evaluating the vigour and quality of *Zinnia elegans* seedlings produced in alternative substrates in trays of different cell sizes, observed little influence on the evaluated characteristics in relation to the container used, indicating that the species does not require large spaces for the production of its seedlings.

There was an increase in leaf number with the increasing proportion of biosolid in the substrate (Figure 1D). As for the leaf area (Figure 1E) and total chlorophyll (Figure 1F), quadratic regression was observed for both characteristics, with means increasing until the proportion of 79.06% and 81.39% of biosolid, respectively. It is also noted that for these characteristics, the increase in the proportion of biosolid in the composition of the substrate proved to be efficient as they outperformed the control treatment (100% subsoil).

Studies shown that an increase in the leaf number per plant correlates directly with an increase in leaf area (Menegatti et al., 2017), as observed in this work. Leaf area is of great importance in the evaluation of seedling quality, since the amount of photoassimilates in the plant is usually proportional to its leaf area and, plants that present high concentration of chlorophyll, are potentially able to achieve higher photosynthetic rates, and consequently better to adapt during the field planting phase (Cavalcante et al., 2016; Afonso et al., 2017).

Shoot dry mass (SDM), root dry mass (RDM) and total dry mass (TDM) exhibited a quadratic response on the regression analysis, with maximum increments in dry biomass of 66.84%, 52.54% and 63.42% respectively, with the increase of biosolid in the substrate (Figures 2A, 2B and 2C, respectively). According to Damasceno et al. (2019), shoot dry mass is an indicator of hardiness in a seedling and the higher efficiency obtained in solar energy uptake by this trait for photosynthesis and photoassimilate production results in a higher total dry weight (Souza et al., 2020)

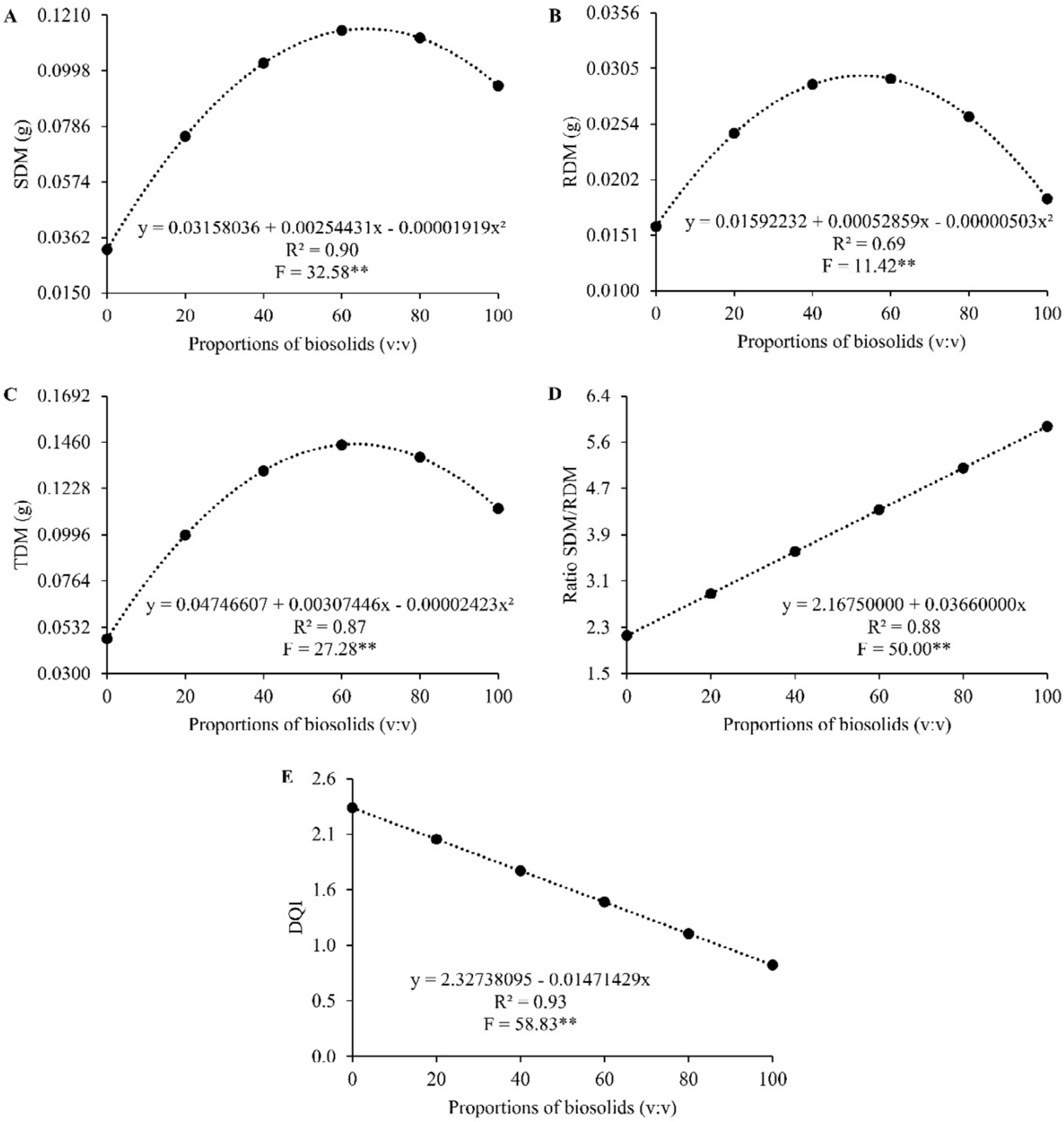

Figure 2. (A) Shoot dry mass - SDM, (B) root dry mass - RDM, (C) total dry mass - TDM, (D) shoot dry mass / root dry mass ratio - SDM/RDM and (E) Dickson Quality Index - DQI of *Zinnia elegans* Jacq. seedlings produced in substrates with different proportions of biosolid.
F-Test: ** Significant at 1% probability. Where: 0 = 100% subsoil; 20 = 20% biosolid + 80% subsoil; 40 = 40% biosolid + 60% subsoil; 60 = 60% biosolid + 40% subsoil; 80 = 80% biosolid + 20% subsoil; 100 = 100% biosolid.

Based on the results obtained for the root length (Figure 1C) correlated with the RDM (Figure 2B), it is possible to understand that there were a greater production in number of root apices by the plant, being in agreement with what was proposed by Afonso et al. (2012), who report that roots with greater amount of dry mass tend to show a greater number of root apices, mainly as a result of the nutritional richness present in the substrate. Furthermore, Pêgo et al. (2019) when evaluating the vigor and quality of *Zinnia elegans* seedlings produced in alternative substrates in trays of different cell sizes, found that the substrate obtained by composting agricultural waste provided the greatest increment in dry mass for the seedlings. Similarly, Barroso et al. (2020) when producing seedlings of the same species in substrates based on carnauba bagasse observed an increase in dry mass with the increasing proportion of compost in the substrates. Thus, it can be seen that the presence of organic compost in the substrates has a direct influence on the accumulation of dry mass for the ornamental species *Zinnia elegans*.

Shoot dry mass / root dry mass ratio (SDM/RDM) fitted for linear regression (Figure 2D). In general, the

SDM/RDM ratio is lower in environments with lower fertility and can be considered a plant strategy to withdraw the maximum nutrients in that condition (Caldeira et al., 2013).

The Dickson Quality Index (DQI) is considered a good indicator of seedling quality since it is an integrated morphological measure that considers the robustness and balance of the distribution of the seedling dry biomass (Abreu et al., 2019). Araújo et al. (2020), who report that the value for this index can vary according to the species, age of the seedling and treatment to which it was submitted, being considered a minimum value of 0.2. In this way it is possible to observe that all treatments produced seedlings of quality (Figure 2E).

According to Pearson's correlation matrix (r), there were significant positive and negative correlations between the analyzed characteristics (Figure 3). The shoot height/stem diameter ratio was the characteristic that correlated least with the others, with a positive correlation only with shoot height, indicating that this species guarantees a greater increase in height in its initial phase of seedling growth. There is also a significant negative correlation between leaf number and root length, indicating that these traits are inversely correlated with each other

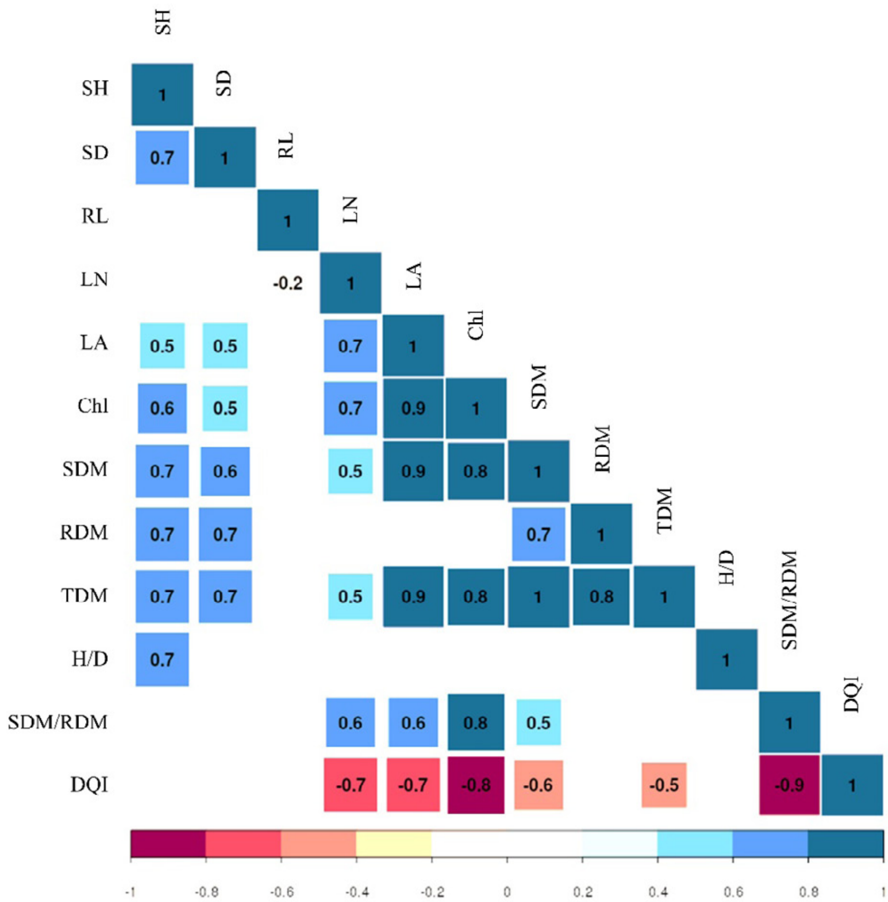

Figure 3. Pearson correlation matrix (r) between the characteristics and quality parameters of *Zinnia elegans* Jacq. seedlings produced in substrates with different proportions of biosolid.
Significant at 5% probability. The blank spaces indicate that there is no significant correlation between variables. SH = shoot height; SD = stem diameter; RL = root length; LN = leaf number; LA = leaf area; Chl = total chlorophyll; SDM = shoot dry mass; RDM = root dry mass; TDM = total dry mass; H/D = shoot height / stem diameter ratio; SDM/RDM = shoot dry mass / root dry mass ratio; and DQI = Dickson Quality Index.

The DQI correlated negatively and significantly with the other characteristics, especially SDM, TDM and SDM/RDM ratio, which are important parameters used to evaluate the quality of seedlings of several species. Therefore, it is understood that the higher the increment for these characteristics the lower the DQI of the seedlings. It should be noted that Barroso et al. (2020) found no significant effect for this same characteristic when producing *Zinnia elegans* seedlings in substrates based on carnauba bagasse, which may indicate that this characteristic has no direct influence on seedling quality for this species. Thus, the DQI should not be the only parameter used to evaluate the quality of *Zinnia elegans* seedlings. Therefore, the characteristics shoot height, stem diameter, leaf number and total chlorophyll should be considered, since they are easy to measure and are non-destructive analyses.

Conclusions

The use of biosolid in the proportion of 60% in the substrate composition proved to be efficient for the initial growth of *Zinnia elegans* seedlings as it showed the best responses for most morphological and seedling quality characteristics.

The destination of sewage sludge in biosolid presents as a viable alternative in the composition of the substrate, with this there is a reduction in the use of the soil to produce seedlings, and also in the optimization of the management, with the form of lighter trays.

Author Contribution

AMBS: Conceptualization, data curation, formal analysis, investigation, methodology, software, visualization and writing-original draft. **GRV:** Conceptualization, data curation, formal analysis, investigation, methodology, software, visualization, writing-review and editing and translation. **GS:** Conceptualization, investigation, methodology, visualization and writing-original draft. **KBF:** Conceptualization, data curation, investigation, methodology, visualization and writing-original draft. **TSC:** Conceptualization, data curation, investigation, methodology, visualization and writing-original draft. **KFLP:** Funding acquisition, methodology, project administration, resources, supervision, validation and writing-review and editing.

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