

Maximizing agricultural sustainability: determining the optimal biosolid dose for germination and initial growth of maize and cowpea

Maximizando a sustentabilidade agrícola: determinando a dose ideal de biossólido para germinação e crescimento inicial de milho e feijão-caupi

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RESUMO

Os resíduos gerados nas atividades industriais muitas vezes são descartados, mas o reaproveitamento desses materiais pode reduzir o impacto ambiental e agregar valor aos produtos. Esta pesquisa tem como objetivo identificar a dose ideal de biossólido resultante da produção de ácido láctico, para utilização como adubo orgânico na germinação de feijão-caupi (*Vigna unguiculata*) e milho (*Zea mays*). O experimento envolveu doze tratamentos: substrato comercial, 100% areia e proporções do biossólido (2,5, 5,0, 7,5, 10,0, 12,5, 15,0, 17,5, 20,0, 22,5 e 25,0%). Foram realizadas avaliações diárias de temperatura e pH e, após dez dias, foram realizadas avaliações morfológicas. Essas avaliações incluíram porcentagem de germinação, plântulas normais e anormais e índice de velocidade de emergência. Os resultados indicaram que o milho apresentou germinação mais resiliente em doses mais elevadas de resíduo biossólido, com menos anormalidades em comparação ao feijão-caupi. Doses superiores a 5% de biossólido tiveram efeitos adversos tanto na germinação quanto no crescimento das plântulas. Para o feijão-caupi, a dose de 2,5% de biossólido não prejudicou significativamente a germinação e o crescimento das plântulas. Porém, para o milho, embora a dose 2,5% não tenha afetado a porcentagem de germinação, diminuiu a velocidade de emergência e o comprimento da raiz.

Palavras-chave: economia circular, agroecologia, plântulas anormais, *Zea mays*, *Vigna unguiculata*

ABSTRACT

Waste generated from industrial activities is often disposed of, but reusing these materials can reduce environmental impact and add value to the products. This research aims to identify the ideal dose of biosolid resulting from the lactic acid production, for use as organic fertilizer in germinating of cowpea (*Vigna unguiculata*) and maize (*Zea mays*). The experiment involved twelve treatments: commercial substrate, 100% sand, and proportions of the biosolid (2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5, 20.0, 22.5 and, 25.0%). Daily assessments of temperature and pH were carried out, and after ten days, morphological evaluations were performed. These evaluations included germination percentage, normal and abnormal seedlings, and the emergency speed index. Results indicated that maize showed more resilient germination at higher doses of biosolid residue, with fewer abnormalities compared to cowpea. Doses exceeding 5% of biosolid had adverse effects on both germination and seedling growth. For cowpea, a 2.5% residue dose did not significantly hinder seedling germination and growth. However, for maize, while the 2.5% residue did not affect the germination percentage, it did decrease the emergency speed and root length.

Keywords: circular economy, agroecology, abnormal seedlings, *Zea mays*, *Vigna unguiculata*

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1. INTRODUCTION

The traditional linear production model, prevalent since the onset of industrialization, involves extracting raw materials, manufacturing goods, consuming them, and then disposing of the waste. This linear process contributes significantly to environmental impacts globally, with industrial residues playing a substantial role. In contrast, the circular economy model advocates for the closure of linear production processes by reintegrating waste into a productive cycle. By doing so, it minimizes environmental disposal and reduces the need for extracting new raw materials (OLIVEIRA et al., 2020).

Biosolid residues, originating from a variety of sources and subjected to different treatment processes, are characterized by their richness in organic matter and essential nutrients. One common source of biosolid is effluent treatment stations (ETS), where the treated waste, known as biosolids, is often utilized in agriculture. Many studies have explored the use of ETS residues in agricultural crops. Repurposing industrial residues, such as tailings, into fertilizers presents a cost-effective solution, saving resources that would otherwise be devoted to disposal (MORAIS et al., 2021; MARCHUK et al., 2023).

Lactic acid is a crucial ingredient in food and beverage products due to its diverse functional qualities. The wide range of applications of lactic acid in the food and beverage sector and the variety of functional benefits offered by food acidulants drive the market. The industry is driven by solid expansion in the food and beverage sector. With evolving consumer behavior and economic development in Asia-Pacific, the region is emerging as a potential market for the food and beverage industry to increase the use of food acidifiers. Lactic acid is also widely used in the preservation industry. For example, it is permitted as a food additive in Europe, United States, Australia, Brazil and New Zealand. Hence, the increasing use of lactic acid in various food manufacturing processes could increase demand across the world (MORDOR, 2024).

Despite the potential benefits, the biosolid from lactic acid production treatment stations remains underexplored as a potential organic fertilizer for use in agriculture. As a result, there is a need for exploratory research to assess its suitability as an organic fertilizer and to investigate potential impacts on agricultural crops at various growth stages.

Cowpea (*Vigna unguiculata* (L.) Walp.) holds significant importance in the Niger, Nigeria and Brazilian market and in the last mentioned country it is primarily concentrated

in the northeast, with increasing cultivation in other regions due to its adaptability and market acceptance. Meanwhile, maize (*Zea mays* L.) is a staple crop, cultivated across various scales of agriculture throughout world (CONAB, 2023).

This study aims to evaluate the viability of using biosolid from an industrial effluent treatment station, specifically one that processes lactic acid, for the production of cowpea and maize crops. The objective is to assess its influence on the germination and initial growth of these plants.

2. MATERIALS AND METHO

Two similar experiments were conducted in a B.O.D. chamber with cowpea seeds (*Vigna unguiculata* (L.) Walp.) cv. 'Poços de Caldas' and maize seeds (*Zea mays* L.) cv. 'AL Bandeirantes'. Cowpea seeds exhibited 95% germination, whereas maize seeds displayed 98% germination, before starting the experiments.

Each experiment comprised the following treatments: commercial substrate; 100% sand; 97.5% sand + 2.5% biosolid; 95% sand + 5% biosolid; 92.5% sand + 7.5% biosolid; 90% sand + 10% biosolid; 87.5% sand + 12.5% biosolid; T8: 85% sand + 15% biosolid; T9: 82.5% sand + 17.5% biosolid; 80% sand + 20% biosolid; 77.5% sand + 22.5% biosolid and; 75% sand + 25% biosolid.

Both experiments utilized a randomized block experimental design with four replications. The experimental units consisted of 500 mL plastic containers filled with the substrates corresponding to each treatment.

The treatments were prepared by blending the biosolid with sand in the specified proportions until complete homogenization. The commercial substrate and 100% sand served as the controls. The biosolid originated from an effluent treatment station in the lactic acid production industry. The biosolid chemical composition was characterized, yielding the following values: pH 6.6; N; P₂O₅; K₂O; Ca; Mg; and C were 37.03, 25.83, 7.01, 157.87, 2.03, and 129.60 g kg⁻¹, respectively, while Fe; Cu; Zn; and Mn were 10408, 50, 656, and 312 mg kg⁻¹, respectively.

After blending the biosolid with sand, cowpea and maize seeds were sown in each experiment, with two seeds per container, at a depth of 2 to 3 cm. The containers were maintained in the B.O.D. chamber under a photoperiod of 16:8 h (light: dark) and a controlled temperature of 25°C ± 2°C, following the Rule for Seed Analysis (RAS, 2009).

The experiments were monitored daily after sowing, with irrigation performed to maintain substrate moisture at optimal levels for seed germination. After ten days of growth, assessments were conducted. Plants were carefully extracted from the containers to preserve root integrity and remove adhered particles. Temperature and pH were measured daily using a measuring device inserted into the substrate or sand and biosolid mixture in all treatments.

Germination percentage was calculated according to Labouriau and Valadares (1976) using the formula $G = (N/A) \times 100$, where G represents germination, N is the total number of germinated seeds, and A is the total number of seeds sown.

Seedlings were classified as normal if they exhibited potential for continued development, regardless of minor imperfections. Normal seedlings displayed well-developed, complete, proportional, and healthy structures. Seedlings with minor imperfections, despite displaying some anomalies, were considered normal due to satisfactory and balanced development (BRAZIL, 2009).

The Emergency Speed Index (ESI) was calculated by counting daily emerged seeds and utilizing the formula $ESI = E1/N1 + E2/N2... + EN/NN$, where ESI represents the Emergency Speed Index, E1, E2, and EN correspond to the number of seedlings counted on the first, second, and last days, respectively, and N1, N2, and NN correspond to the number of days from sowing to the first and second days of counting, respectively (MAGUIRE, 1962).

Seedling length was assessed at the finish of the experiment. Root volume was determined using a known volume of water in a beaker, and aerial parts and roots were dried in a greenhouse with forced air circulation at 65°C for 72 hours. Samples were weighed on an analytical balance to determine dry matter mass of the aerial parts and roots.

The obtained data were subjected to analysis of variance, and means were compared using the Duncan Test at a 5% probability level, employing the statistical software System for Statistical and Genetic Analysis (SAEG, 2007).

3. RESULTS AND DISCUSSION

3.1. Temperature and pH

In cowpea and maize experiments, the overall temperature fluctuation between the lowest and highest values measured was 2°C (Figures 1A and 1B). In cowpea experiment, was found that the commercial substrate and treatments with 15% and 22.5% biosolid did

not significantly differ from each other ($p < 0.05$). The highest average temperature, approximately 26°C, was observed in the treatment with 20% biosolid residue, although this value did not significantly differ from the other treatments (Figure 1A). In maize substrates, control treatments (commercial substrate and 100% sand) yielded the lowest values, with commercial substrate and treatments with 100% sand, 2.5%, 7.5%, and 22.5% biosolid not differing significantly from each other ($p < 0.05$), and 100% sand showing no difference from the commercial substrate, 2.5%, 5%, 7.5%, 22.5%, and 25% biosolid treatments (Figure 1B).

Cowpea experiment, regarding pH levels, doses of 2.5%, 5%, 7.5%, 10%, 12.5%, and 25% biosolid did not significantly differ from each other ($p < 0.05$). Similarly, the commercial substrate and treatments with 15%, 17.5%, 20%, and 22.5% biosolid did not show significant differences in pH values ($p < 0.05$), recording the lowest values overall (Figure 1C). However, in maize experiment, the variation between the lowest and highest pH values was 2.1, indicating a greater variation compared to the cowpea experiment (Figure 1C and 1D). In terms of average comparisons, the treatment with 100% sand exhibited the highest pH value (6.5), surpassing all other treatments (Figure 1D), consistent with the observations in cowpea experiments (Figure 1C).

In various studies involving sludge, it has been noted that the residue can indeed impact the pH of the substrate. When the residue is treated with lime, it tends to increase the pH value, whereas when untreated, it may decrease the pH due to nitrification processes occurring in sewage sludge (NASCIMENTO et al., 2004; NOGUEIRA et al., 2006). However, when utilizing biosolid waste from an effluent treatment station in lactic acid production without prior treatment, there was no observed interference with pH significant enough to negatively affect the germination and development of cowpea and maize seedlings.

In a study with maize and biosolid from a sewage treatment station after treatment and stabilization, it was found that increasing the biosolid dose from 1 kg/portion to 4 kg/portion resulted in decreased germination rates, dropping from 92% at the lowest dose to 83% at the highest dose (COSTA et al., 2019). It's crucial to highlight that for successful germination; the substrate must retain moisture and readily provide necessary nutrients for initial seedling development. Organic matter content and structure also play pivotal roles in this effectiveness (CANESIN and BARBOSA, 2017).

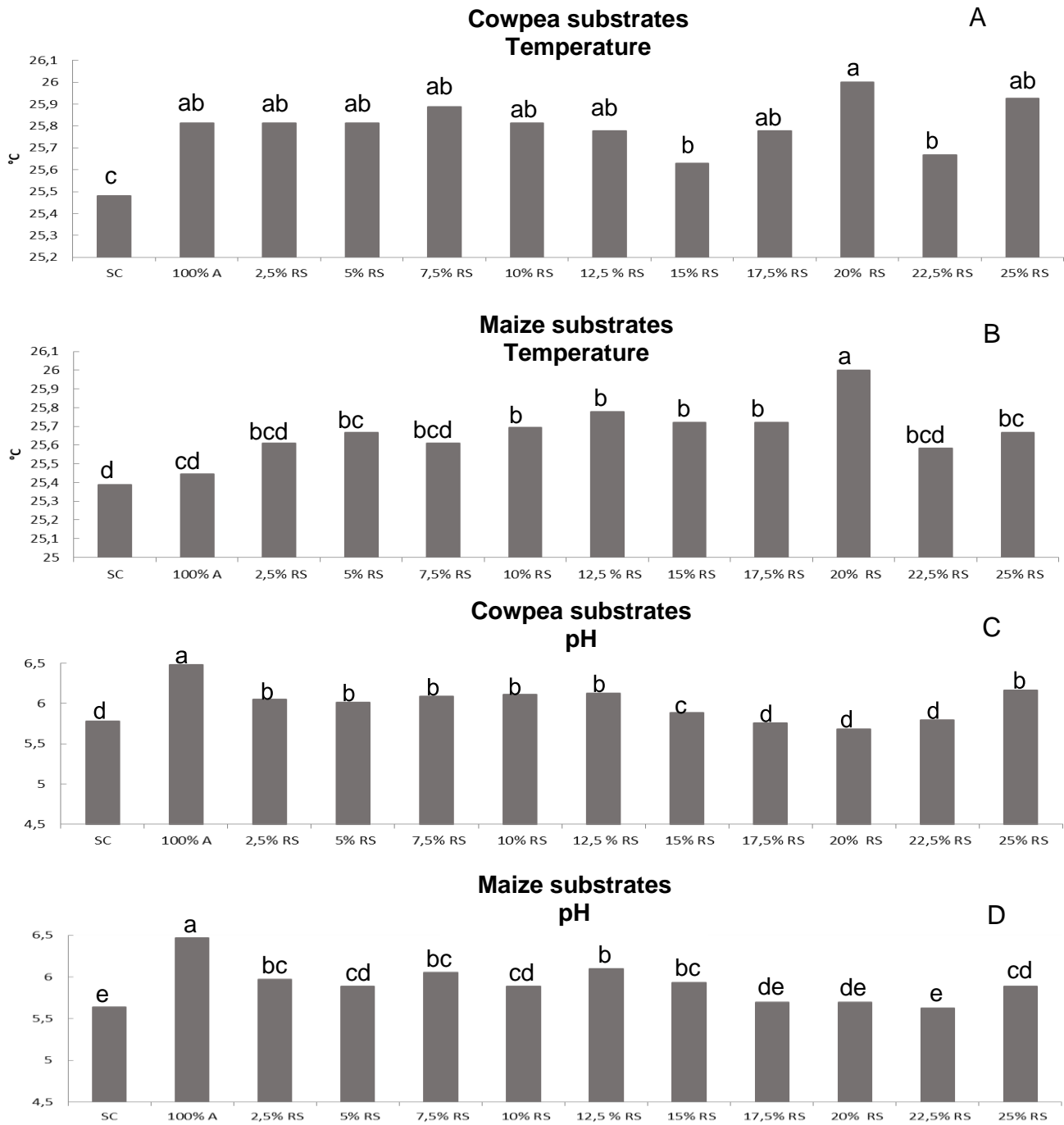


Figure 1. Temperature and pH considering commercial substrate (SC), 100% sand (100% A) and biosolid doses (RS) in cowpea (A, C) and maize (B, D) experiments. Columns followed by the same letters do not differ from each other by Duncan's Test at 5% probability.

3.2. Germination, ratio normal / abnormal seedlings and emergency speed index (ESI)

Cowpea bean germination reached 100% in the control treatments (commercial substrate and sand) and in treatments containing 2.5% and 5.0% biosolid (Figure 2A).

However, doses of 7.5% and 10% biosolid led to approximately a 50% decrease in germination, while with a dose of 12.5% biosolid residue, only 20% of the seeds germinated. Doses of biosolid exceeding 15% resulted in seed rotting and failure to germinate (Figure 2A). No seed germination was observed in treatments which corresponded to 15%, 17.5%, 20%, 22.5%, and 25% biosolid indicating the threshold doses for the use of this biosolid as a substrate for cowpea germination (Figure 2A).

Maize germination reached 100% in the 100% sand treatment. The 2.5% biosolid dose resulted in 87% germination, similar to that obtained with the commercial substrate. However, doses of 5% and 7.5% biosolid led to approximately a 25% decrease in germination. With the 12.5% biosolid dose, there was a decrease of over 60% in germination. In the treatment with 10% biosolid residue, only 25% of the seeds germinated, while treatments with 15% and 17.5% biosolid exhibited only 13% germination (Figure 2B).

No maize germination was observed in doses 20%, 22.5%, and 25% biosolid residue, indicating a threshold concentration for the use of this biosolid as a substrate (Figure 2B).

Notably, the amplitude of daily temperature variation across treatments did not seem sufficient to account for the observed effects on cowpea and maize seeds germination (Figures 1A, 1B, 2A and 2B), indicating no direct relationship between substrate temperature and germination. Additionally, no relation was observed between pH values and the lack of germination in treatments containing 20%, 22.5%, and 25% biosolid (Figures 1C, 1D, 2A and 2B).

None of the treatments achieved 100% normal cowpea seedlings; the treatment with 100% sand exhibited 50% normal seedlings, whereas the treatment with 2.5% biosolid showed 66.7% normal seedlings. Treatments with 7.5% and 12.5% biosolid solely produced abnormal seedlings, with proportions of 50% and 16.7%, respectively (Figure 1C).

The treatments with commercial substrate, 100% sand, and 2.5% biosolid yielded 87.5% normal maize seedlings. Conversely, treatments with 5%, 7.5%, 12.5%, 15%, and 17.5% biosolid resulted in 12.5% abnormal seedlings, with the latter two treatments not producing any normal seedlings. In contrast, all germinated seeds in the treatments with commercial substrate and 2.5% and 10% biosolid developed into normal seedlings. Notably, treatments with commercial substrate and 2.5% biosolid yielded three times more normal plants than the treatment with 10% biosolid (Figure 1D).

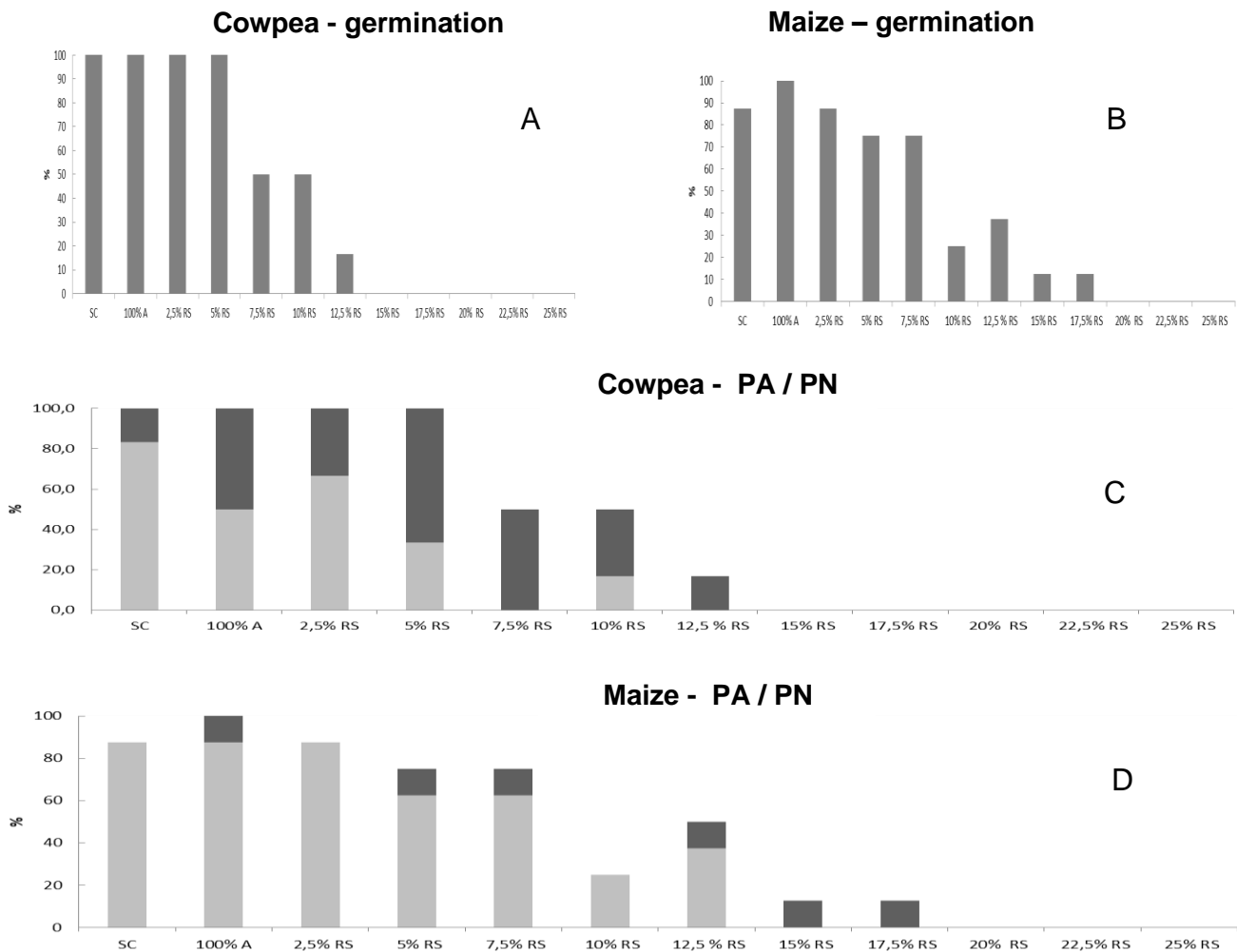


Figure 2. Percentages of germination and, abnormal - PA and normal - PN seedlings of cowpea and maize considering commercial substrate (SC), 100% sand (100% A) and biosolid doses (RS).

According to Madejón et al. (2015), seed germination is considered a critical parameter in evaluating the phytotoxicity of organic compounds. In a study conducted with ETA Sludge (a water station) in the cultivation of guandu beans (*Cajanus cajan*) and millet (*Pennisetum glaucum*), it was observed that the percentage of germination of guandu beans showed satisfactory results with an average of 90% in almost all treatments. In contrast, millet did not display significant differences in this parameter across the tested treatments (BITENCOURT et al., 2020).

It was observed that the concentration of biosolid in the substrate inversely correlated with the speed of cowpea seed germination (Figure 3A); in other words, treatments with higher biosolid concentrations exhibited delayed emergence. However, doses of 2.5% and

5% biosolid demonstrated intermediate ESI values compared to those of the commercial substrate and sand (controls), suggesting that these biosolid concentrations do not adversely impact the emergence rate of cowpea seeds.

Higher doses of biosolid led to lower ESIs from maize, with the exception of the 12.5% biosolid dose, which exhibited a higher ESI than the 10% biosolid dose (Figure 3B). Even at the lowest dose (2.5% biosolid), ESI decreased and was lower than those observed in the commercial substrate and 100% sand treatments.

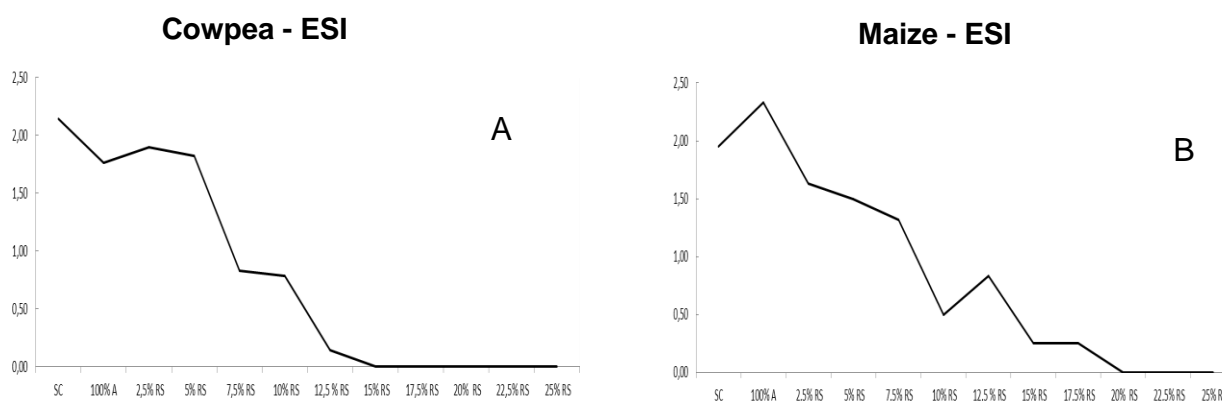


Figure 3. Emergency Speed Index (ESI) considering commercial substrate (SC), 100% sand (100% A) and biosolid doses (RS) in cowpea and maize.

3.3. Dry matter and length of aerial part

Cowpea presented a higher value of aerial part dry matter (DMAP) with a dose of 5% of biosolid, compared to controls, and this dose did not present a significant difference in relation to the dose of 2.5% of biosolid. Conversely, all other doses, including 2.5% biosolid residue, did not significantly differ from the control treatments (Figure 4A).

For maize DMAP, doses of 2.5% and 5% biosolid did not differ ($p < 0.05$) from the commercial substrate and 100% sand treatment. However, the 7.5% biosolid dose resulted in DMAP comparable to the 100% sand control, while doses of 10% and 12.5% biosolid led to approximately three times lower DMAP compared to maize sown in 100% sand. Further, doses of 15% and 17.5% biosolid resulted in an even greater decrease in DMAP (Figure 4B).

Regarding the length of cowpea aerial part (APL), it was found that doses of 2.5%, 5%, 7.5%, 10%, and 12.5% biosolid did not exhibit differences ($p < 0.05$) from the controls

(Figure 4C). For maize, APL there was a significant decrease ($p > 0.05$) from the 7.5% biosolid dose compared to controls (commercial substrate and 100% sand). APL of maize seedlings from the 7.5% biosolid dose was less than half of that observed in the commercial substrate and 100% sand controls (Figure 4D).

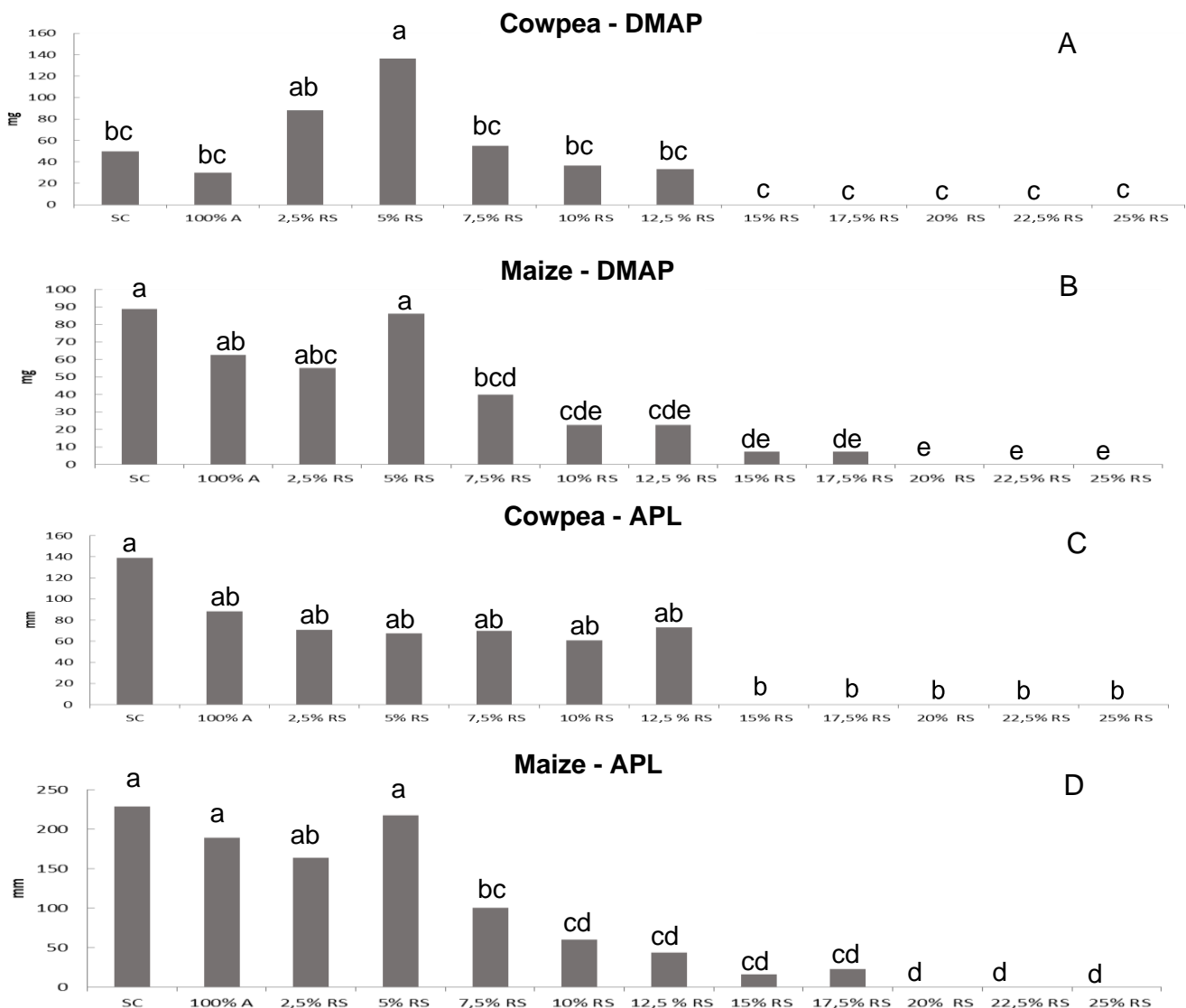


Figure 4. Dry matter of the aerial part – DMAP and aerial part length – APL considering commercial substrate (SC), 100% sand (100% A) and biosolid doses (RS) in cowpea and maize germination tests. Columns followed by the same letters do not differ from each other by Duncan's Test at 5% probability.

3.4. Dry matter, length and volumes of roots

Commercial substrate, 100% sand, 2.5%, 5%, 7.5%, and 10% biosolid doses did not show differ ($p < 0.05$) in dry root matter (DRM) of cowpea (Figure 5A). In maize DRM, doses

of 2.5% and 5% biosolid did not differ ($p < 0.05$) from the commercial substrate and 100% sand treatment, while the 12.5% biosolid dose resulted in DRM comparable to the 100% sand control (Figure 5A). Conversely, doses of 7.5%, 10%, 15%, and 17.5% biosolid led to more than a threefold decrease in maize DRM compared to maize sown in 100% sand (Figure 5B).

For cowpea root length (RL), doses of 2.5% and 5% did not differ ($p < 0.05$) from the control (100% sand) but resulted in RL lower than that observed with the commercial substrate. Doses of 7.5% and 10% biosolid exhibited RLs lower than the controls (Figure 5C). The commercial substrate yielded the longest RL in maize seedlings, followed by the 100% sand treatment. However, all doses of biosolid resulted in decreases of more than 50% in RL compared to the commercial substrate and 100% sand. Doses of 2.5% and 5% of biosolid did not differ from each other ($p < 0.05$), while from the 7.5% biosolid dose, lower RL values were observed ($p > 0.05$) (Figure 5D).

Regarding cowpea root volumes (RV), the averages of the control treatments (commercial substrate and 100% sand) and the 2.5% biosolid dose were the largest. However, the 2.5% dose resulted in a root volume that did not differ significantly from doses of 5.0%, 7.5%, and 10% biosolid (Figure 5E). As for maize RV, a significant decrease ($p > 0.05$) was observed from the 7.5% biosolid dose. Compared to controls (commercial substrate and 100% sand), this decrease in RV exceeded 50% from the 7.5% biosolid dose. Conversely, doses of 2.5% and 5% biosolid resulted in RV that did not differ ($p < 0.05$) from the commercial substrate and 100% sand treatment (Figure 5F).

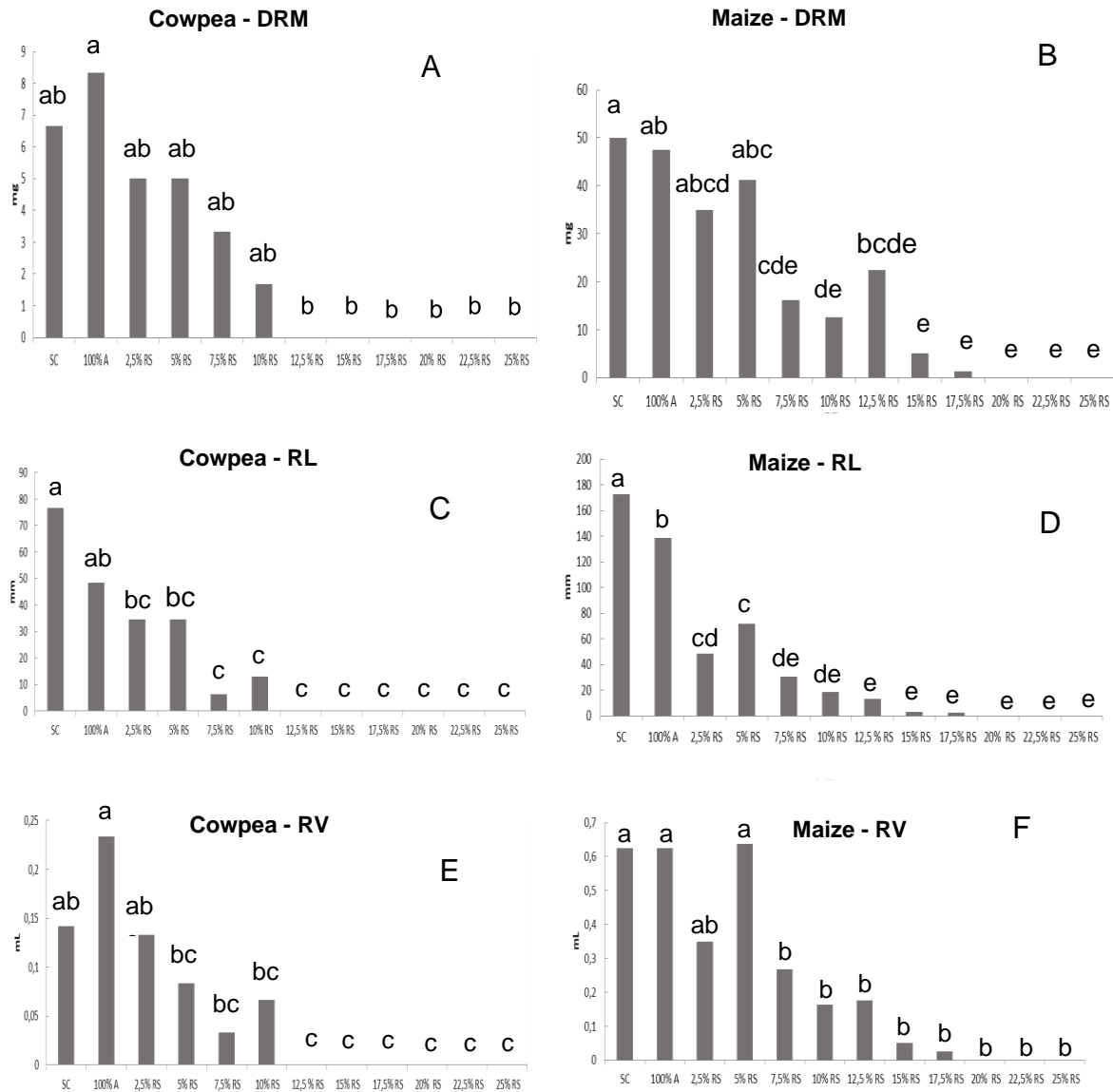


Figure 5. Dry root matter – DRM, root length - RL and root volume – RV considering commercial substrate (SC), 100% sand (100% A) and biosolid doses (RS) in cowpea and maize germination tests. Columns followed by the same letters do not differ from each other by Duncan's Test at 5% probability.

Considering the cowpea and maize, it is noteworthy that in variables related to the aerial part and roots, 2.5% and 5% biosolid doses exhibited notable performance across all analyzed variables. In a comprehensive analysis, it was observed that doses of 2.5% and 5.0% biosolid maintained germination at 100% in cowpea (Figure 1A), and the emergency speed was comparable to that of the treatment with 100% sand (Figure 2A). Additionally, these doses did not result in a decrease ($p < 0.05$) in dry matter and length of the aerial part and roots compared to the treatment with 100% sand (Figures 4A, 4C, 5A and 5C). However,

the biosolid 5% dose led to a higher proportion of abnormal plants (Figure 1B) and a lower root volume (Figure 5E) compared to the treatment with 100% sand.

In maize, 2.5% biosolid dose maintained germination and the number of normal plants similar to the commercial substrate (Figure 1C and 1D). In general, 2.5% and 5% biosolid doses did not differ ($p < 0.05$) from the control treatments and consistently yielded among the highest averages in most of the variables analyzed (Figures 4B, 4D, 5B and 5F). However, for ESI and root length did the control with commercial substrate stand out (Figures 2B and 5D).

Consequently, the 2.5% biosolid dose emerged as the most promising, as it yielded germination and biometric characteristics of cowpea seedlings comparable to those of the control with 100% sand (Table 1). However, for maize, it is advisable to conduct a new experiment by evaluating a dosage of less than 2.5% of biosolid.

Tabela 1. Maximum biosolid doses (%) added to sand that did not affect negatively both cowpea and maize germination and initial growth

Characteristic	Cowpea	Maize
Germination	5,0	2,5
Normal plants	2,5	2,5
ESI*	5,0	0,0
Dry matter of aerial part	12,5	5,0
Length of aerial part	12,5	5,0
Dry matter of roots	10,0	5,0
Length of roots	5,0	0,0
Root volume	2,5	5,0

Legenda: ESI – Emergency Seed Index

Studies by Liu et al. (2014) utilizing biosolid ETS as a substrate for tomato and lettuce germination indicated that the presence of soluble salts in biosolid ETS could inhibit seedling germination and growth. However, various methods exist to mitigate this issue, such as mixing with underground or raw soil, vermiculite, agricultural residue, or undergoing a water elution process (LIU et al., 2014; ZALLER, 2007).

Similarly, Cai et al. (2010) reported inhibition of seedling germination and growth in experiments using sewage sludge composted with cucumber, tomato, and pepper crops. They found that composted sewage sludge with higher salt content inhibited aerial part length and tomato biomass, while pepper showed inhibition of biomass index. Lower salt

content resulted in smaller stem diameters, heights, biomass, and seedling indexes in tomatoes and peppers.

Bitencourt et al. (2020) observed increased root growth for guandu beans with a mixture treatment of soil, ETA sludge, bagasse, and vinasse, while millet showed higher values only in the control treatment with soil. Toxic compounds present in the substrate primarily affect germination and root growth, as noted by Mata et al. (2010).

It's important to note that a 2.5% biosolid dose would equate to an application of 50 cubic meters of biosolid per hectare if incorporated at a depth of 20 cm. However, further parameters must be considered before recommending this application. Nonetheless, this study suggests that even below this dose, there will be no negative effects on the germination of cowpea seeds.

4. FINAL CONSIDERATIONS

In conclusion, for cowpea beans, the optimal dose appears to be 2.5% biosolid residue, as it did not significantly impair germination or seedling growth. However, for maize, while the 2.5% biosolid dose did not affect the percentage of germination, it did lead to decreased germination speed and root length.

Doses exceeding 5% of biosolid residue, derived from effluent treatment stations in the lactic acid production industry, have been found to be detrimental to the germination and growth of both cowpea and maize seedlings.

These findings underscore the importance of careful consideration when utilizing lactic acid's biosolid from industrial effluent treatment stations as a substrate for seed germination and seedling growth. Lower doses, such as 2.5%, may offer a balance between utilizing waste materials and ensuring successful plant growth, particularly for cowpea. Further research into optimal application methods and potential mitigation strategies may enhance the utilization of such residues in agricultural practices while minimizing negative impacts on plant growth and development.

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