

EFFICACY OF BREWER'S SPENT YEAST CONCENTRATIONS
AND APPLICATION METHODS ON *Coffea arabica* SEEDLING
DEVELOPMENT

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Abstract

Brewer's spent yeast (BSY) contains *Saccharomyces cerevisiae*, which is a byproduct that may be used as a biofertilizer and plant growth promoter. Improving the understanding of the ideal BSY concentration and application method for coffee seedling development will enable more targeted application strategies. We hypothesized that a given BSY concentration and application method would improve the development of coffee seedlings by adding sources of organic matter containing yeast. This hypothesis was tested by assessing the development parameters of *Coffea arabica* var. Typica (Cramer variety) using different concentrations (0, 2.35, 4.71, 9.40, 11.75 x 10⁸ *S. cerevisiae* cells mL⁻¹) and two application methods (foliar-FA and soil-SA). Our analysis revealed that BSY foliar application was more efficient than soil application in improving coffee seedling development. Foliar application at 2.3 x 10⁸ *S. cerevisiae* cells mL⁻¹ increased fresh shoot weight (2.35 times), fresh root weight (4.22 times), dry shoot weight (2.23 times), and dry root weight (3.62 times) in coffee plants compared to the negative control (sterile solution). Our findings provide evidence of waste reuse as an alternative method for developing coffee seedlings through the addition of materials rich in organic matter and yeast. They also align with the Sustainable Development Goals (SDGs), demonstrating strategies that support sustainable agriculture and circular economy practices.

Keywords: Agro-industrial by-products. *Coffea arabica* L. Plant growth promotion. *Saccharomyces cerevisiae*.



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1. Introduction

In recent years, there has been a rapid growth in beer consumption and production, making it the third beverage most consumed in the world, reaching 186 million hectoliters in 2021 (Piva et al. 2021; Fan et al. 2023; Ciocan et al. 2023). Beer production generates a large amount of waste, which may become an environmental liability if not properly managed. Conversely, brewer's spent yeast (BSY) represents an alternative revenue stream for producers due to its richness in monosaccharides and polysaccharides (Mussatto et al. 2006). It offers potential applications in human and animal nutrition (McCarthy et al. 2013; Chetrariu and Dabija 2023). Additionally, BSY is regarded as a plentiful and renewable substrate suitable for biorefinery processes (Djukić-Vuković et al. 2016) and agricultural applications, as it is rich in organic matter and yeast (Mata et al. 2019; Wang et al. 2021).

Yeast-containing waste has represented an important aspect of sustainable agricultural research, as yeast promotes plant growth (Fernandez-San Millan et al. 2020). This is attributed to different mechanisms, namely the production of active substances such as amino acids, phytohormones, vitamins, and NH_3 ; the solubilization of inorganic phosphate or zinc; and iron sequestration via siderophores (De Souza et al. 2019; Fernandez-San Millan et al. 2020). It also highlights the role of yeast as an effective biocontrol agent in combating plant diseases by inhibiting pathogen colonization (Tenório et al. 2019; Ramos-Garza et al. 2023; Sipiczki 2023). *Saccharomyces cerevisiae* (Desm.) Meyen is the yeast contained in BSY (Giannakou et al. 2021). It stands out as a promising species (Texeira et al. 2021; Sipiczki 2023) for plant development (Freimoser et al. 2019; Cabrini et al. 2019; Ramos-Garza et al. 2023).

Coffee seedling production is an important issue in agriculture because it is one of the main beverages consumed worldwide (Lu et al. 2022). It is also considered a primary crop in more than 80 countries (Fonseca et al. 2022) and plays a fundamental role in the economic livelihood of millions of people worldwide (Zhang et al. 2019). However, coffee seedling production has been threatened by nutrient deficiency (Fonseca et al. 2022) and diseases (Duong et al. 2020; Júnior et al. 2020), increasing the costs associated with the rising need for agricultural pesticide management and application (Ventura et al. 2017). The restricted access to technology among coffee growers underscores the need for exploring alternative methods for producing high-quality coffee seedlings while reducing the reliance on chemical products (Martínez et al. 2022).

Previous studies demonstrate the potential effectiveness of using fungi as growth promoters for coffee seedlings in a phosphorus-unlimited substrate, with increases of up to 13% in root dry mass and 15% in root volume (Araújo et al. 2020). Despite the increasing interest in sustainable agricultural practices, the potential of BSY containing *S. cerevisiae* to enhance the growth of plant seedlings remains unexplored. Given the relevance of minimally invasive agricultural practices for the environment, understanding and establishing the conscious use of microorganisms over synthetic inputs is becoming increasingly urgent. Additionally, finding use for the large amount of waste produced by craft breweries contributes to circular economy principles and reinforces the development of ecologically sound agricultural practices. Thus, considering the existing gaps in BSY use in large-scale crops, the limited understanding of its potential (Millán et al. 2020; Vargas et al. 2024), and the richness in yeast and organic matter, BSY emerges as a promising sustainable alternative for coffee seedling production. Based on this, the present study hypothesizes that different BSY (*S. cerevisiae*) concentrations and application methods enhance the growth of coffee seedlings.

2. Materials and Methods

Brewer's spent yeast (BSY) characterization

The BSY used in this study was derived from the fermentation process, with *Saccharomyces cerevisiae* as the fermentation agent in substrates supplemented with malt and hops. It was sourced from a craft brewery located in Garanhuns, Pernambuco, Brazil. The waste was analyzed for yeast cell quantification (Johnson and Curl, 1972), yielding values of 12.9×10^7 CFU g^{-1} . Solutions containing BSY with *S. cerevisiae* were prepared at a concentration of 4.71×10^8 *S. cerevisiae* cells mL^{-1} .

The chemical attributes of BSY were determined and measured using standard laboratory protocols (Table 1).

Table 1. Characterization of the chemical properties of brewer's spent yeast used in the experiment.

pH	OM	P	S-SO ₄ ²⁻	Ca ²⁺	Mg ²⁺	K ⁺	TOC	
(H ₂ O)	(%)	----- (mg L ⁻¹) -----						
4.02	6	10	<0.11	<0.059	<0.1	252	3.49	
N	Fe ²⁺	Mn ²⁺	Cu ²⁺	Zn ²⁺				
----- (mg L ⁻¹) -----								
1.740	<0.014	17	1.54	4.2				

OM = organic matter; TOC = total organic carbon.

Effect of BSY concentration and application methods on coffee seedling development.

Different volumes of BSY (0, 10, 20, 40, and 50 mL) were diluted in sterile distilled water to a final volume of 200 mL, yielding concentrations of 0, 2.35, 4.71, 9.40, and 11.75 × 10⁸ *S. cerevisiae* cells mL⁻¹. An aliquot of 20 mL from each solution was applied per seedling/sample using two methods: foliar application (FA) and soil application (SA).

The experiment was conducted at the Federal University of Agreste de Pernambuco, Garanhuns, Pernambuco, Brazil (8°56'18.6" S; 36°28'57.9" W). The climate is classified as Cwa (Köppen classification), with a mean precipitation of 1300 mm yr⁻¹ and an annual mean temperature of 20 °C. The experimental design was completely randomized, arranged in a 5 × 2 factorial scheme. The first factor was the crude volume of BSY (0, 10, 20, 40, 50 mL). The second factor was the application method, comprising FA and SA with four replications.

Coffee seeds (*Coffea arabica*) were sown using an indirect method in germinators containing a 15–20 cm layer of sand distributed at a density of 1 kg m⁻². Microcosms were prepared using typical Neosol collected from the 0–20 cm surface layer of a native forest fragment. Table 2 details the physicochemical properties of the soil. The soil was placed into polyethylene bags with a capacity of 1 kg each and amended with sugarcane filter cake as an organic matter source (Total Nitrogen = 1.37%, Phosphorus = 1.69%, Potassium = 0.24%).

The seedlings were transplanted 60 days after sowing, when all plants had fully developed cotyledon leaves, commonly referred to as “jaguar ears.” The application of the BSY solution began 75 days after sowing, coinciding with the appearance of the first pair of true leaves in all seedlings.

In FA treatments, 20 mL of BSY (concentrations: 0, 2.35, 4.71, 9.40, and 11.75 × 10⁸ *S. cerevisiae* cells mL⁻¹) solution was sprayed over the entire leaf surface of the coffee seedlings until complete coverage. In SA treatments, 20 mL of the BSY solution was applied directly to the soil. Standard regional coffee cultivation practices were adopted throughout the experimental period.

The following growth parameters were measured 90 days after sowing: seedling height, number of leaves, stem diameter, root length, root volume, leaf area, fresh weight of the shoot (FWS), fresh weight of the root (FWR), dry weight of the shoot (DWS), and dry weight of the root (DWR).

Statistical analyses

Data were analyzed using R software, version 4.2.3 (R Core Team 2023). Analysis of variance (ANOVA) was conducted using a completely randomized design with a double factorial arrangement. Assumptions of normality were tested using the Shapiro-Wilk test. Variable means with significant variances were compared using Fisher's least significant difference (LSD) test. A significance level of 5% ($\alpha = 0.05$) was employed in all statistical tests, applying Bonferroni correction to prevent Type I error. Principal component analysis (PCA) was performed to explore correlations between variables and treatment influences, utilizing tools from the ‘factoextra’ package in R. Regression models and graphs were adjusted using the ‘ggpubr’ and ‘ggpmisc’ libraries.

3. Results

Considering the variables of coffee seedling growth, the exploratory multivariate analysis revealed that two principal components explained 80% of the total data variation (Figure 1A). The first principal component (dimension 1) accounted for 65.7% of this variation, while the second explained 14.7% (Figure 1B). The variables FWS, DWS, and seedling height were the most influential in explaining this variation.

In the multivariate model, the FA of BSY with 2.3×10^8 cells of *S. cerevisiae* mL⁻¹ significantly enhanced coffee seedling development. This treatment formed a distinct cluster without overlap compared to the control group (Figure 1B). The cluster corresponding to the control treatment was located in the opposite quadrant relative to the vectors associated with the variables of coffee seedling growth. This antagonistic positioning suggests that BSY application significantly improved the development of Arabica coffee seedlings compared to the control treatment.

The analysis revealed that shoot fresh and dry weights are the primary variables significantly influenced by the application of BSY containing *S. cerevisiae*, suggesting a robust response to the treatment. It highlighted significant improvements in growth variables, as plant height considerably increased, as well as root volume. These changes indicate that BSY effectively enhanced coffee seedling development above and below the ground (Figure 1C).

Furthermore, root and shoot dry weights significantly changed after BSY application. These alterations underscore the effectiveness of BSY in enhancing Arabica coffee plant vigor and biomass (Figure 1C).

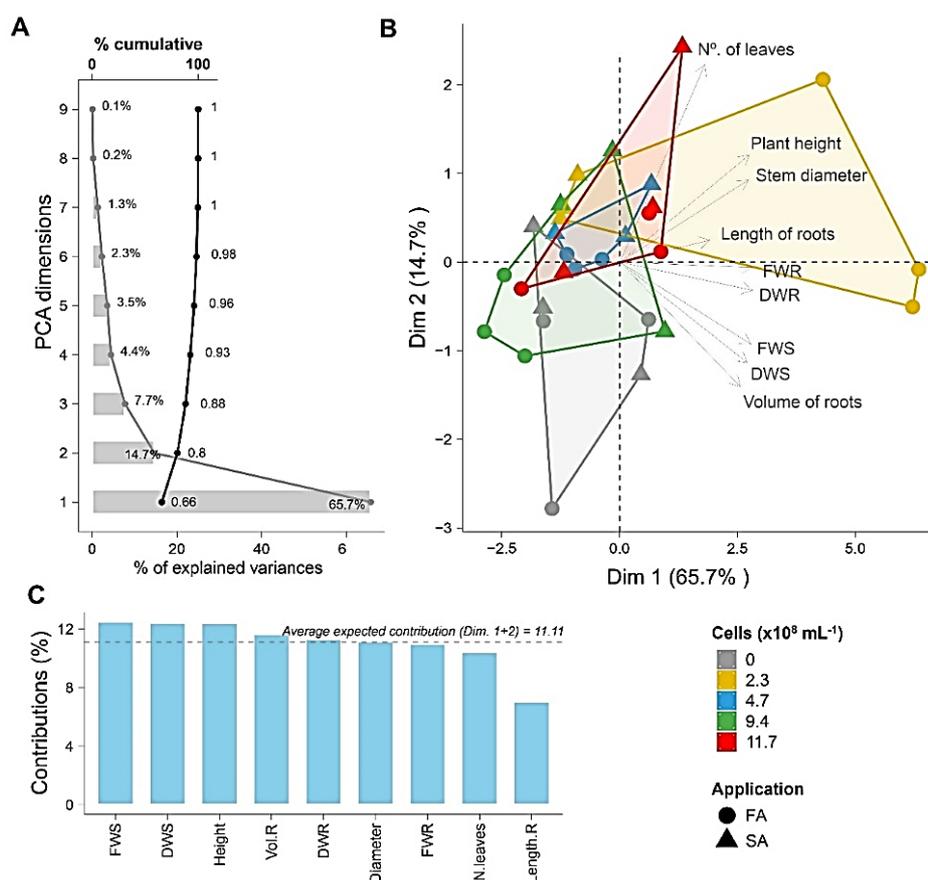


Figure 1. Principal component analysis (PCA) demonstrating the contribution of plant variables in the first collection cycle. (a) PCA biplot demonstrating groupings according to the treatments (different colors). (b)

Scree plot demonstrating the proportion of variance explained by each principal component combined with a cumulative explanation. (c) Graph of the average contribution expected by each soil and plant variable for the three main components; the variables were ranked in descending order from left to right according to the degree of explanation of multivariate ordering. Abbreviations: FWS - fresh weight of the shoot, FWR - fresh weight of the root, DWS - dry weight of the shoot, DWR - dry weight of the root, FA - foliar application, SA - soil application.

The variance analysis indicated a significant interaction between the application method (FA) and BSY concentration for most growth variables, except for the number of leaves (Table 2). This finding reinforces that the growth parameters of Arabica coffee seedlings are significantly affected by two key factors: BSY concentration and application method.

Table 2. Variance analysis of cell concentrations and yeast application methods for phenotypic variables of coffee plants.

Variable	mean	sd	Appl. (F1)	Conc. (F2)	F1*F2
			(p-value)		
FWS (g)	3.5	1.9	0.031	0.080	0.000
FWR (g)	1.2	1.0	0.010	0.001	0.000
DWS (g)	1.0	0.5	0.015	0.059	0.001
DWR (g)	0.3	0.2	0.011	0.004	0.000
Root length (cm)	13.2	4.4	0.175	0.000	0.019
Root volume (cm ³)	1.4	1.4	0.008	0.000	0.013
Plant height (cm)	13.6	3.4	0.291	0.203	0.002
Stem diameter (cm)	0.2	0.1	0.447	0.026	0.002
N. of leaves (uni.)	6.2	0.8	0.125	0.021	0.676
Clo A (uni.)	26.6	7.4	0.043	0.018	0.002
Clo B (uni.)	6.1	2.8	0.412	0.000	0.033
Clo A+B (uni.)	28.5	7.2	0.249	0.636	0.027
Leaf area (cm ²)	85.7	39.9	0.156	0.126	0.001

Abbreviations: sd – standard deviation, FWS – fresh weight of the shoot, FWR - fresh weight of the root, DWS – dry weight of the shoot, DWR - dry weight of the root.

Following the ANOVA guidelines and justifying the PCA outcomes, Fisher's LSD test also demonstrated that the FA of 2.3×10^8 *S. cerevisiae* cells mL⁻¹ more effectively enhanced shoot fresh and dry weights of Arabica coffee seedlings (Figure 2). This treatment increased shoot fresh weight (2.35 times), root fresh weight (4.22 times), shoot dry weight (2.23 times), and root dry weight (3.62 times) in coffee plants, compared to the negative control (sterile solution) applied via FA.

Conversely, coffee plants that received different BSY concentrations through SA did not exhibit significant differences in seedling development. This suggests that the method of BSY application plays a crucial role in its effectiveness. These variables did not show a significant fit with the values calculated by the linear and quadratic models.

The FA of BSY at a concentration of 2.3×10^8 *S. cerevisiae* cells mL⁻¹ also yielded the highest means for plant height (1.59 times), stem diameter (1.45 times), leaf area (2.06 times), root length (1.45 times), and root volume (2.39 times) compared to the negative control using the same application method (Figure 3). Hence, this specific BSY concentration used through FA significantly enhances plant growth parameters. Similarly, there was no influence of foliar concentration in SA, and these variables did not show significant adjustments to linear and quadratic models.

In foliar treatments, the root length and volume, as well as chlorophyll A and B contents, showed significant correlations ($p < 0.05$) to values predicted by quadratic models (Figure 4). The estimated maximum root length was observed at a cellular concentration of 2.2×10^8 *S. cerevisiae* cells mL⁻¹ (Figure 4B), which closely aligns with the second level of the standard treatment concentration (2.3×10^8 *S. cerevisiae* cells mL⁻¹). However, in FA treatments, the root volume (Figure 4C) and chlorophyll A and B concentrations (Figure 4D and E) decreased with cell density, reaching minimum values at respective 11.7 , 7.7 , and 9.0×10^8 *S. cerevisiae* cells mL⁻¹. This result limited the estimated value within the tested range ($0 - 11.7 \times 10^8$ *S. cerevisiae* cells mL⁻¹). In SA treatments, only plant height showed a similar response, showing a positive linear trend according to cell concentrations (Figure 4A).

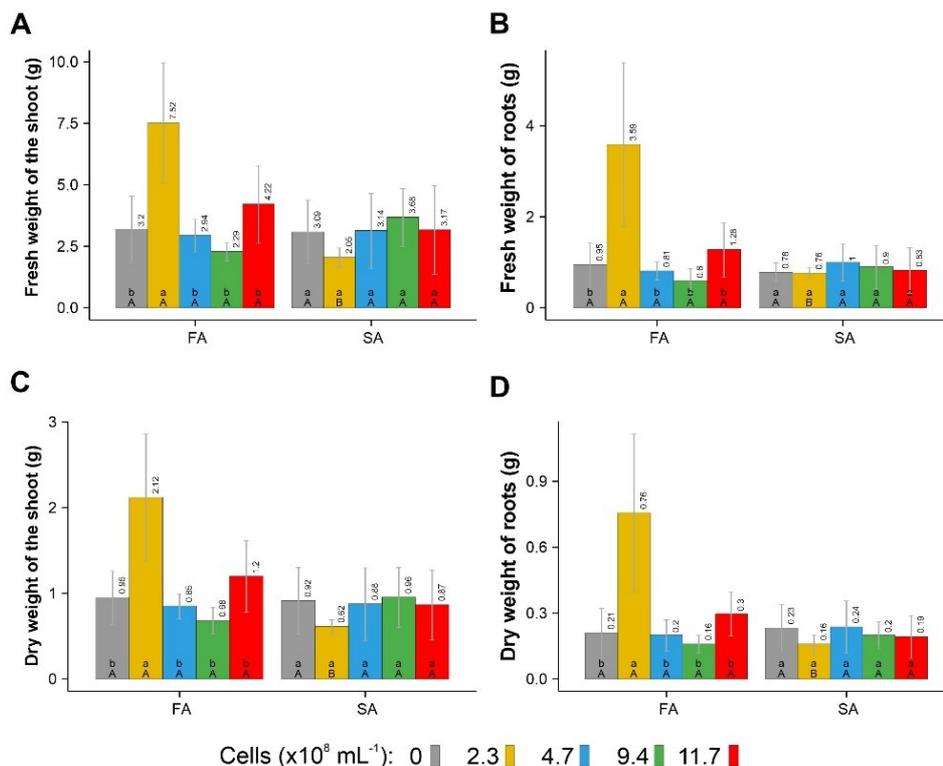


Figure 2. Mean fresh and dry weights of roots and shoots of coffee seedlings according to yeast application methods and cellular concentrations. Only treatments followed by different lowercase (in the same application mode) or uppercase (at the same concentration) letters differed, according to the LSD test with Bonferroni correction ($p < 0.05$). FA - foliar application; SA - soil application.

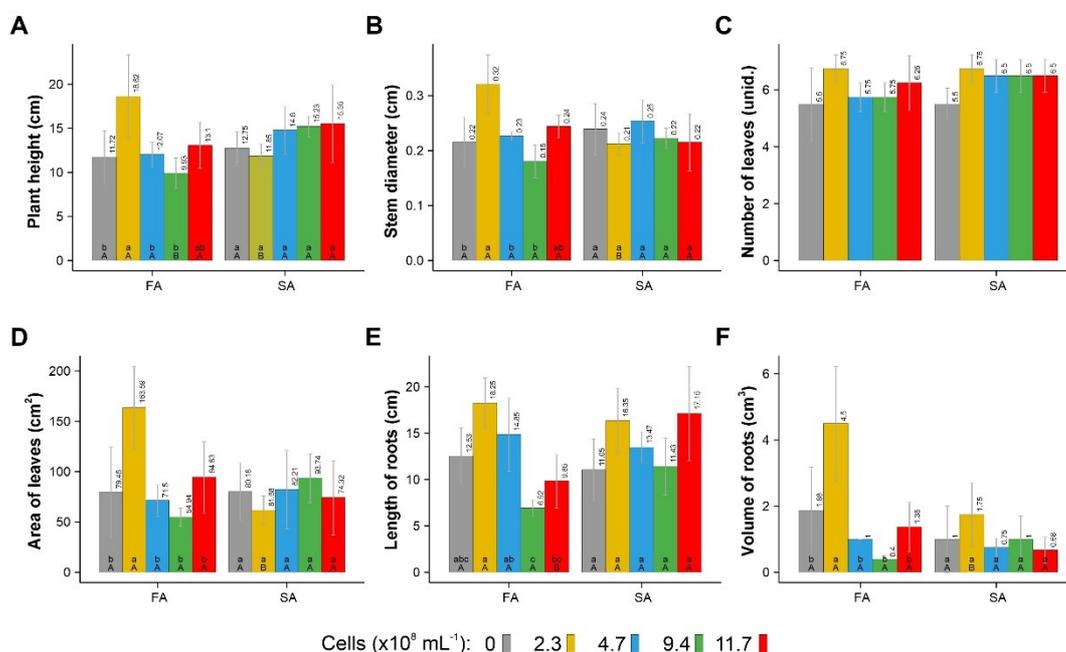


Figure 3. Mean growth-elongation variables of coffee seedlings according to yeast application methods and cellular concentrations. Only treatments followed by different lowercase (in the same application mode) or uppercase (at the same concentration) letters differed, according to the LSD test with Bonferroni correction ($p < 0.05$). FA - foliar application; SA - soil application.

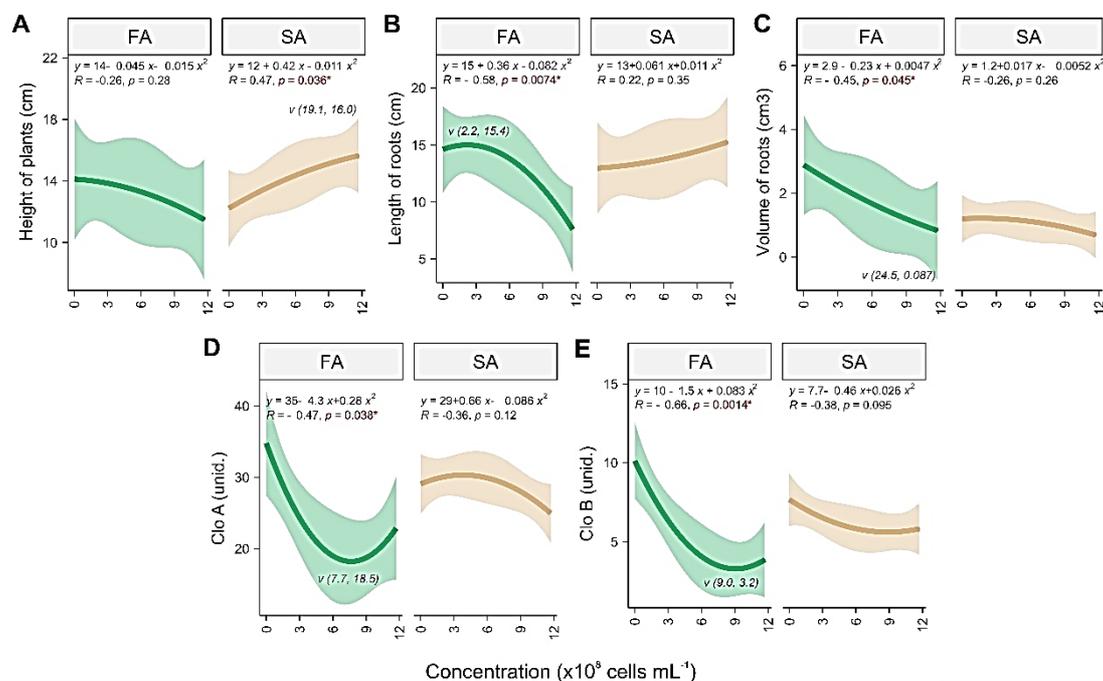


Figure 4. Regression analysis of the variables that were significantly correlated to yeast concentrations. The coordinates of vertices were reported in the significant models, indicating the cell concentration where the corresponding variable reached its theoretical maximum or minimum value. The filled area around the curve indicates the 95% confidence interval. FA - foliar application; SA - soil application.

4. Discussion

We assessed the potential efficacy of different concentrations and application methods of brewer's spent yeast (BSY) containing *Saccharomyces cerevisiae* in coffee seedling development. Typically, BSY comprises minerals and several nutrients, such as proteins, vitamins, polyphenols, antioxidants, β -glucans, mannoproteins, and enzymes (Jaeger et al. 2020). During the fermentation process, yeast releases phosphorus and potassium (Hesham and Mohamed 2011), which are instrumental in creating formulations from these residues to act as plant growth enhancers.

Several studies have suggested that yeast is highly promising as a plant growth promoter, owing to its ability to produce numerous beneficial compounds, such as vitamins, amino acids, phytohormones, enzymes (Mukherjee et al. 2020; Kapoor et al. 2021; Ramos-Garza et al. 2023; Sipiczki 2023), monosaccharides, and polysaccharides (Mussatto et al. 2006).

Our study demonstrated that the foliar application (FA) of BSY at 2.3×10^8 *S. cerevisiae* cells mL^{-1} significantly enhanced the development of coffee seedlings and that soil application (SA) did not yield similar results. This pattern may be associated with the amount of yeast applied, given that yeast interactions in soil are significantly more complex than those occurring on foliage. This complexity is due to the interaction of the soil's biological, chemical, and physical properties, as well as among soil aggregates and with the soil's microfauna, promoting competition (Botha 2011). These results confirm that nutrient absorption and utilization by plants vary significantly based on the application method of yeast extract, whether foliar or soil-based (AL-Huqail 2023).

The substantial increases in above-ground (height, stem diameter, leaf area) and below-ground (root length, volume) characteristics suggest a holistic improvement in coffee seedling development using BSY. These findings demonstrate the effectiveness of yeast in promoting growth, potentially improving overall plant health and productivity, as observed in lettuce and maize (Cabrinini et al. 2019; Ramos-Garza et al. 2023). The presented results are attributed to the nutrients in BSY, such as phosphorus, potassium, manganese, copper, and zinc (Table 1).

The principal component analysis (PCA) demonstrated the formation of at least three groups based on the application methods and concentrations of BSY. The lowest concentration (2.3×10^8 *S. cerevisiae* cells mL^{-1}) is located in the same quadrant as the growth variable vectors. The higher concentrations did not yield

proportionally better results, suggesting that the applied amount is not directly correlated to positive outcomes. This phenomenon is associated with the nutrient absorption curve in coffee seedlings, as observed by Neves et al. (2007) and Prado et al. (2008). According to these authors, nutrient uptake occurs up to a certain peak, and any increase in dosage beyond this peak does not confer additional advantages to the crop.

Therefore, this study on BSY application methods and concentrations highlights the potential of biofertilizers in sustainable agriculture. The varied responses from coffee seedlings underscore the complexity of yeast-soil interactions, emphasizing the need for precise applications. Considering that agriculture tends toward eco-friendly alternatives, our findings contribute insights into optimizing biofertilizer use for enhanced plant health and productivity. Ongoing research and refinement of application strategies are crucial for advancing sustainable and resilient agricultural practices.

5. Conclusions

This study assessed the potential of brewer's spent yeast (BSY) containing *Saccharomyces cerevisiae* as a valuable resource to enhance coffee seedling development. Our analysis revealed that the foliar application of BSY at 2.3×10^8 *S. cerevisiae* cells mL⁻¹ significantly improved growth parameters in *Coffea arabica* compared to the soil application and negative control. Our findings provide a better understanding of waste reuse as an effective alternative to promote coffee seedling development by incorporating materials rich in organic matter and yeast. Finally, these findings provide valuable insights into the development of strategies that support sustainable agriculture and circular economy practices that will benefit coffee producers, the beer industry, and the environment.

Authors' Contributions: SILVA, E.L.D.: acquisition of data, analysis and interpretation of data, drafting the article, critical review of important intellectual content; de BARROS, J.A.: analysis and interpretation of data, drafting the article, critical review of important intellectual content; COSTA, D.P.: conception and design, analysis and interpretation of data, drafting the article; NEVES, R.L.: analysis and interpretation of data, drafting the article; TELINO-JUNIOR, W.R.: analysis and interpretation of data, drafting the article; MEDEIROS, E.V.: interpretation of data, drafting the article, critical review of important intellectual content. All authors have read and approved the final version of the manuscript.

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