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Coffee processing residue extracts on the germination and vigor of maize seeds under water stress

Extractos de residuos del procesamiento de café en la germinación y vigor de semillas de maíz bajo estrés hídrico

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Abstract

Maize (*Zea mays* L.) is one of the most produced cereals in the world, however, water stress impairs the germination and vigor of its seeds. Agro-industrial residues as the husk and pulp obtained from the processing of coffee fruits (*Coffea arabica* L.) have high concentrations of total phenolic compounds, substances with antioxidant activity, which, if applied exogenously to plants, can reduce the effects of water stress. Thus, the objective of this study was to evaluate different doses of coffee processing residue extract on the physiological quality of maize seeds under water stress. The experiment was carried out in a completely randomized design, with four replications, in a 5 × 3 factorial scheme, with 5 doses of extract from the husk and pulp of coffee fruits: 0, 2, 4, 8, and 16 ml of extract per kg of seeds^{-1}, at 3 different osmotic potentials: 0.0, -0.2, and -0.4 MPa, on the maize variety IPR 164. The characteristics evaluated were first germination count, germination, abnormal seedling, non-germinated seeds, length, and dry matter seedling. Although the extract from coffee fruit processing residues, including husk and pulp, reduced the percentage of abnormal seedlings and non-germinated seeds of the IPR 164 maize variety under water stress conditions (osmotic potential of -0.4 MPa), it did not demonstrate, in the manner it was used, potential as a biostimulant, as it did not alter germination and reduced the initial development of the seedlings.

Keywords: biostimulant, coffee husk, coffee pulp, physiological quality, *Zea mays* L.

Resumen

El maíz (*Zea mays* L.) es uno de los cereales más producidos en el mundo, sin embargo, el estrés hídrico perjudica la germinación y el vigor de sus semillas. Los residuos agroindustriales como la cáscara y pulpa obtenidas del procesamiento de los frutos del café (*Coffea arabica* L.) tienen altas concentraciones de compuestos fenólicos totales, sustancias con actividad antioxidante que, aplicadas exógenamente a las plantas, pueden reducir el estrés hídrico. Así, el objetivo de este estudio fue evaluar el efecto de diferentes dosis de extracto de residuos de procesamiento de café en la calidad fisiológica de semillas de maíz bajo estrés hídrico. El experimento se realizó en un delineamiento enteramente aleatorizado, con cuatro repeticiones, en un esquema factorial 5 × 3, con 5 dosis de extracto de cáscara y pulpa de frutos de café: 0, 2, 4, 8 y 16 ml de extracto por kg de semillas^{-1} a 3 potenciales osmóticos diferentes: 0.0, -0.2 y -0.4 MPa, en la variedad de maíz IPR 164. Las características evaluadas fueron primer conteo de germinación, germinación, plántula anormal, semillas no germinadas, longitud y materia seca de plántula. La aplicación del extracto de los residuos del procesamiento de frutos de café, incluyendo cáscara y pulpa, aunque redujo el porcentaje de plántulas anormales y semillas no germinadas de la variedad de maíz IPR 164 bajo condiciones de estrés hídrico (potencial osmótico de -0,4 MPa), no demostró, en la forma en que se utilizó, potencial como bioestimulante, ya que no alteró la germinación y redujo el desarrollo inicial de las plántulas.

Palabras claves: bioestimulante, cáscara de café, calidad fisiológica, pulpa de café, *Zea mays* L.

Introduction

Maize (*Zea mays* L.) is one of the most produced cereals in the world, and it is a subsistence crop in various regions, especially among vulnerable populations. In addition to its role as food source, maize grains are used in several other sectors, particularly in animal protein production, where they serve as the primary ingredient in many feeds.

Brazil is one of the world's largest producers and exporters of maize grains. Its wide geographical distribution and the ability to cultivate maize at different times of the year, combined with the effects of climate change, have increased the likelihood of abiotic stresses during cultivation, in particular low temperatures and water shortages (USDA, 2022).

Abiotic stresses arise from natural environmental factors or are influenced by intense human activity. Water has become one of the most frequent and harmful stresses because it can cause damage in virtually all stages of cultivation, leading to significant production losses (Parkash and Singh, 2020). When present during the germination process, it decreases the germination speed and percentage, as well as the vigor of the seedlings and, consequently, of the plants, affecting the establishment of the optimal cultivation stand and, consequently, the productivity (Marcos Filho, 2015).

According to Ferreira *et al.* (2019) and Sousa *et al.* (2023), water is one of the most important factors for the germination of maize seeds because its presence triggers a series of essential biological and physiological processes. Water stress causes major changes in the germination process which, depending on the severity, leads to a drastic reduction in the germination percentage and time, as well as in the initial development of the seedlings due to changes in cell turgidity and the increase in reactive oxygen species.

These deleterious effects of water stress can be minimized by antioxidant agents, which consist of substances capable of regenerating the affected substrate or significantly preventing the oxidation process (Hasanuzzaman *et al.*, 2021). These antioxidant agents can be produced endogenously by the plant itself, but it can also benefit from an exogenous application, such as through biostimulation (Chen *et al.*, 2022; Drobek *et al.*, 2019).

Biostimulants can be derived from different sources, such as bacteria, fungi, algae, and plants. Plant-based biostimulants, primarily produced through enzymatic hydrolysis (Drobek *et al.*, 2019), have been shown to effectively increase productivity, enhance nutritional levels (Kocira, 2019), and provide protection against biotic and abiotic stresses (Panfili *et al.*, 2018) in various crops.

When selecting a vegetable source for use as a biostimulant, it is important to consider not only the concentrations of bioactive compounds but also their availability and environmental impact. In this context, recycling agricultural residues is both advantageous and necessary due to their abundance and the fact that they are usually discarded improperly, leading to harmful accumulation in ecosystems (Publia *et al.*, 2021).

Brazil, as the world's largest coffee producer and exporter, is certainly one of the largest producers of residues from its production and processing, especially residues derived from the processing of coffee fruits, such as the husk and pulp. These residues contain high levels of total phenolic compounds and possess strong antioxidant capacity, making them an important source of antioxidant compounds and a potential biostimulant (Machado *et al.*, 2023).

However, few research has been carried out in this area, particularly regarding the use of seeds as a treatment source and the investigation of their effects, which is crucial to understand optimal application doses and interactions with abiotic stress conditions, such as water stress. Thus, the objective of the study was to evaluate different doses of coffee processing residue extract on the physiological quality of maize seeds under water stress.

Materials and methods

The research was conducted at the Seed Analysis Laboratory at the State University of Minas Gerais, in Ituiutaba, Brazil. Arabica coffee (*Coffea arabica* L.) fruits were obtained from a property in the municipality of Araguari, Minas Gerais, Brazil, and stored in a freezer for approximately one week for later processing.

Husk and pulp from mature coffee fruits were obtained by manually peeling and pulping the fruit and were used as residues for extraction. These residues were dried in a kiln with forced air circulation at 60 °C until they reached a constant mass, with daily weighing. They were then ground, stored under refrigeration, and kept away from light.

The extraction of residues was performed using 1 g of sample and 20 ml of distilled water, with constant stirring for 2 h at 150 RPM, followed by centrifugation for 10 min at 3000 RPM. The determination of total phenolic compounds, used for characterizing the extract, was performed using the Folin-Ciocalteau colorimetric method, according to Kumazawa *et al.* (2002) and Singleton *et al.* (1999), using the standard curve of gallic acid. The reading was performed at 760 nm in a spectrophotometer, and the content of total phenolic compounds was 383.4 mg EAG L^{-1}.

The experimental design was completely randomized with four replications, in a 5 × 3 factorial scheme. The treatments consisted of five doses of coffee processing residue extract and three different osmotic potentials. The maize variety IPR 164 was used.

Initially, the seeds were soaked at different doses of the extract (0 ml, 2 ml, 4 ml, 8 ml, and 16 ml of extract per kg of seeds^{-}1) and, later, dried at room temperature. For the simulation of water stress, a PEG 6000 polyethylene glycol solution was used at three different osmotic potentials (0.0, -0.2, and -0.4 MPa) established by the Van't Hoff equation.

The germination test consisted of four replicates of 50 seeds per treatment wrapped in three sheets of substrate paper (Germitest type), two as base and one to cover, which where moistened with different PEG 6000 solutions at a rate of 2.5 times its dry weight. Afterwards, they were kept in a germination chamber of Biochemical Oxygen Demand (BOD) type at a constant temperature of 25 °C. The evaluations followed the Rules for Seed Analysis (Brazil, 2009). Following aspects were evaluated:

First germination count (FGC): corresponding to the accumulated percentage of normal seedlings four days after the application of the test. Results were expressed as percentages.

Germination (GER): corresponding to the accumulated percentage of normal seedlings from the fourth to the seventh day after the application of the test. Results were expressed as percentages.

Abnormal seedlings (AS) and non-germinated seeds (NGS): number of abnormal seedlings and non-germinated seeds (dead or dormant) present at the seventh day after the installation of the germination test. Results were expressed as percentages.

Seedling length: during the germination test, ten normal seedlings were randomly selected from each treatment in their respective replications. Shoot length (SL) and root length (RL) measurements took place on the fourth day with the aid of a ruler, and were performed in centimeters. The results were expressed in mm seedling^{-1}.

Seedling dry matter: the seedlings used in the length test were sectioned into shoot and root parts, placed in paper bags, and then taken to a kiln with forced air circulation at 60 °C until they reached a constant weight. Subsequently, they were weighed on a precision scale (error = 0.0001). Results were expressed as shoot dry mass (SDM) and root dry mass (RDM). The measurements were performed in grams and the results expressed in mg seedling^{-1}.

The data were subjected to Hartley's homogeneity tests at 5 % probability of error, with the AS and NGS variables transformed using the arcsine function. Subsequently, an analysis of variance was performed, and when significance was found ($p < 0.05$), polynomial regressions up to the second degree were applied. Analyzes were conducted using the Sisvar program.

Results

The significance of the stress x extract interaction for the variables abnormal seedlings, non-germinated seeds, and root dry mass was assessed using analysis of variance. The extract factor was significant only for root length, while the stress factor was significant for all variables studied. The coefficient of variation ranged from 1.59 % (non-germinated seeds) to 22.84 % (root dry mass), and the general mean for the germination percentage was 89.11 % (Table 1).

Table 1. Mean square values for maize seedlings of the IPR 164 variety treated with different concentrations of coffee residue extract (0, 2, 4, 8, and 16 ml per kg of seeds-1) at three different osmotic potentials (0.0, -0.2, and -0.4 MPa)

Causes of variation	D.F.	Mean square							
		FGC (%)	GER (%)	AS	NGS	SL (mm)	RL (mm)	SDM (mg)	RDM (mg)
Extract	4	62.90^{ns}	24.52^{ns}	0.0008^{ns}	0.0019*	2.43^{ns}	39.50*	0.33^{ns}	1.32^{ns}
Stress	2	19598.32*	1190.64*	0.0698*	0.0095*	792.63*	783.51*	57.21*	69.94*
Extract x Stress	8	73.37^{ns}	16.12^{ns}	0.0022*	0.0015*	2.09^{ns}	18.45^{ns}	0.66^{ns}	2.39*
Error	45	45.10	21.08	0.0010	0.0006	1.24	12.92	0.62	0.89
CV (%)	---	11.33	5.15	2.09	1.59	11.03	13.94	20.35	22.84
Overall average	---	59.28	89.11	1.51	1.52	10.10	25.79	3.86	4.14

$^{ns:}$ Not significant, *: Significant at 5% probability of error by the F test.

FGC: first germination count, GER: germination, AS: abnormal seedlings, NGS: non-germinated seeds, SL: shoot length, RL: root length, SDM: shoot dry mass, RDM: root dry mass.

There was a linear reduction in the percentage of normal seedlings observed in the first germination count as water stress increased. The -0.1 MPa reduction in osmotic potential decreased approximately 15.62 % among normal seedlings at the first count (Figure 1). For germination, the best fit to the data with respect to water stress was a quadratic model, with the maximum response occurring at -0.08 MPa, resulting in approximately 96 % germination (Figure 2).

A linear increase in the percentage of abnormal seedlings in response to water stress was observed as the osmotic potential decreased, under conditions of 4 and 8 ml of extract per kg of seeds^{-1}. For conditions with 0, 2, and 16 ml of extract per kg of seeds^{-1}, the best-fitting models were quadratic, with minimum points at -0.1, -0.07, and -0.08 MPa, respectively. A drastic increase in the percentage of abnormal seedlings was observed as these potentials decreased (Figure 3).

Considering the variations in the concentrations of coffee waste extract according to the percentage of abnormal seedlings, no significant adjustments were observed for the osmotic potentials of 0, and -0.2 MPa. However, for the condition at -0.4 MPa, a quadratic adjustment was observed (R^2 = 93 %), with the minimum response point at 10.5 ml of extract for kg of seeds, with a subsequent increase (Figure 4).

The response observed for the percentage of non-germinated seeds is consistent with that for abnormal seedlings. Considering the response percentage of non-germinated seeds to water stress, a linear increase was observed as the osmotic potential decreased for the condition of 16 ml of extract per kg of seeds^{-1}. For the conditions of 0 and 8 ml of extract per kg of seeds^{-1}, the best fitting models were quadratic, with minimum points at -0.19 and -0.15 MPa, respectively. For doses of 2 and 4 ml of extract, no significant adjustments were found (Figure 5).

No significant adjustments were found for the osmotic potentials of 0 and -0.2 MPa in response to variations in the concentrations of coffee residue extract on the percentage of non-germinated seeds. For the -0.4 MPa condition, the best fit was a quadratic model, with the minimum response point at 2.62 ml of extract per kg of seeds^{-1}, with a subsequent increase in the percentage of non-germinated seeds (Figure 6).

Seedling lengths showed a quadratic fit with minimum response points of -0.30 and -0.39 MPa for shoot length (Figure 7) and root length (Figure 8), respectively, against water stress. Among the seedling lengths, root length was the only one that showed significant effects after the variation of doses of the coffee residue extract, with a linear reduction of 0.20 mm for each ml of the extract added (Figure 9).

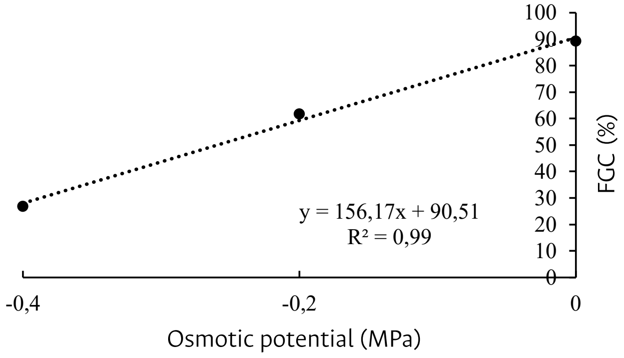

Figure 1. First germination count (FGC) of maize seeds of the variety IPR 164 at three different osmotic potentials.

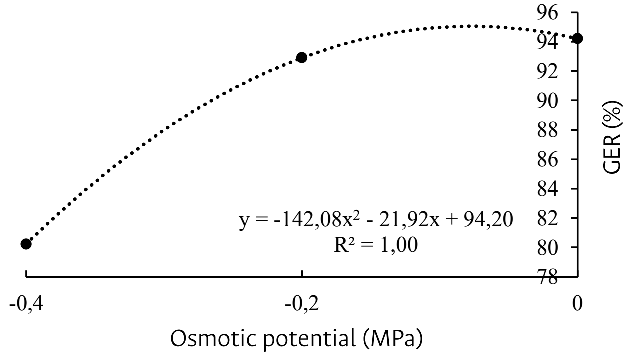

Figure 2. Germination (GER) of maize seeds of variety IPR 164 at three different osmotic potentials.

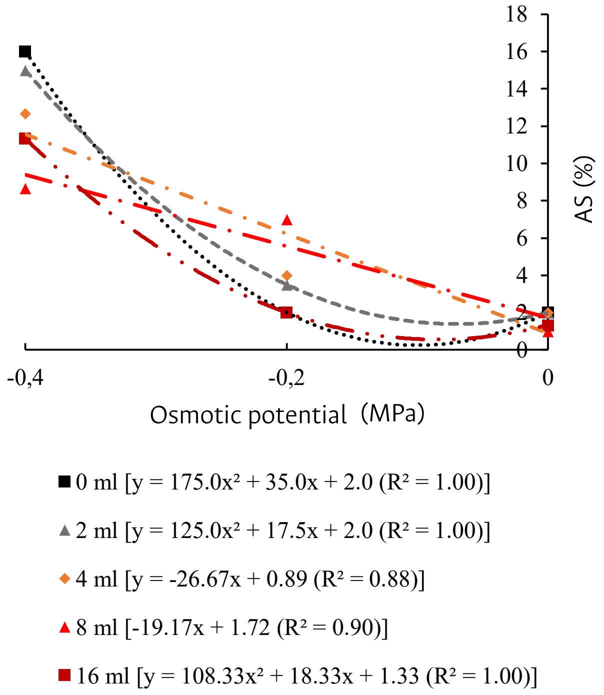

Figure 3. Breakdown of the interaction of abnormal seedlings (AS) of maize seeds of the IPR 164 variety treated with different concentrations of coffee residue extract (0, 2, 4, 8, and 16 ml per kg of seeds^{-1}) at three different osmotic potentials.

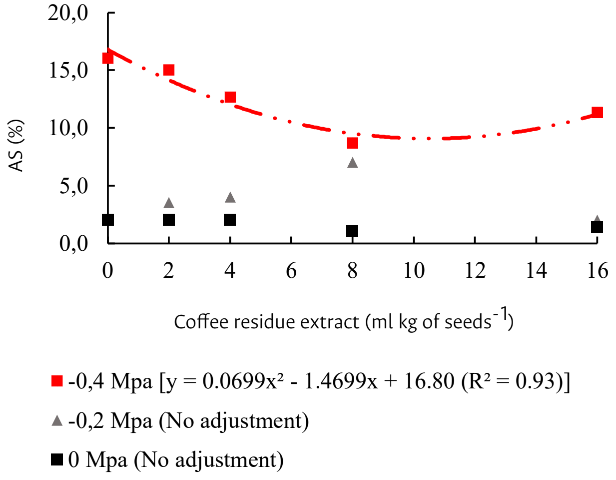

Figure 4. Breakdown of the interaction of abnormal seedlings (AS) of maize seeds of the IPR 164 variety treated with different concentrations of coffee residue extract (0, 2, 4, 8, and 16 ml per kg of seeds^{-1}) at three different osmotic potentials.

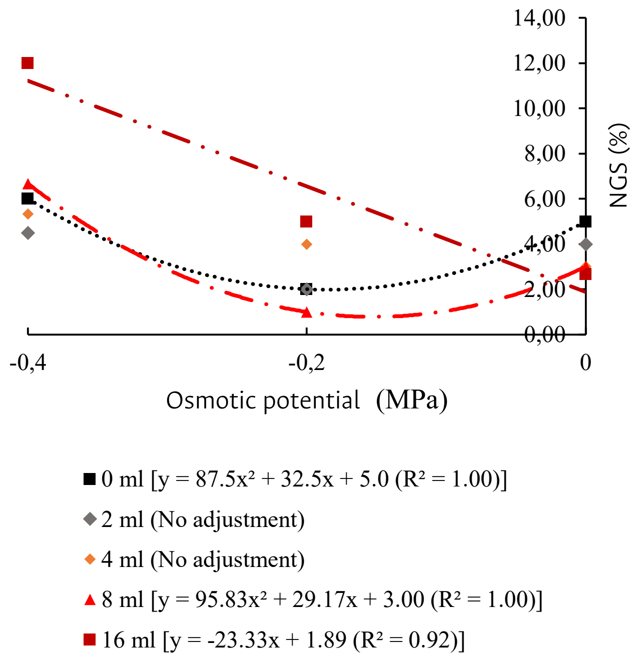

Figure 5. Breakdown of the interaction of non-germinated seeds (NGS) of maize variety IPR 164 treated with different concentrations of coffee residue extract (0, 2, 4, 8, and16 ml per kg of seeds^{-1}) at three different osmotic potentials.

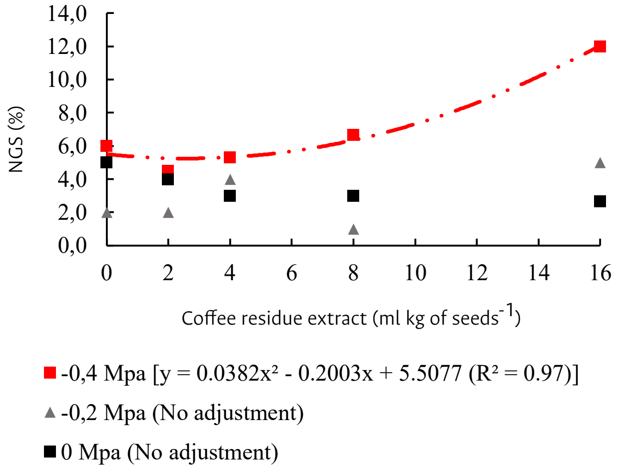

Figure 6. Breakdown of the interaction of non-germinated seeds (NGS) of maize variety IPR 164 treated with different concentrations of coffee residue extract (0, 2, 4, 8, and 16 ml per kg of seeds^{-1}) at three different osmotic potentials.

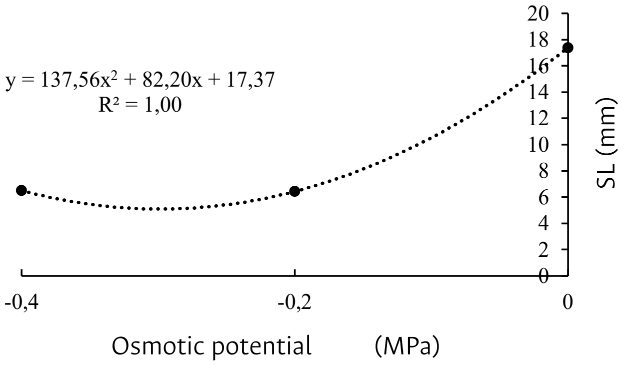

Figure 7. Shoot length (SL) of maize seedlings of variety IPR 164 at three different osmotic potentials.

Root dry mass showed a quadratic adjustment, with the minimum response point at -0.28 MPa, in response to variations in osmotic potential (Figure 10). For root dry mass, the adjustments were quadratic with minimum response points at -0.31 MPa without using the extract, and -0.34 MPa for seeds treated with 16 ml of coffee residue extract per kg of seeds. For the other extract doses used, a linear reduction in root dry mass was observed as the osmotic potential decreased (Figure 11).

For root dry mass, a quadratic adjustment was observed, with a maximum response point at 9.75 ml of coffee residue extract per kg of seeds^{-1} when subjected to a water stress of -0.2 MPa. For the other stress conditions (0 and -0.4 MPa), no significant adjustments were observed in relation to variations in extract doses (Figure 12).

Discussion

According to the Ministry of Agriculture, the minimum percentage for a lot to be considered as maize seeds in Brazil is 85 %, which was met by the sample used in the study with an average germination of 89.11 % (Table 1).

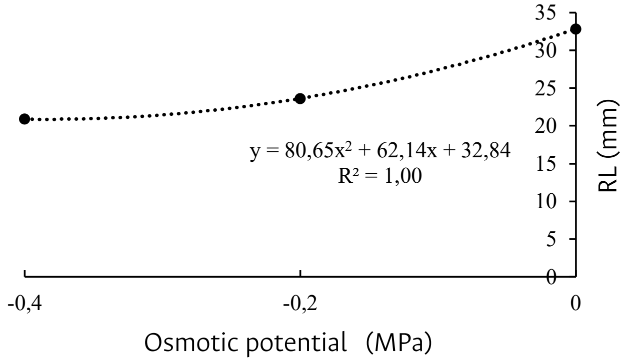

Figure 8. Root length (RL) of maize seedlings of variety IPR 164 at three different osmotic potentials.

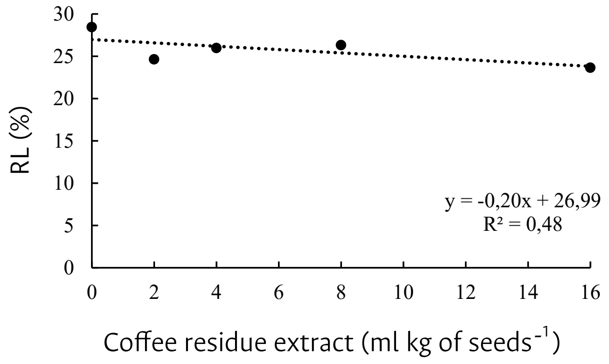

Figure 9. Root length (RL) of maize seedlings of the IPR 164 variety treated with different concentrations of coffee residue extract (0, 2, 4, 8, and 16 ml per kg of seeds^{-1}).

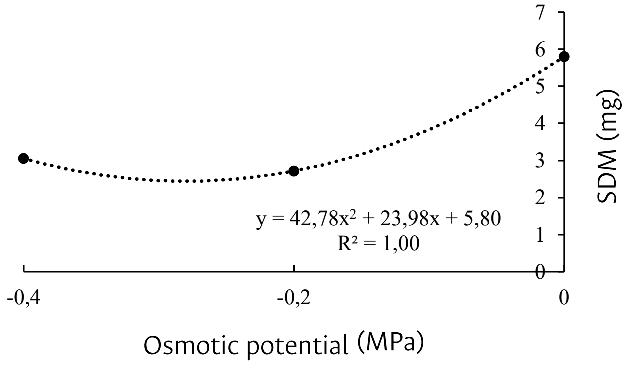

Figure 10. Shoot dry mass (SDM) of maize seedlings of variety IPR 164 at three different osmotic potentials.

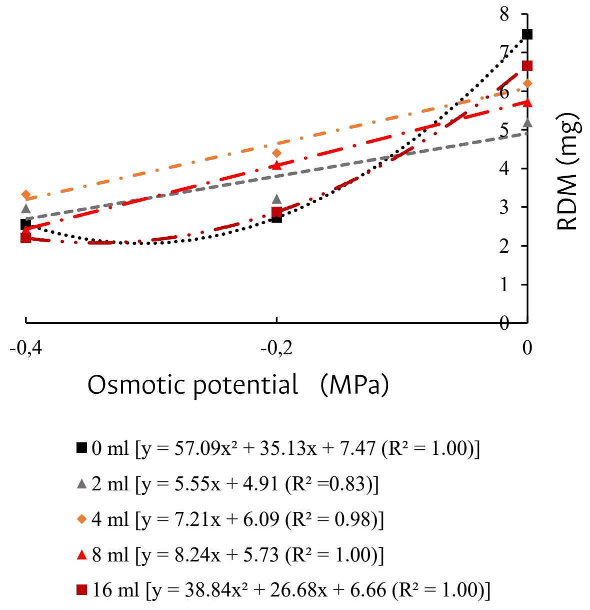

Figure 11. Root dry mass (RDM) of maize seedlings of variety IPR 164 at three different osmotic potentials.

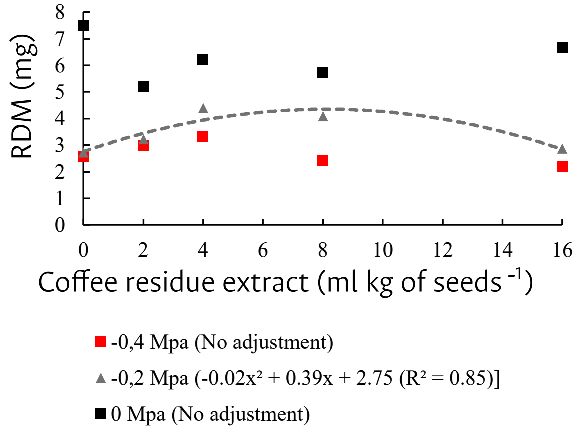

Figure 12. Breakdown of the interaction for root dry mass (RDM) of maize seedlings of variety IPR 164 treated with different concentrations of coffee residue extract (0, 2, 4, 8, and 16 ml per kg of seeds^{-1}) at three different osmotic potentials.

For Marcos Filho (2015), the adverse effects of lack of water on the germination process of seeds result in the reduction of their germination and vigor. This explains the reductions in the first germination count (Figure 1) and germination (Figure 2), and the increase in the percentage of abnormal seedlings (Figure 3) and non-germinated seeds (Figure 5) at osmotic potentials below -0.1 MPa.

Sousa *et al.* (2023) also found a reduction in the speed and germination percentage, and an increase in the number of abnormal seedlings and non-germinated seeds for the maize hybrid KWS 9960 when subjected to water stress simulated by PEG 6000. These authors reported the absence of normal seedlings at osmotic potentials of -0.3 MPa, which shows the damage caused by water stress in maize seed germination.

Ferreira *et al.* (2019) studied the effect of water stress on two popcorn cultivars (BRS Ângela and IAC 125), and reported reductions in germination of both cultivars, at osmotic potentials lower than -0.1 MPa.

Shoot and root length showed minimum response points at -0.3 and -0.39 MPa, respectively (Figures 7 and 8), as a response to the reduction in osmotic potential. After this point, length values tended to stabilize. These results were similar to those obtained for shoot and root dry mass (Figures 10 and 11).

Contrary to the results obtained in this study, Ferreira *et al.* (2019) and Sousa *et al.* (2023) in maize, and Nunes *et al.* (2020) in sorghum, found that seedling lengths decreased with the decreasing osmotic potentials. This can be explained by the fact that these authors used more drastic osmotic potentials than in the present study (-0.9 MPa, -0.9 MPa, and -0.6 MPa, respectively).

For Hasanuzzaman *et al.* (2021), the reduction in most attributes related to germination under water stress is associated with the loss of cell turgidity and the increased production of free radicals, such as reactive oxygen species (ROS). The increase in ROS causes serious damage to the plasma membrane, which may explain the increase of abnormal seedlings and non-germinated seeds.

The use of coffee residue extract influenced the percentage of abnormal seedlings, non-germinated seeds, root length (RL) and root dry mass (RDM) (Table 1). For all these variables, except for RL, the extract only showed effects under conditions of water stress.

The use of 10.5 ml and 2.62 ml of extract for each kg of seeds reduced the percentage of abnormal seedlings (Figure 4) and non-germinated seeds, respectively (Figure 6), at the osmotic potential of -0.4 MPa. According to Kausar *et al.* (2022), water stress causes damage to the cell structure, which results in anomalies and, subsequently, cell death, mainly because of oxidative stress that can be minimized by antioxidant substances.

Residues from coffee fruit processing, such as husk and pulp, have high concentrations of total phenolic compounds and antioxidant activity (Chen *et al.*, 2021; Machado *et al.*, 2023). The residues used for the extract in the present study contained 383.4 mg EAG L^{-1} of total phenolic compounds, which is consistent with the findings of these authors.

However, root length (Figure 9) was negatively affected by the extract, both under presence or absence of water stress. Some authors report negative effects on the germination and initial development of seedlings of some crops, such as pumpkin (Braga and Pasin, 2020) and lettuce (Sant'anna *et al.*, 2017), after the use of coffee residue extracts. Such authors attributed these results to possible allelopathic substances present in coffee fruits, however, these effects seem to depend on the species and the dose used, since in the present study, the first germination count and germination were not affected using the extract (Table 1).

Conclusions

Although the application of extract from coffee fruit processing residues, including husk and pulp, reduced the percentage of abnormal seedlings and non-germinated seeds in the IPR 164 maize variety under water stress conditions (osmotic potential of -0.4 MPa), it did not show potential as a biostimulant in its current form, as it neither improved germination nor supported the initial development of the seedlings.

Acknowledgments

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