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Use of Fava d'Anta extract in maize cultivation under saline stress

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ABSTRACT

Extract of Fava d’Anta (*Dimorphandra mollis* Benth) alleviates the deleterious effects of saline water. This study evaluates gas exchange and the initial growth of maize under saline stress and the use of Fava d’Anta extract. The experimental design was a randomized block design in a 5 × 4 factorial scheme with three replications. The first factor consisted of five levels of electrical conductivity of irrigation water (ECw = 1.0, 1.5, 3.0, 4.0, and 5.0 dS m^{-1}), and the second factor consisted of four foliar application frequencies of Fava d’Anta extract: F1 – no application; F2 – application every 10 days; F3 – application every 5 days; and F4 – daily application. Increasing salinity of the irrigation water reduced plant height, stem diameter, photosynthesis, stomatal conductance, transpiration, shoot dry mass, and root dry mass. The different application frequencies of the Fava d’Anta extract influenced plant height, photosynthesis, and shoot dry mass. Interactive effects were observed, with better results for the combinations S2 × F2, S3 × F2, and S3 × F2 for photosynthesis, stomatal conductance, and transpiration, respectively. These findings indicate that foliar application of Fava d’Anta extract every 10 days during the vegetative stage has the potential to mitigate the deleterious effects of saline water on maize. Daily application of the extract mitigated the harmful effects of irrigation water with EC of 4.0 dS m^{-1} on gas exchange parameters.

Keywords: *Dimorphandra* mollis Benth, salinity, *Zea mays* L.

Uso do extrato da Fava d’Anta no cultivo do milho sob estresse salino

RESUMO

O extrato da Fava d’Anta (*Dimorphandra mollis* Benth), alivia os efeitos deletérios da água salobra. Neste sentido, objetivou-se avaliar as trocas gasosas e crescimento inicial da cultura do milho sob estresse salino e utilização do extrato da Fava D’anta. O delineamento experimental foi em blocos ao acaso, em esquema fatorial 5 x 4, com três repetições, referentes. O primeiro fator foi constituído por cinco condutividade elétrica da água de irrigação (CEa = 1,0; 1,5; 3,0; 4,0 e 5,0 dS m^{-1}) e frequências de aplicação via foliar de extrato de Fava D’anta (F1 – sem aplicação do extrato; F2 – aplicação a cada 10 dias, F3 – aplicação a cada 5 dias e F4 – aplicação diária. O aumento da salinidade da água de irrigação causou redução nas variáveis altura, diâmetro do colmo, fotossíntese, condutância estomática, transpiração, biomassa seca da parte aérea e do sistema radicular. As diferentes frequências de aplicação do extrato da Fava d’Anta influenciaram as variáveis altura da planta, fotossíntese e massa seca da parte aérea. O efeito interativo ocorreu com melhores resultados nas combinações S2 x F2, S3 x F2 e S3 x F2 para fotossíntese, condutância estomática e transpiração, respectivamente; evidenciando que a aplicação do extrato de Fava d’Anta a cada 10 dias, durante o ciclo vegetativo, demonstra potencial de mitigar os efeitos deletérios da água salobra no milho. O extrato, aplicado diariamente, mitigou os efeitos danosos da água de irrigação com CE de 4,0 dS m^{-1}, no que se refere às trocas gasosas.

Palavras-chave: *Dimorphandra mollis* Benth*;* salinidade, *Zea mays* L.

1. INTRODUCTION

Maize (*Zea mays*) is of great economic importance due to its diverse uses, ranging from animal feed to high-tech industries. The use of maize grain as animal feed represents the largest share of global consumption of this cereal, approximately 70% worldwide (Duarte and Garcia, 2021). Currently, maize ranks second in Brazil's grain production, with around 125 million tons produced (CONAB, 2023).

However, every year, thousands of hectares of irrigated land in the semi-arid region are becoming unsuitable for cultivation due to soil and water salinization, resulting in decreased crop productivity and revealing the harmful consequences of salinity (morphological, physiological, and productive) on maize development (Lacerda *et al.*, 2022; Santos *et al.*, 2020; Sousa *et al.*, 2022).

One approach to mitigate these effects is the use of biostimulants. The use of biostimulants in agriculture is increasingly integrated, aiming to modify plant physiological processes and optimize productivity (Yakhin *et al.*, 2017). According to Costa (2020), rutin, a bioflavonoid found in the fruits of Fava d’Anta, shows potential for application in various areas, as reported in several studies. In Brazil, the main source of rutin is Fava d’Anta, whose fruits contain higher levels of this compound compared to the flowers of the Chinese species (Costa *et al.*, 2022).

According to Filizola (2013), fava d’anta is found in several regions of Brazil, in the states of Goiás, Minas Gerais, Federal District, Mato Grosso, Mato Grosso do Sul, São Paulo, Maranhão, Tocantins, Piauí, Bahia, Pernambuco and Ceará. This plant has economic and social relevance to the communities where it occurs, which led to it being listed among the “Native Species of the Brazilian Flora of Current or Potential Economic Value” (Brasil, 2016).

Few studies have been conducted to investigate the beneficial and/or deleterious effects of flavonoids such as rutin, quercetin, and kaempferol on cultivated plants. Research is even more scarce when it comes to analyzing the effects of these compounds, derived from plant extracts, on plants under saline stress. However, in existing literature, Fava d’Anta extract has emerged as a promising alternative for mitigating salinity stress.

In this context, this study evaluates gas exchange and initial growth of maize under saline stress and the application of Fava d’Anta extract (*Dimorphandra mollis* Benth).

2. MATERIAL AND METHODS

The experiment was conducted under full sun at the experimental area of Universidade Federal do Ceará, Campus do Pici, Fortaleza, Ceará, Brazil (3°45’ S; 38°33’ W), from July to November 2023.

The experimental design was a randomized block design in a 5 × 4 factorial scheme with three replications. The treatments consisted of five levels of irrigation water electrical conductivity (ECw = 1.0, 1.5, 3.0, 4.0, and 5.0 dS m^{-1}) and four foliar application frequencies of Fava d’Anta extract: F1 – no application; F2 – application every 10 days; F3 – application every 5 days; and F4 – daily application.

The substrate used in the experiment was characterized through physicochemical analysis carried out in a soil analysis laboratory. The chemical analysis of the substrate yielded the following results: pH in water = 7.72; N = 0.17%; P = 585.0 mg dm^{-3}; K = 1,170.0 mg dm^{-3}; Ca = 3.45 cmolc dm^{-3}; Mg = 3.22 cmolc dm^{-3}; Fe = 121.0 mg dm^{-3}; Mn = 87.2 mg dm^{-3}; Cu = 0.86 mg dm^{-3}; Zn = 48.0 mg dm^{-3}; Al = 0.0 cmolc dm^{-3}; and 27.47 g kg^{-1} of organic matter.

The crop used was maize, cultivar BRS 3046 (Saboroso). In addition to its high appeal, BRS 3046 has good green ear yield. Sowing was carried out in 18-liter pots. The pots were filled with a mixture of sandy soil, coarse sand, and cattle manure in a 5:3:2 ratio.

Fertilization management was based on the substrate analysis and fertilization recommendations for maize: 30 kg ha^{-1} of N, 40 kg ha^{-1} of P_2O_5, and 15 kg ha^{-1} of K_2O (Rodrigues, 2013). Both basal and top-dressing fertilizations were performed. Urea (45% N) was used as the nitrogen source; triple superphosphate (44% P_2O_5) as the phosphorus source; and potassium chloride (62% K_2O) as the potassium source. The maximum NPK dose per pot was 23.6 g for basal application and 13.2 g for top-dressing.

The saline irrigation solutions were prepared using NaCl, following the methodology of Rhoades *et al.* (2000), in which the electrical conductivity (EC) is obtained from the relationship between EC and salt concentration ($mmol_c$ L^{-1} = EC × 10). Only NaCl was used to prepare saline solutions. Ten days after sowing (DAS), the application of the different saline water treatments began, along with daily monitoring of water requirements per treatment, determined using drainage microlysimetry.

Irrigation was performed manually daily. From the average drained volume per pot/microlysimeter (Vdi) and the volume applied per pot/microlysimeter (Vci), the actual water volume used per plant per treatment (Vui), in milliliters per day, was calculated according to Equation 1:

$$Vui = Vci - Vdi \tag{1}$$

The volume replaced per treatment (Vri) daily was always 20% higher than the predicted water consumption, to ensure excess drainage, following the principles of the drainage lysimeter method (Bernardo *et al.*, 2019), as shown in Equation 2.

$$Vri = 1.2 \times Vui \tag{2}$$

Between May and July, Fava d’Anta pods were collected from plants found in the Cerrado regions of Ceará and Maranhão. To ensure a higher yield of rutin in the Fava d’Anta fruits, immature pods with a yellowish hue and greater dry mass and thickness were collected (Costa, 2020).

After drying, the pods were ground using an industrial grinder until a fine powder was obtained. For extract preparation, the proportion used was 30 grams of Fava d'Anta powder per 1 liter of filtered water preheated to 60°C. The extract was then filtered using a simple mesh sieve to retain larger particles. To preserve the extract and prevent fungal growth, the antifungal agent methylparaben was added at a concentration of 1 g of the chemical powder per 1 liter of Fava d'Anta extract.

Once the extract was prepared, a sample was analyzed using ultra-performance liquid chromatography coupled with photodiode array detection and mass spectrometry (UPLC-PDA-MS) to identify and quantify the compounds present. Six components were identified, including the glycosylated flavonoids: rutin, quercetin hexoside, kaempferol hexose-deoxyhexoside, and kaempferol hexoside; and the proanthocyanidins: procyanidin B dimer and (epi) gallocatechin-(epi) gallocatechin. Rutin concentration was estimated by comparing the peak areas of the rutin in the 20 ppm standard solution and the sample. The rutin concentration in the extract was estimated to be 260 ppm (260 mg L^{-1}).

During the experiment, a standard dosage of 20 mL of Fava d'Anta extract was applied according to the treatment frequencies.

At 56 DAS, the following physiological variables were evaluated: net photosynthesis – A (mmol m^{-2} s^{-1}), transpiration – E (mmol m^{-2} s^{-1}), stomatal conductance – gs (mmol m^{-2} s^{-1}), and internal CO_2 concentration – Ci (ppm). These were determined using a portable infrared gas analyzer – IRGA (Model LCi, ADC BioScientific, UK). Measurements were taken between 09:00 and 11:00 a.m., using an artificial radiation source of 1500 μmol m^{-2} s^{-1} under ambient temperature, humidity, and CO_2 conditions. During measurements, the central part of the leaf was placed directly into the IRGA chamber for gas exchange readings.

At the same time, growth variables were evaluated: plant height (measured with a measuring tape, in cm) and stem diameter (measured with a digital caliper, in mm), approximately 1.0 cm above the base of the plant.

At the end of the experimental cycle, to determine dry biomass, fresh weights of the root and shoot portions of the maize plants were recorded (on 10/14/2023). Then, dry biomass was obtained by weighing the shoots and roots dried in paper bags in a greenhouse oven (located adjacent to the experimental area) for 15 days until a constant weight was reached.

The experimental data were subjected to analysis of variance. When significant according to the F-test, regression analyses were performed to assess the effects of different saline water concentrations on the evaluated variables. The statistical analyses were conducted using the ASSISTAT 7.7 BETA software (Silva and Azevedo, 2016).

3. RESULTS AND DISCUSSION

From the analysis of variance (Table 1), a significant ($p < 0.01$) isolated effect of both the different electrical conductivities of the irrigation water (ECw) and the application of Fava d'Anta extract (F) was observed on plant height (PH). For stem diameter (SD), a significant effect was observed only in response to salinity. Regarding shoot and root dry biomass of maize plants, the different salinity levels significantly ($p < 0.01$) influenced all variables analyzed. As for the frequencies of Fava d'Anta extract application, a significant effect ($p < 0.01$) was observed for shoot dry biomass at the 5% significance level. No interaction effect (S × F) was detected for the variables under investigation.

Table 1. Summary of analysis of variance for plant height (PH), stem diameter (SD), shoot dry mass (SDM), and root dry mass (RDM) in maize plants at 56 DAS, as a function of different electrical conductivities of irrigation water and foliar application frequencies of Fava d'Anta extract.

Source of variation	Mean square				
	DF	PH	SD	SDM	RDM
Salinity (S)	4	7035.60**	51.47**	37956.25**	3837.22**
Fava D'anta (F)	3	386.17*	11.08^{ns}	3867.42*	313.20^{ns}
S x F	12	91.01^{ns}	6.71^{ns}	1258.39^{ns}	131.19^{ns}
CV (%)	-	7.58	9.21	22.79	39.5

DF - Degrees of freedom; CV - Coefficient of variation ns, ** and *: not significant and significant at 1 and 5% of probability, respectively, by the F test.

The increase in the electrical conductivity of the irrigation water linearly reduced plant height (Figure 1A). This scenario was certainly due to the osmotic effect caused by the increasing presence of soluble salts in the soil. According to Dias *et al.* (2016), in saline soils, the soluble salts in the soil solution increase water retention forces due to the osmotic effect, thus reducing water absorption by the plant.

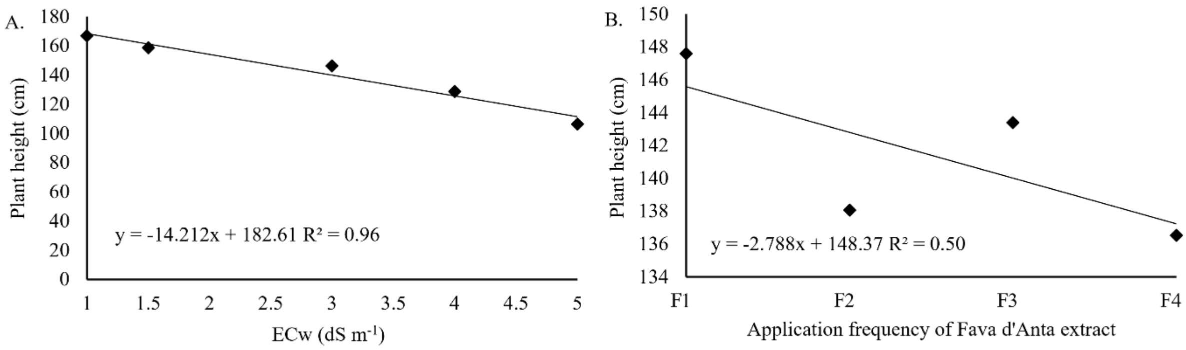

Figure 1. Plant height as a function of different electrical conductivities of irrigation water (A) and application frequencies of Fava d'Anta extract (B).

Evaluating the effect of salt stress on maize growth, Zahra *et al.* (2020) concluded that salinity stress affected plant height. Similarly, Santos *et al.* (2020) found that the electrical conductivity of irrigation water negatively influenced maize plant growth.

When analyzing separately the influence of fava d'anta extract application frequencies on maize plant height (Figure 1B), it was observed that plants that did not receive the extract grew taller than those treated with the extract. On average, plants that received daily applications (F4) ended the experimental cycle 7.5% shorter compared to those that were not exposed to the substance (F1) at any point during the experiment. However, among the plants that received the extract, those under the five-day application frequency (F3) showed the highest average heights (143.40 cm).

On the other hand, plants that received the fava d'anta extract daily may have suffered toxic effects from one or more substances contained in the extract, such as rutin, since each 20 mL application provided 5.2 mg of rutin, totaling 239.2 mg of this compound throughout the experimental cycle.

Salt stress negatively affected stem diameter (Figure 2). A quadratic polynomial equation best fit the data, with the highest values recorded in plants irrigated with water of 1.5 dS m^{-1} electrical conductivity (25.42 mm). Salinity can reduce the water potential in the soil, and in response to this reduction, plants tend to perform osmotic adjustment, which may lead to hormonal and nutritional changes (Taiz *et al.*, 2024).

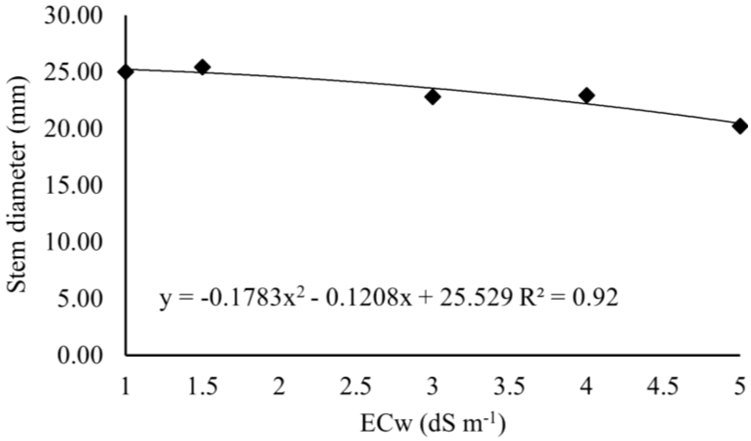

Figure 2. Stem diameter as a function of different electrical conductivities of irrigation water.

This scenario may be due to diminished photosynthesis (reduction in photoassimilates) caused by stomatal closure as a form of the plant's "physiological defense" mechanism in response to the osmotic effect resulting from increasing concentrations of NaCl in the irrigation water. According to Atta *et al.* (2023), Na^+ and Cl^- ions, when absorbed and accumulated in plant tissues at excessive concentrations from the soil, cause cytotoxicity that can eventually lead to leaf burn, reduced growth, and ultimately plant death. Several studies have shown a reduction in maize stem diameter with increasing electrical conductivity of irrigation water (Lacerda *et al.*, 2022; Li *et al.*, 2019; Oliveira *et al.*, 2016; Sousa *et al.*, 2016).

A quadratic polynomial equation best fit the decreasing trend of shoot dry mass (SDM) in response to the electrical conductivity of irrigation water (Figures 3A/B).

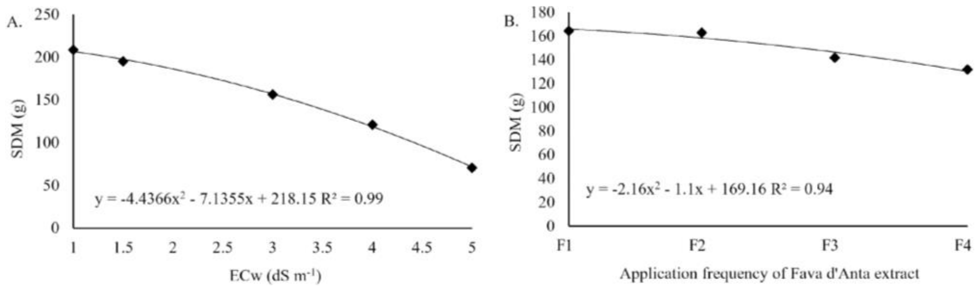

Figure 3. Shoot dry mass as a function of different electrical conductivities of irrigation water (A) and application frequencies of Fava d'Anta extract (B).

This result may be related to the reduction in osmotic potential caused by the increased concentration of soluble salts in the soil solution, which directly affects water absorption by plants and, consequently, biomass production. Evaluating maize development under different levels of irrigation water salinity, Santos *et al.* (2020) reported a decrease in shoot dry mass with increasing electrical conductivity of irrigation water. Similarly, Du *et al.* (2022) concluded that soil salinity stress can inhibit, among other variables, the accumulation of dry matter in maize crops.

Under the foliar extract application frequency of every 10 days, maize plants reached higher shoot dry mass (162.89 g) among those that received the Fava d'anta extract; however, daily application had a negative effect on this variable (Figure 3B). Possibly, the combination of high application frequency, compound concentration, and foliar dosage resulted in this effect. Evaluating the influence of single superphosphate doses on the growth and biomass accumulation of sweet sorghum plants irrigated with saline water, Sá *et al.* (2018) concluded that increased water salinity affects plant growth and dry matter accumulation.

As shown in Figure 4, the quadratic polynomial model best fit the data, with a maximum root dry mass (RDM) value of 50.84 g at an ECw of 0.22 dS m^{-1}. Salinity stress may have caused a nutrient uptake imbalance in maize plants, reducing root surface area and decreasing the density and length of root hairs (Arif *et al.*, 2019), which negatively impacted essential elements for normal root growth (Atta *et al.*, 2023).

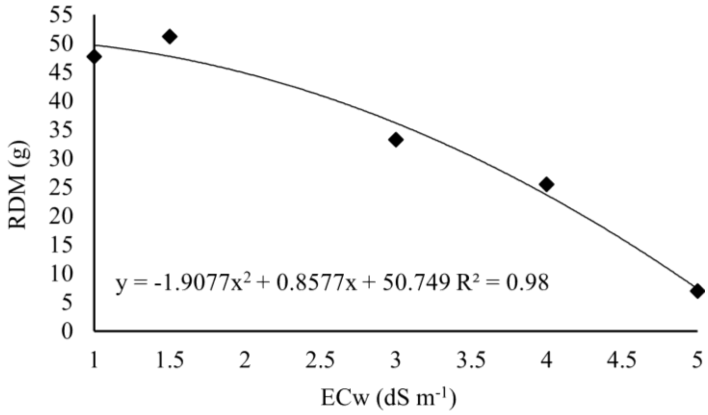

Figure 4. Root dry mass as a function of different electrical conductivities of irrigation water (A) and application frequencies of Fava d'Anta extract (B).

The plants reached the highest root dry mass value (50.84 g) when irrigated with water at ECw = 1.5 dS m^{-1}. Furthermore, comparing the plants that received water with lower electrical conductivities (S1 = 1.0 dS m^{-1}) to those that received the highest EC levels (S5 = 5.0 dS m^{-1}), a 583.09% reduction in RDM was observed. Studies worldwide have also shown negative effects of irrigation water salinity on dry biomass in maize crops (Li *et al.*, 2019; Blanco *et al.*, 2008; Zahra *et al.*, 2020).

Investigating the effect of irrigation water salinity on maize, Santos *et al.* (2020) observed a decrease in root dry mass with increasing electrical conductivity of the irrigation water.

According to the analysis of variance (Table 2), there was a significant effect ($p<0.01$) of the interaction between electrical conductivity of irrigation water (ECw) and Fava d'anta extract (FD) application on photosynthesis rate (A), stomatal conductance (gs), and transpiration rate (E), all show significance at the 1% probability level.

Table 2. Summary of analysis of variance and means for photosynthesis (A), stomatal conductance (gs), transpiration (E), and internal CO_2 concentration (Ci) in maize plants at 56 DAS, as a function of different electrical conductivities of irrigation water and frequencies of Fava d'anta extract application.

Source of variation	Mean square				
	DF	A	gs	E	Ci
Salinity (S) dd(S)(S)	4	299.68**	0.08**	18.35**	1720.95^{ns}
Fava D'anta (F)	3	98.51*	0.00^{ns}	1.13^{ns}	1794.24^{ns}
S x F	12	131.23**	0,02**	4.72**	1375.42^{ns}
CV (%)	-	20.59	28.30	16.26	26.65

DF - Degrees of freedom; CV - Coefficient of variation ns, ** and *: not significant and significant at 1 and 5% of probability, respectively, by the F test.

The interaction between the different electrical conductivities of irrigation water and the frequencies of application of Fava d'Anta extract on photosynthesis in corn plants indicates that, under certain combinations of these factors, it is possible to mitigate the effects of salinity (Figure 5).

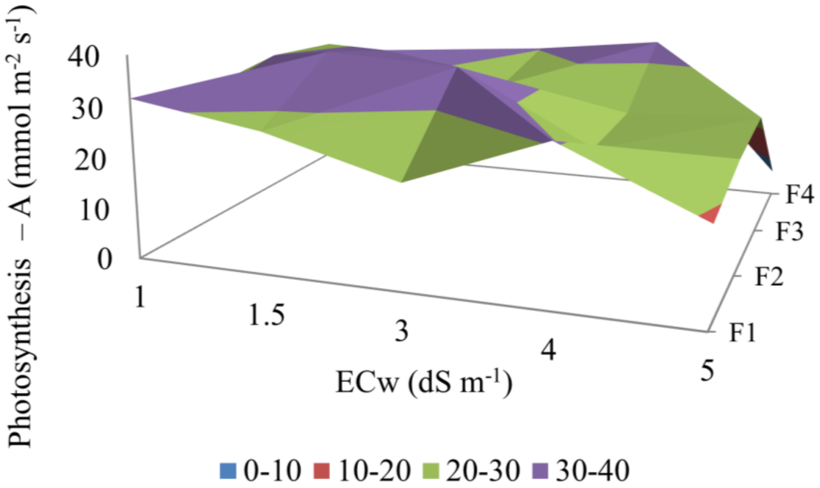

Figure 5. Photosynthesis as a function of different electrical conductivities of irrigation water and application frequencies of Fava d'Anta extract.

In general, regardless of the extract application frequency, maize plants under salt stress exhibited reduced photosynthetic activity, with minimum values reaching 5.19 μmol m^{-2} s^{-1}. It is well known that one of the main harmful effects of using saline water for irrigation is the reduction in photosynthesis (Magalhães *et al.*, 2021; Atta *et al.*, 2023).

The interaction results indicate the existence of both lower and upper thresholds for photosynthetic performance. For instance, when maize plants received daily applications of Fava d'anta extract (F4), those irrigated with water of lower electrical conductivity showed reduced photosynthetic rates (27.97 and 19.82 μmol m^{-2} s^{-1}), suggesting that daily application may have exerted a deleterious effect under these conditions.

Conversely, under EC = 4.0 dS m^{-1}, daily applications of the extract appeared to act positively. Under this condition, plants exhibited the highest photosynthetic rates (33.26 μmol m^{-2} s^{-1}) compared to other application frequencies. The flavonoids present in the Fava d'anta extract may have mitigated the adverse effects of saline water on maize plants, as these compounds are a diverse group of secondary metabolites known to play a wide range of biological roles, including stress protection (Singh *et al.*, 2021). Moreover, according to Frasca *et al.* (2020), biostimulants can promote and modulate efficient responses in metabolic and physiological processes to reduce damage caused by abiotic stresses, such as photosynthetic inhibition.

Regarding stomatal conductance (gs), a similar trend was observed: increasing water salinity led to a reduction in gs values in maize plants (Figure 6). Plants irrigated with saline water at EC = 3.0 dS m^{-1} and treated with the extract every 10 days showed 117.83% higher stomatal conductance compared to plants that did not receive the extract (F1) under the same salinity level. A similar response was observed in plants irrigated with water of EC = 1.5 dS m^{-1}.

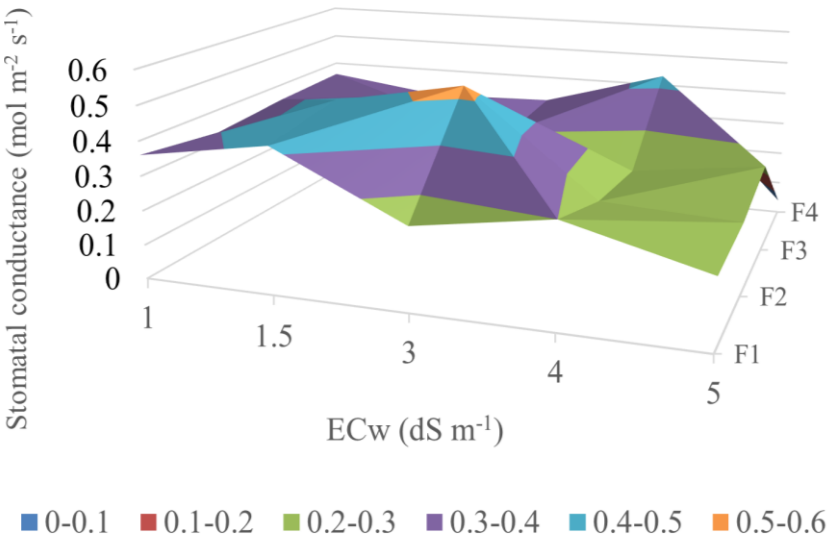

Figure 6. Stomatal conductance as a function of different electrical conductivities of irrigation water and application frequencies of Fava d'Anta extract.

As observed in the photosynthesis analysis, it is worth highlighting the apparent positive influence of Fava d'anta extract on the stomatal conductance of maize plants under saline water irrigation. Similarly, among plants irrigated with EC = 4.0 dS m^{-1}, those that received daily foliar applications of the extract exhibited the highest stomatal conductance values.

In contrast, for plants irrigated with EC = 1.5 dS m^{-1}, the most effective application frequency was F2 (every 10 days). There seems to be a direct relationship between application frequency and extract efficiency, where higher saline concentrations demand more frequent applications of the extract for greater efficacy.

In addition to rutin, other compounds such as quercetin and kaempferol, also present in the extract, likely contributed positively by enhancing the plants' tolerance to salt stress. These compounds effectively protect plants under stress conditions by regulating specific hormones and secondary metabolites (Jan *et al.*, 2022).

Stomatal closure induced by salt stress can decrease the availability of carbon dioxide within the leaves and the partial pressure of CO_2 in the intercellular spaces or substomatal cavity, thereby leading to photosynthetic inhibition (Kamran *et al.*, 2020). Among other evaluated variables, Lacerda *et al.* (2022) concluded that irrigation water with EC = 2.0 dS m^{-1} reduced stomatal conductance in maize plants.

Salt stress also decreased transpiration rates; however, this reduction was less pronounced when the extract was applied every 10 days (Figure 7).

The response surface model provided the best fit to the data, highlighting the positive impact of Fava d'anta extract in mitigating the effects of saline irrigation water (at ECw = 1.5, 3.0, and 5.0 dS m^{-1}). Maize plants that received the extract every five days exhibited 4.80%, 18.96%, and 35.88% higher transpiration, respectively, compared to plants that did not receive the extract.

This effect is most likely due to the action of rutin, quercetin, and kaempferol, which act as antioxidant agents and enhance plant tolerance to various biotic and abiotic stresses (Singh *et al.*, 2021; Jan *et al.*, 2022).

According to Taiz *et al.* (2024), the reduction in leaf water potential caused by osmotic stress leads to stomatal closure, which in turn decreases stomatal conductance and consequently reduces transpiration.

An increase of 40.63% in transpiration was observed in comparison to plants that did not receive the extract under the same ECw. Compounds present in the extract, such as rutin and quercetin, may have contributed positively. In fact, the beneficial effects of these flavonoids on the photosynthetic performance of plants such as tomato and wheat have been reported in scientific studies (Gorni *et al.*, 2022; Jańczak-Pieniążek *et al.*, 2021).

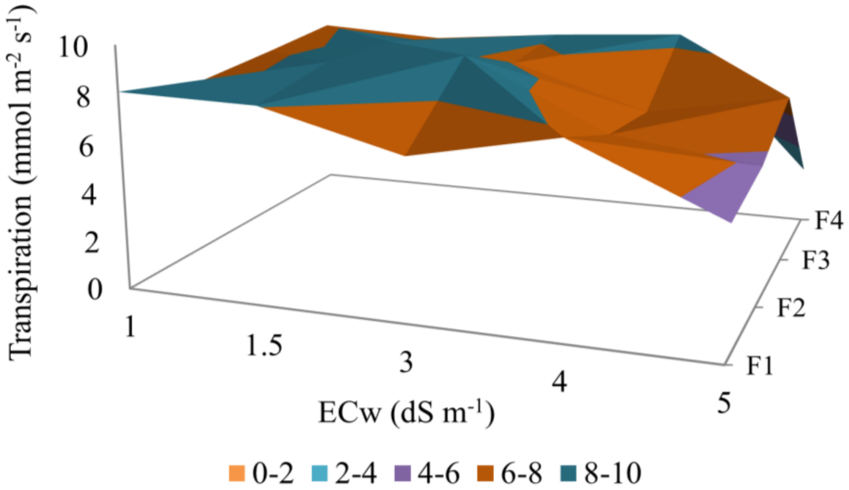

Figure 7. Transpiration as a function of different electrical conductivities of irrigation water and application frequencies of Fava d'Anta extract.

4. CONCLUSIONS

The increase in the electrical conductivity of irrigation water led to reductions in both morphological (plant height and stem diameter) and physiological variables (photosynthesis, stomatal conductance, and transpiration) of maize.

Different application frequencies of Fava d'anta extract significantly influenced the results, showing an inverse relationship with variables such as plant height, photosynthesis, and shoot dry mass. In general, higher application frequencies were associated with reduced plant development.

Daily application of Fava d'anta extract mitigated the harmful effects of irrigation water with an electrical conductivity of 4.0 dS m^{-1}, particularly with respect to gas exchange processes.

Overall, the best results for photosynthesis, stomatal conductance, and transpiration were obtained under the combinations S2 × F2, S3 × F2, and S3 × F2, respectively. These findings suggest that the application of 20 mL of Fava d'anta extract every 10 days during the vegetative cycle has the potential to alleviate the deleterious effects of saline water in maize cultivation. However, further studies are recommended, especially regarding the optimal frequency, dosage, and method of application of Fava d'anta extract in plants.

5. DATA AVAILABILITY STATEMENT

Data availability not informed.

6. ACKNOWLEDGMENTS

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