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Gas exchange dynamics and leaf water potential in sorghum intercropped with prickly pear and irrigated with reused water[1]

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ABSTRACT

The reuse of treated domestic wastewater is a promising alternative in semiarid regions of the world. This study aimed to assess the intercropping of sorghum (*Sorghum sudanense*) with prickly pear (*Opuntia stricta*) cultivated under irrigation depths (80, 100 and 120 % of the crop evapotranspiration - ETc) with reused water, in soil with and without mulch. A randomized block design was used, in a 3 (irrigation depths) x 2 (with and without mulch) factorial scheme, with four replications. The leaf gas exchange, water potential and chlorophyll fluorescence were assessed. The use of mulch in the intercropping between prickly pear and sorghum positively influenced the gas exchange and photosynthetic efficiency, resulting in increases of up to 156, 186 and 14 %, respectively for net photosynthesis, transpiration and photosynthetic efficiency, in addition to increasing the water potential. The irrigation depths affected the quantum yield and water potential, with the 100 % irrigation depth standing out. Considering the performance of the physiological variables, it is possible to indicate as positive the adoption of reused water and mulch.

KEYWORDS: *Sorghum sudanense*, *Opuntia stricta*, abiotic stress, domestic wastewater.

RESUMO

Dinâmica de trocas gasosas e potencial hídrico foliar em sorgo consorciado com palma e irrigado com água de reuso

O reuso da água oriunda de esgoto doméstico tratado é uma alternativa promissora em regiões semiáridas do mundo. Objetivou-se avaliar o consórcio de sorgo (*Sorghum sudanense*) com palma (*Opuntia stricta*), utilizando-se lâminas de irrigação (80, 100 e 120 % da evapotranspiração da cultura - ETc) com água de reuso, em solo com e sem cobertura morta. Utilizou-se delineamento em blocos casualizados, em esquema fatorial 3 (lâminas de irrigação) x 2 (com e sem cobertura), com 4 repetições. Foram avaliadas as trocas gasosas foliares, potencial hídrico e fluorescência da clorofila. O uso da cobertura morta em plantio consorciado entre palma e sorgo influenciou positivamente nas trocas gasosas e eficiência fotossintética, resultando em aumentos de até 156, 186 e 14 % para fotossíntese líquida, transpiração e eficiência fotossintética, respectivamente, além de promover aumento do potencial hídrico. As lâminas de irrigação influenciaram no rendimento quântico e no potencial hídrico, com destaque para a lâmina de 100 %. Considerando-se a performance das variáveis fisiológicas, é possível indicar como positiva a adoção de água de reuso e cobertura morta.

PALAVRAS-CHAVE: *Sorghum sudanense*, *Opuntia stricta*, estresse abiótico, esgoto doméstico.

INTRODUCTION

Agriculture is the economic activity that consumes the freshest water on the planet, reaching rates of around 70 % (Peng et al. 2019). On the other hand, the scarcity of water sources for agriculture in some regions, primarily in arid and semiarid climates, makes reused water a sustainable alternative to solve this problem (FAO 2017).

Sustainability and a decline in environmental impacts have heightened the debate on the Agenda 2030, whose main goal is sustainable development (United Nations 2015). The United Nations (2015) has established 17 sustainable development goals (SDG), and agricultural practices play a key role in meeting some of them.

The adoption of agricultural production systems using treated wastewater is directly related to the SDG 2, 6 and 12 in several ways, with SDG 2 (zero hunger) aimed at ending hunger, achieving food security and improving nutrition through sustainable agriculture; SDG 6 (clean water and sanitation) related to agricultural practices involving water resource management, including reducing water pollution

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from runoff and improving water-use efficiency; and SDG 12 (responsible consumption and production) associated with promoting sustainable agriculture and food systems, including reducing the use of harmful chemicals (FAO 2023).

Treated wastewater used in irrigation could be an alternative to conserve available high-quality water (Ungureanu et al. 2020) and help to restore degraded soils (Hussain et al. 2018), and may reflect in an increased agricultural yield in marginal soils. Additionally, the use of reclaimed water from domestic sewage treatment plants can benefit the soil physical, chemical and biological attributes, since, even after secondary treatment, its chemical-physical composition still differs from other water sources commonly used in irrigation (Ofori et al. 2021, Mishra et al. 2023).

Mulching is another promising practice to mitigate the effects of water stress in semiarid environments and protect the bare soil, providing benefits such as weed control and organic matter deposition, mitigating the impact of solar radiation, improving soil moisture distribution and reducing water losses due to evaporation (Jiménez et al. 2017, Alves et al. 2022).

Sorghum (*Sorghum sudanense*) is a high-energy multipurpose grass with considerable digestibility, yield and adaptation to environments, used for green cutting, silage, grazing, animal and human feed (Silva et al. 2021). It is characterized by high production in low-fertility areas, tolerance to water regimes, high sprouting capacity and salt tolerance (Reza et al. 2019, Carvalho 2020). Furthermore, it has been cultivated with reused water in several regions due to its adaptability to environmental stresses, and uses less water than traditional crops, such as maize (Carvalho 2020, Chaganti et al. 2020).

Prickly pear (*Opuntia stricta*) is adapted to different soil and climate conditions. This cactus species has a high water, mineral, fiber, carbohydrate and energy content for animals, making it an alternative for forage supply, primarily during periods of drought. The crassulacean acid metabolism, characteristic of the prickly pear, allows the plant to reduce water loss to the environment, making it more efficient in water use, when compared to C3 and C4 metabolism plants (Epifânio 2019).

Sorghum-prickly pear intercropping is widely studied in semiarid regions, demonstrating a good fresh matter production, with more than 60 t ha^{-1} (Diniz et al. 2017). Additionally, variations in irrigation depth indicate a potential for increased profits from this agricultural system, due to the high tolerance of these crops to water stress (Alves et al. 2020, Silva et al. 2023).

Among the parameters used for indirect crop assessment, gas exchange and chlorophyll fluorescence stand out as non-destructive evaluations that serve as stress indicators (Geetika et al. 2019, Sonawane & Cousins 2020). Moreover, the study of water potential in plants is widely disseminated, since it assesses water status, making it suitable for analyzing crops under abiotic stress conditions.

As such, the aim of this study was to assess sorghum plants intercropped with prickly pear, using irrigation with treated wastewater and mulch to identify alternative management practices aligned with the SDG and suited to the semiarid regions in Brazil.

MATERIAL AND METHODS

The experiment was conducted at the experimental station for domestic sewage treatment and reuse of the Mutuca district, in the municipality of Pesqueira, Pernambuco state, in the Brazilian semiarid region (8º16’50.94”S and 36º34’17.63” W), where the climate is classified as BSh, according to the Köppen-Geiger climate classification (Alvares et al. 2013). The soil is classified as Sodic Eutric Planosol (IUSS 2022).

The sewage treatment plant is gravity fed and receives approximately 3,000 L day^{-1} of wastewater from 150 households. The domestic sewage undergoes preliminary treatment with bar screens, followed by an up-flow anaerobic sludge blanket reactor combined with an upward-flow anaerobic filter. This process generates treated wastewater for irrigation.

The experiment was conducted with intercropping of sorghum (IPA SUDAN 4202) and prickly pear (elephant ear). The latter was planted in March 2021, and sorghum in April 2022. The experimental design was randomized blocks, with a 3 x 2 factorial scheme (three irrigation depths x mulching and non-mulching), and four replications. The irrigation depths for reused water were calculated based on the crop evapotranspiration (ETc - 80, 100 and 120 %) for sorghum and applied via drip irrigation, which was installed at the time of sorghum planting. Additionally, the crop was assessed with and without mulch. The reference evapotranspiration (ETo) for sorghum was estimated using the Penman-Monteith method (FAO 56), obtained with data from the automatic weather station located in the study area. Rainfall was measured from April to October 2022 (Figure 1).

The prickly pear was planted in a single row with 0.2 m between plants and 1 m between rows, while the sorghum was planted between prickly pear rows at a distance of 0.5 m, with 12 sorghum and 5 prickly pear plants m^{-1}. The plots consisted of four single rows of prickly pear and four of sorghum, being each plot 3 m long and 5 m wide, totaling 15 m^2 $plot^{-1}$, with 4 replications (Figure 2).

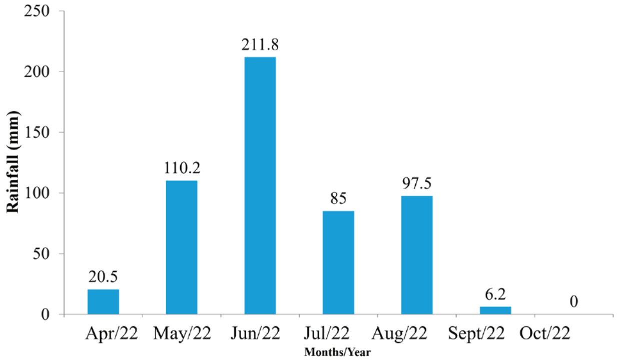


Figure 1. Rainfall from April to October 2022, in the experimental area (Mutuca, Pesqueira, Pernambuco state, Brazil).

The mulch was applied after sorghum planting, composed of species found in the experimental area such as creeping grass (*Urochloa mosambicensis*), sandbur (*Cenchrus echinatus*), goosefoot grass (*Eleusine indica*) and chichá leaves (*Sterculia striata*), with a predominance of creeping grass and sandbur. About 8,000 kg ha^{-1} of mulch were applied to the soil.

Disturbed soil samples were collected using a Dutch auger at depths of 0-10, 10-20 and 20-40 cm. Undisturbed samples were also collected using Uhland auger and volumetric rings (height = 50 mm; diameter = 50 mm).

The physical characterization consisted of the following attributes: granulometric analysis using the densimeter method, soil bulk density obtained by the volumetric cylinder method, and total porosity based on the direct method (Teixeira et al. 2017) (Table 1).

The soil chemical characterization was carried out using the following analyses: pH in water, electric conductivity of the saturation extract, P, K and Na according to Teixeira et al. (2017), total organic carbon quantified by oxidizing organic matter using potassium dichromate (Yeomans & Bremmer 1988) (Table 2).

The leaf gas exchange was measured using leaves from the middle third of the plants between 8:30 and 11:30 a.m., in August (sampling I) and October 2022 (sampling II). The net photosynthesis, transpiration and stomatal conductance were

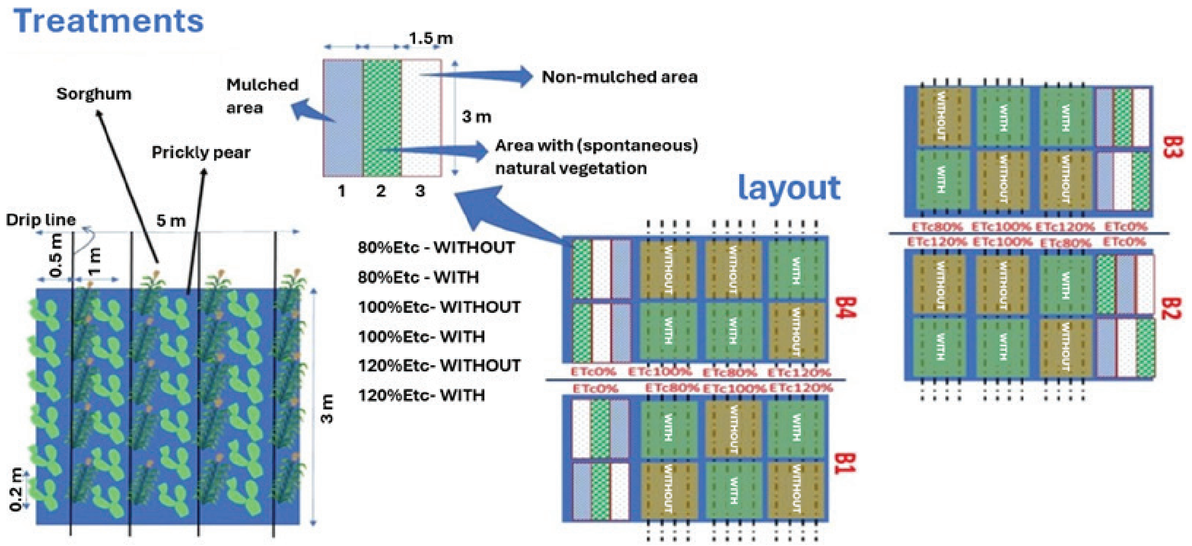


Figure 2. Layout of the experimental unit.

Table 1. Granulometric analysis and particle density of the research area soil.

Layer cm	Sand	Silt	Clay	WDC	FD %	SD	PD	Textural class
	g kg^{-1}					g cm^{-3}		
0-10	734	119	147	5.3	96	1.3	2.6	Sandy-loam
10-20	724	107	169	6.8	96	1.5	2.6	Sandy-loam
20-40	704	118	178	7.1	96	1.7	2.6	Sandy-loam

WDC: water-dispersible clay; FD: flocculation degree; SD: soil density; PD: particle density.

Table 2. Chemical analysis of the research area soil.

Layer cm	pH (H_2O)	ECse Sd m^{-1}	P Mg dm^{-3}	K	Na	TOC	N
				$cmol_c$ dm^{-3}		g kg^{-1}	
0-10	7.42	0.96	117.75	1.5	1.8	14.89	4.63
10-20	7.91	1.72	94.22	1.4	1.9	11.35	3.16
20-40	8.12	2.44	51.22	1.3	2.1	7.26	2.22

ECse: electrical conductivity of the saturation extract; P: phosphorus; K: potassium; Na: sodium; TOC: total organic carbon; N: nitrogen.

analyzed using the Infra Red Gas Analyzer (IRGA - Licor LI6400XT, LICOR® Inc., Lincoln, NE, USA), and the instantaneous water-use efficiency was calculated (Oliveira et al. 2017).

Parameters related to maximum chlorophyll fluorescence (F_m) and variable fluorescence (F_v) were analyzed using a fluorometer (Fluorpen Model FP-100) (Martins et al. 2020) on the same dates and times as gas exchange measurements, with one assessment in August and another in October. The leaves were adapted to darkness, using leaf clips, for 30 min, after which chlorophyll fluorescence was measured. The maximum quantum yield of the photosystem II was determined by calculating the F_v/F_m ratio.

The leaf water potential was evaluated in August and October, using the Scholander pressure chamber (Model 1515D, PMS Instrument Company) in plant samples collected before dawn. After the leaf collection, readings were taken directly at the experimental site. The leaf material was subjected to constant pressure until sap was observed oozing from the petiole (Leal et al. 2020).

The Shapiro-Wilk test ($p \leq 0.05$) was used to test data normality. Comparisons within and between treatments were performed via Anova. When Anova showed significance ($p \leq 0.05$), the mean values were compared using the Scott-Knott test ($p \leq 0.05$). The treatments were compared independently in each sampling period (August and October) and for the reused water irrigation depths, and the data were analyzed using the Sisvar software version 5.6 (Ferreira 2019).

RESULTS AND DISCUSSION

No significant differences ($p \leq 0.05$) were observed in the assessment of net photosynthesis in sampling I (Figure 3A); however, in sampling II, a significant difference ($p \leq 0.05$) was observed between the mulched and non-mulched treatments, but no significant effect ($p \leq 0.05$) was found for the different reused water irrigation depths (Figure 3B). The higher values in the mulched treatment indicate that mulch helps sorghum to achieve a higher net photosynthesis rate.

In the first sampling, the low differentiation of factors may be related to the different sampling times, since August 2022 (sampling I) recorded a rainfall of 975 mm, representing an accumulation of 259 %, when compared to the regional average (APAC 2022). During the second sampling, there was no rainfall (0 mm), causing an opposite effect to that of sampling I, that is, a greater contribution of the soil cover factor to the analyzed variables.

In the second sampling, mulching resulted in increases of 156, 154 and 142 % for net photosynthesis at the ETc levels of 80, 100 and 120 %, respectively, when compared to the non-mulched crop. The soil cover promotes moisture retention and lowers the soil temperature (Chang et al. 2020), conditions under which plants tend to increase photosynthesis. In addition, sorghum shows an increased photosynthetic pigment content and production under mulched conditions (Abd El-Mageed et al. 2018).

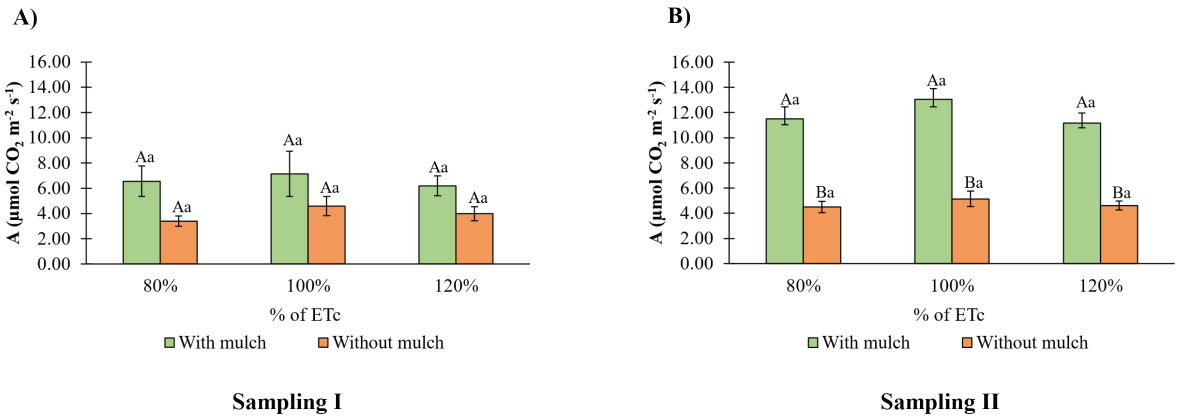


Figure 3. Net photosynthesis (A) of sorghum irrigated with reused water irrigation depths, with and without mulch, in sampling I (A) and sampling II (B). Means followed by the same lowercase letter are not significantly different among the irrigation depths. Different uppercase letters indicate significance between the mulched and non-mulched treatments, according to the Scott-Knott test ($p \leq 0.05$).

As observed for net photosynthesis, the crop transpiration showed significant differences ($p \leq 0.05$) only during sampling II, and only in relation to mulching (Figures 4A and 4B).

Abiotic factors such as higher temperatures and salt stress reduce transpiration in sorghum plants (Feijão et al. 2011), with salt stress often associated with the indiscriminate application of reused water, due to its higher salt concentration, when compared to other water sources (Chaganti et al. 2020). Although soil cover promotes favorable growing conditions, such as increased soil moisture and reduced water loss through evaporation, it may also increase sorghum transpiration, maintaining overall water loss (Zhang et al. 2020). This was observed during the sampling II, where mulch promoted increases of 186, 158 and 153 % in transpiration for the ETc levels of 80, 100 and 120 %, respectively, when compared to soil with no cover.

The sorghum transpiration was not affected by irrigation depths, similarly to the results found by Moreira et al. (2013), who studied sorghum cultivars and observed that transpiration was not affected at irrigation depths of 80, 60 and 40 % of the field capacity, for the Ramanda and BRS 501 cultivars. These results may be justified by the greater adaptation of C4 plants to hot and dry climates, with some of these adaptations being smaller stomatal

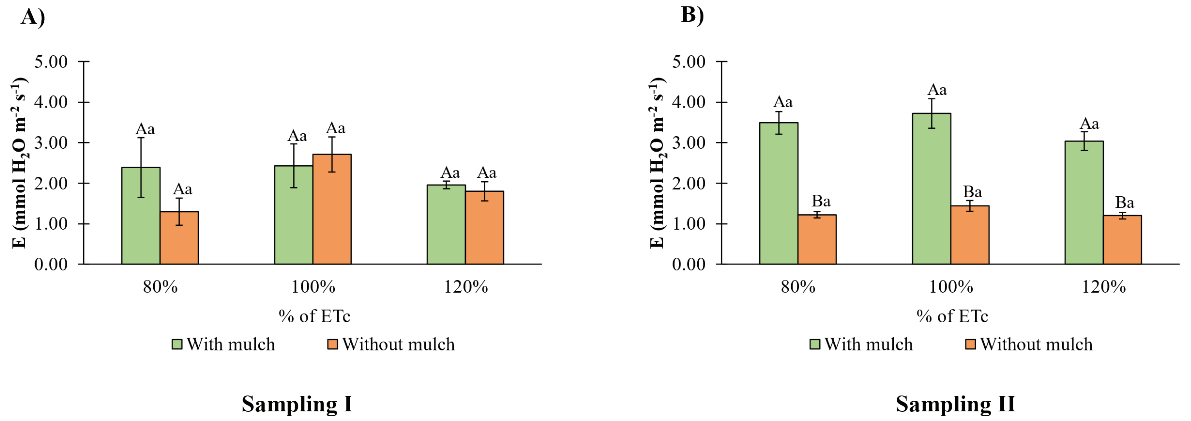


Figure 4. Transpiration (E) of sorghum irrigated with reused water irrigation depths, with and without mulch, in sampling I (A) and sampling II (B). Means followed by the same lowercase letter are not significantly different among the irrigation depths. Different uppercase letters indicate significance between the mulched and non-mulched treatments, according to the Scott-Knott test ($p \leq 0.05$).

opening and the absence of photorespiration (Killi et al. 2020).

For stomatal conductance, significant differences ($p \leq 0.05$) were observed between mulched and non-mulched treatments in sampling I, where the latter obtained the lowest values at 120 % of irrigation, averaging 0.19 mol m^{-2} s^{-1} (Figure 5A). No differences were found in any comparisons during sampling II (Figure 5B).

Among the main mechanisms in plants tolerant to abiotic stress, osmotic adjustment contributes to reducing water loss to the atmosphere while maintaining stomatal opening and cell turgor (Pimentel 2004). This mechanism is widely reported in the literature, in relation to sorghum under water stress, including other evapotranspiration mechanisms and the presence of cuticular wax on leaves (Yahaya & Shimelis 2021).

Sorghum is highly tolerant to water stress, and although high stomatal conductance results are used as a parameter for selecting more tolerant genotypes (Geetika et al. 2019), when studying a single genotype, they may not show significant differences between control and water stress conditions (Sonawane & Cousins 2020), as observed for most treatments assessed during the samplings I and II.

Although less reported in the literature, the excess of water stress can also affect crops, and reductions in gas exchange, including stomatal conductance, have been reported for sweet sorghum under water excess (Zhang et al. 2016). This was observed in the sampling I for the 120 % irrigation treatment with mulching, which exhibited the lowest stomatal conductance value.

The maximum fluorescence showed a significant effect ($p \leq 0.05$) between irrigation depths and mulched and non-mulched treatments in the first sampling period, with mulching at 100 % of the ETc irrigation obtaining the highest mean value (Figure 6A).

In the sampling II, a significant difference ($p \leq 0.05$) was found between mulched and non-mulched treatments, where the latter at 100 and 120 % of the ETc irrigation exhibited the highest maximum fluorescence values, when compared to the other treatments (Figure 6B). Reductions in the maximum fluorescence (F_m) may indicate stress in sorghum (Yoo et al. 2014). Abiotic stresses can alter the initial (F_0) and maximum fluorescence due to possible damage or loss of efficiency in the plant's photosynthetic apparatus (Zhang et al. 2019, Stefanov et al. 2023).

The maximum quantum yield of the photosystem II (F_v/F_m) for sorghum showed a significant difference ($p \leq 0.05$) between irrigation depths and mulched and non-mulched treatments in the sampling I, with mulching at 100 and 120 % of the ETc irrigation showing the highest F_v/F_m values of 0.75 and 0.65, respectively (Figure 7A).

In the sampling II, a significant difference ($p \leq 0.05$) was observed between mulched and non-mulched treatments. Increases of 9 and 14 % were obtained in the treatment with mulch at 80 and 120 % of the ETc irrigation, when compared to the treatments without mulch (Figure 7B).

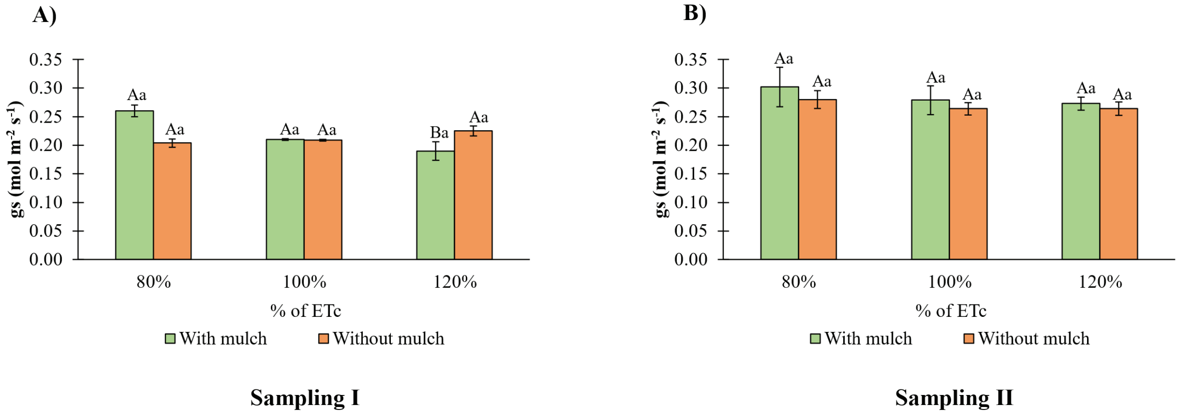
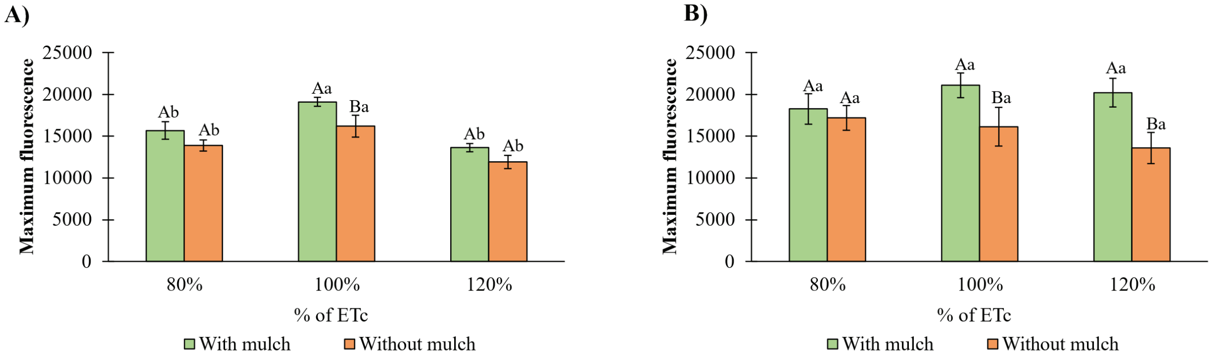


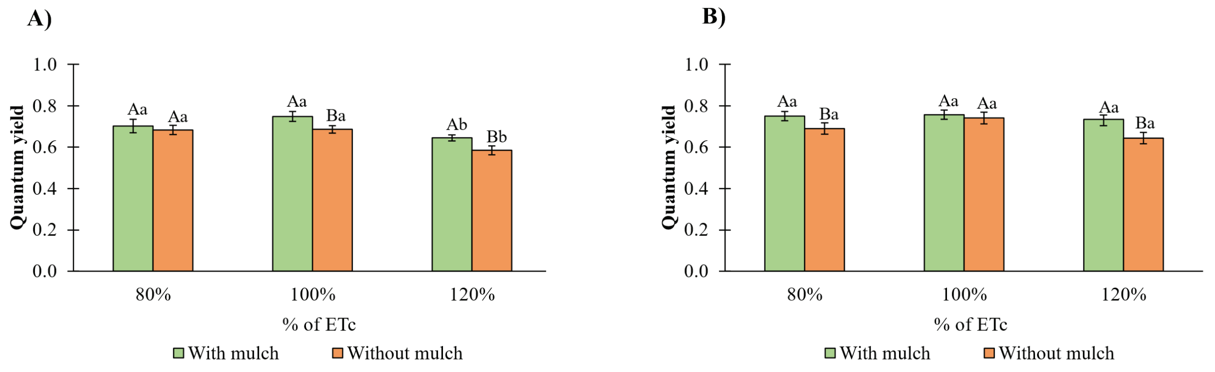
Figure 5. Stomatal conductance (gs) of sorghum irrigated with reused water irrigation depths, with and without mulch, in sampling I (A) and sampling II (B). Means followed by the same lowercase letter are not statistically different among the irrigation depths. Different uppercase letters indicate significance between the mulched and non-mulched treatments, according to the Scott-Knott test ($p \leq 0.05$).



Sampling I

Sampling II

Figure 6. Maximum fluorescence (F_m) of sorghum irrigated with reused water irrigation depths, with and without mulch, in sampling I (A) and sampling II (B). Means followed by the same lowercase letter are not statistically different among the irrigation depths. Different uppercase letters indicate significance between mulched and non-mulched treatments, according to the Scott-Knott test ($p \leq 0.05$).



Sampling I

Sampling II

Figure 7. Maximum quantum yield of the photosystem II (F_v/F_m) of sorghum irrigated with reused water irrigation depths, with and without mulch, in sampling I (A) and sampling II (B). Means followed by the same lowercase letter are not statistically significant among the irrigation depths. Different uppercase letters indicate significance between mulched and non-mulched treatments, according to the Scott-Knott test ($p \leq 0.05$).

Both the water excess and water stress can damage the plant's photosynthetic apparatus due to, among other factors, the accumulation of reactive oxygen species. In the case of water excess, this is mainly due to low root oxygen absorption, while with water stress it can cause metabolic imbalances due to the loss of cellular integrity, with the photosynthetic apparatus being one of the main sites affected by the production and accumulation of reactive oxygen species (Sachdev et al. 2021).

Water excess may have occurred during the first sampling period (rainy season), when the plants were irrigated at 120 % of the ETc, while water stress may have occurred in the 80 % irrigation treatment without mulching, during the second sampling period (no rainfall). Another factor to consider is the continuous application of reused water, which can promote soil salinization (Chaganti et al. 2020), especially when applied to uncovered soil. This is more likely only during the second sampling period, due to the longer treatment time.

Mulching mitigates water stress and reduces salt accumulation in the soil (Wang et al. 2022), given that soil salinity is a potential problem with the

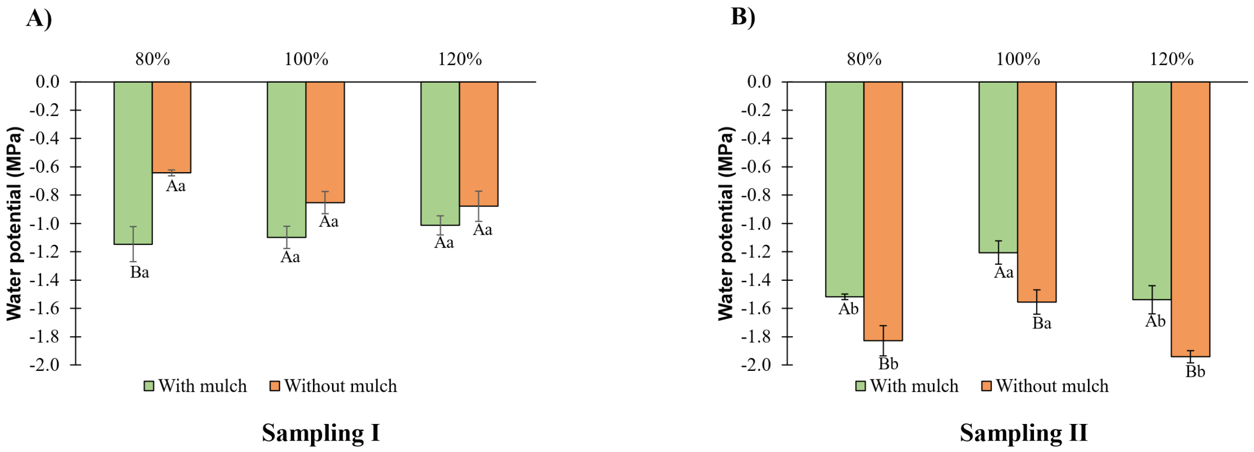


Figure 8. Leaf water potential of sorghum irrigated with reused water irrigation depths, with and without mulch, in sampling I (A) and sampling II (B). Means followed by the same lowercase letter are not statistically significant among the irrigation depths. Different uppercase letters indicate significance between mulched and non-mulched treatments, according to the Scott-Knott test ($p \leq 0.05$).

indiscriminate application of reused water (Rahman et al. 2022). Increased chlorophyll a, b, carotenoids and relative water content in sorghum cultivated with mulch have also been observed by Abd El-Mageed et al. (2018).

The sorghum leaf water potential showed a significant difference ($p \leq 0.05$) at 80 % of the ETc irrigation for sampling I, between mulched and non-mulched treatments, with averages of -1.146 and -0.643 MPa, respectively (Figure 8A).

The non-mulched treatments in sampling II (Figure 8B) were significantly different ($p \leq 0.05$) at all irrigation depths. The lowest water potential values were found at 120 and 80 % of irrigation without mulch, indicating that these plants were more stressed, when compared to those submitted to mulching at 100 % of irrigation.

The sampling II took place in a rain-free month in the region, resulting in more negative water potential values, when compared to the sampling I, and lower water potential in mulched *versus* non-mulched plants, once again indicating stress attenuation through mulching and a possible osmotic adjustment between the assessment periods. Mulching is more effective in dry periods, because it mitigates the effects of water stress on crops (Souza et al. 2022). According to Lavinsky et al. (2015), a low soil water availability decreases the leaf water potential, what may cause water stress. Additionally, plants subjected to abiotic stresses, such as drought and/or salinity, reduce the leaf water potential and relative water content, because these factors are closely related to water availability (Leal et al. 2020, Monteiro et al. 2021).

CONCLUSIONS

1. The use of mulch in intercropping between prickly pear and sorghum positively influenced the gas exchange (net photosynthesis and transpiration) and maximum quantum yield of the photosystem II (Fv/Fm). However, different irrigation depths did not affect gas exchange, indicating that the irrigation, even at 80 % of the crop evapotranspiration (ETc), did not stress the sorghum plants;
2. The maximum fluorescence and quantum yield were positively influenced by mulching and 100 % of the ETc;
3. Under low rainfall conditions, the sorghum water potential is influenced by mulch and 100 % of the ETc, with the lowest values found in this treatment, indicating reduced stress.

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