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CROP PRODUCTION

Combining silicon, organic matter, and *Trichoderma harzianum* to mitigate salt stress in forage sorghum

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ABSTRACT. Salt stress is a major abiotic factor limiting plant growth worldwide, particularly in arid and semiarid regions where excessive groundwater use in irrigation leads to high salt concentrations. To address this issue, this study investigated the efficacy of silicon, either alone or in combination with *Trichoderma harzianum* and organic matter, in mitigating salt stress in forage sorghum. The experiment took place in a saline Fluvisol in Parnamirim, a semiarid region of Pernambuco, Brazil, and followed a randomized block design with five treatments and four replicates: sorghum (control); sorghum + Si; sorghum + Si + OM (organic matter); sorghum + Si + T (*T. harzianum*); and sorghum + Si + T + OM. Sorghum plants were assessed over three cycles (initial cut and two regrowths) from June 2021 to April 2022. The combined treatments of Si + OM, Si + T, and Si + T + OM increased plant growth by 42.17, 35.49, and 27.51%, respectively, compared to the control. Similarly, these treatments led to biomass accumulation gains of 39.42, 40.44, and 31.77% in sorghum plants relative to the control. Silicon alone did not yield significant growth or biomass accumulation improvements. The application of silicon in conjunction with *T. harzianum* and/or organic matter shows promise in enhancing forage sorghum growth under saline stress conditions in semiarid regions.

Keywords: *Sorghum sudanense*; salinity; fungus; semiarid.

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Introduction

Salt stress is a primary constraint on food production in arid and semiarid regions worldwide (Munns, 2002). In the Brazilian semiarid area, irrigation water with high salt concentrations is commonly used due to the limited availability of good quality water. Therefore, it is crucial to explore methods to mitigate saline stress on plants and promote plant growth.

Silicon has been identified as a key element in attenuating salt stress and has gained interest in crop production (Cantuário, Luz, Pereira, Salomão, & Re-Bouças, 2014; Dhiman et al., 2021). It enhances plant tolerance to abiotic stresses, such as soil salinity, by regulating photosynthesis, activating antioxidant enzymes, forming a silica barrier in roots to reduce the salt passage to shoots, and increasing osmoregulator production to adjust root osmotic potential with soil (Coskun, Britto, Huynh, & Kronzucker, 2016). When combined with other mitigating factors, silicon's capacity for stress mitigation can be improved (Kaloterakis, van Delden, Hartley, & De Deyn, 2021; Chakma et al., 2022). However, studies on silicon's effectiveness, when combined with other mitigators in reducing salt stress in plants, are limited.

Animal-origin organic matter improves soil quality and provides more favorable cultivation conditions for plant growth under saline and salt stress. Organic conditioners (manure) reduce the soil's exchangeable sodium percentage (ESP) by releasing Ca^{2+} and Mg^{2+} ions that replace Na+ in the exchange complex (Pessoa et al., 2022). Organic matter also enhances soil fertility, water retention, and aggregation, positively affecting water infiltration and salt leaching (Miranda et al., 2018). It is typically low-cost and readily available on most rural properties (Huang et al., 2022).

Trichoderma harzianum (Rifai) is a fungus that forms a symbiotic relationship with plant roots, releasing various compounds that improve plant response to abiotic stresses, particularly salinity (Afzal, Basra, Farooq,

& Nawaz, 2006). It enhances root growth, antioxidant enzyme activity, osmoregulator production, and photosynthetic rates, which contribute to plant salinity tolerance (Zhang et al., 2019).

While the literature has reported the isolated effects of these salt stress mitigators, further research is needed to assess the efficacy of their interactions (Yin et al., 2016; Gupta et al., 2021b; Oliveira et al., 2022). We hypothesize that combining organic matter and/or *T. harzianum* with silicon may enhance its mitigating effect, improving forage sorghum performance in saline environments in semiarid regions.

In the Brazilian semiarid, forage supply depends on climatic conditions, which are often unfavorable. Forage sorghum (*Sorghum sudanense* [Piper] Stapf) is a viable alternative, as it is tolerant of the region's edaphoclimatic conditions (Jardim et al., 2020). Sorghum is moderately tolerant to salt stress but susceptible during seedling and reproductive stages (Maswada, Djanaguiraman, & Prasad, 2018; Soni et al., 2021). Given this context, investigating ways to mitigate saline stress on forage sorghum in semiarid regions is essential to meet the region's forage demands.

This study aims to evaluate the effectiveness of silicon, applied alone or combined with *T. harzianum* and organic matter, in mitigating salt stress and enhancing forage sorghum growth.

Material and methods

Experimental area

The experiment took place in the field at the Irrigated Agriculture Station of Parnamirim, part of the Federal Rural University of Pernambuco (UFRPE), situated in Parnamirim, Pernambuco State (PE), in the semiarid region of Brazil (latitude 8°5'08" S, longitude 39°34'27" W, and an altitude of 390 m) (Figure 1). Before the experiment, the area was occupied by banana crops irrigated with water from the Brígida River. The soil in the area is classified as a saline Fluvisol (Table 1) (Embrapa, 2013).

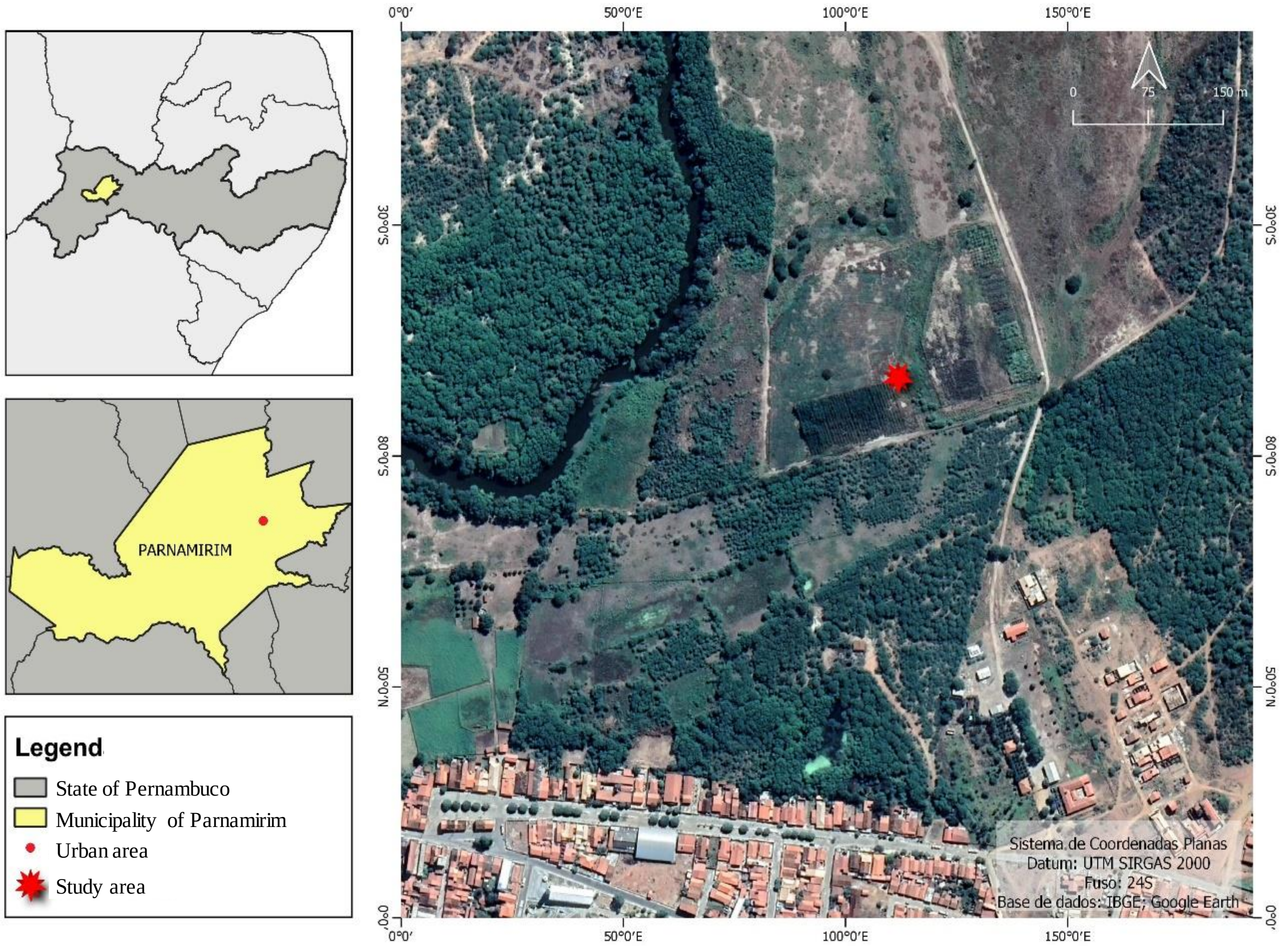

Figure 1. Location of the experimental field in Parnamirim (PE), a semiarid region of Brazil.

Table 1. Chemical and textural characterization of soil in the experimental area.

Soil property	Soil layer (cm)			
	0-10	10-20	20-40	40-60
EC (dS m^{-1})	3.56	6.21	7.22	5.77
pH	6.05	6.09	6.16	6.32
OM (g kg^{-1})	25.08	19.35	18.00	11.75
P (mg dm^{-3})	28.40	19.73	14.83	9.75
K^+ ($cmol_c$ dm^{-3})	0.51	0.34	0.24	0.14
Na^+ ($cmol_c$ dm^{-3})	0.30	0.37	0.52	0.82
Ca^{2+} ($cmol_c$ dm^{-3})	9.06	8.54	8.79	9.98
Mg^{2+} ($cmol_c$ dm^{-3})	5.29	5.40	5.21	5.53
H+Al ($cmol_c$ dm^{-3})	0.96	0.89	0.76	0.60
SB ($cmol_c$ dm^{-3})	15.14	14.65	14.93	16.48
CEC ($cmol_c$ dm^{-3})	16.10	15.54	15.69	17.08
ESP (%)	1.86	2.38	3.31	4.80
Sand (%)	14.20	10.30	11.30	10.92
Silt (%)	72.12	75.62	71.62	75.48
Clay (%)	13.68	14.08	17.08	13.60

EC – electrical conductivity; pH – hydrogen potential; OM – organic matter; SB – sum of base; CEC – cation exchange capacity; ESP – exchangeable sodium percentage.

The region's climate is classified as semiarid type *BSwh'*, which is hot and dry, with summer rainfall and dry winters, according to the Köppen classification. The average rainfall in the region is 431.8 mm, concentrated primarily between December and April (Rodrigues, Rodrigues, Silva, & Galvão, 2019). The municipality's average temperature is 26°C.

Treatments and experimental design

The experiment employed a randomized block design with five treatments and four replications. Treatments tested the effectiveness of *Trichoderma harzianum* (T) and organic matter (OM) in enhancing silicon's (Si) performance in mitigating salt stress in forage sorghum (*Sorghum* sudanense), cv. Sudan 4202. The treatments included: sorghum without any salt attenuator (control); sorghum + Si; sorghum + Si + OM; sorghum + Si + T; and sorghum + Si + T + OM. The experiment covered a total area of 20.0 x 16.5 m, with individual plots measuring 4 x 4 m, and useful plots (where plants were collected and evaluated) measuring 2 x 2 m. The adopted spacing was 0.50 m between rows, with ten plants per linear meter.

Three sorghum cycles (the first cut, followed by two regrowths) took place between June 2021 and April 2022, spanning ten months. During this time, sorghum growth responses to the application of salt attenuators were evaluated in both the dry and rainy seasons (Figure 2). No rain occurred between sowing and the first cut (1^{st} cycle), while the second and third cycles experienced accumulated precipitation of 176.5 mm and 252 mm, respectively.

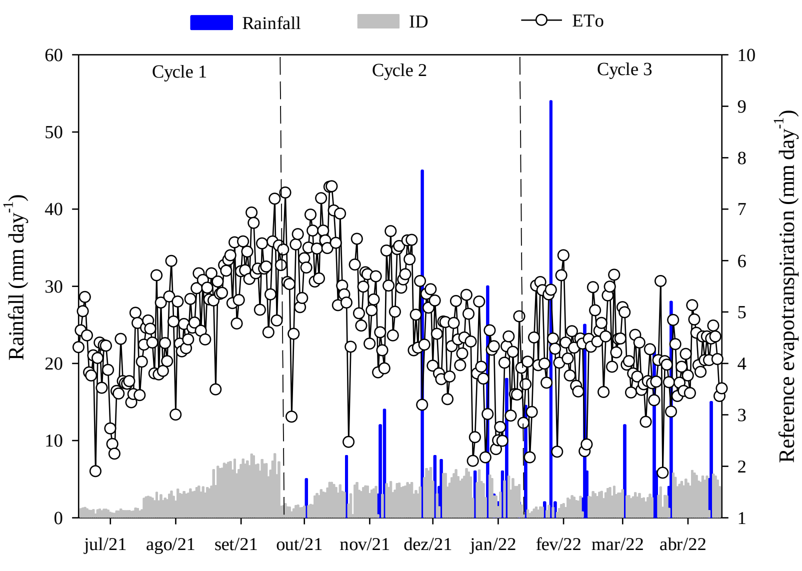

Figure 2. Meteorological conditions in the municipality of Parnamirim (PE) during the experimental period. ID - Irrigation depth (mm day^{-1}); ETo - Reference evapotranspiration (mm day^{-1}).

Irrigation was conducted using a drip irrigation system with an efficiency of 96%. Each dripper had a flow rate of 1.06 L h^{-1}, with emitters spaced at 40 cm and an application interval of 48 hours, based on the total replacement of crop evapotranspiration (ETc) (Allen, Pereira, Raes, & Smith, 1998). Reference evapotranspiration (ETo) was determined using the Penman-Monteith model adapted by FAO-56 (Allen et al., 1998). Meteorological data were collected from an automatic INMET station (National Institute of Meteorology [INMET], 2023), located 50 km from the experimental area. Rainfall data were collected using a manual rain gauge at the experiment site.

The water used for irrigation originated from an artesian well (Table 2) and was classified as C_4S_1 - posing a very high risk of promoting soil salinization and a low risk of sodification, according to Richards (1954).

Table 2. Chemical properties of irrigation water during the experimental period.

pH	EC	Ca^{2+}	Mg^{2+}	K^+	Na^+	Cl^-	HCO^{3-}	SO_4^{2-}	B	Cu	Fe	Mn	Zn	SAR
-	cm^{-1}	---------- mmol$_c$ L^{-1} ----------							---------- mg L^{-1} ----------					(mmol$_c$ L^{-1})$^{0.5}$
7.23	3.12	9.29	9.23	0.12	11.73	26.38	5.70	0.76	0.11	0.02	0.02	0.03	0.20	3.85

pH – hydrogen potential; EC – electrical conductivity; SAR – sodium adsorption ratio.

T. harzianum was obtained from the commercial product Trichodermil SC I306, with a solution concentration of 12.5 mL L^{-1}, as recommended by the manufacturer. Using a manual backpack pump, 39.06 mL of the mixture was applied per linear meter along the planting line. Two soil applications were carried out - the first at sowing and the second 30 days after the initial application.

Potassium silicate served as the silicon source in this study. It is a fertilizer containing at least 10% K^+ in the form of K_2O and 10% silicon. Two soil applications were performed – the first one week after sowing, the second 15 days later – and two foliar applications – the first 15 days after the soil applications and the second 15 days after the first foliar application. The spray concentrations were 5 mL L^{-1} and 10 mL L^{-1} for soil and foliar applications, respectively, according to the manufacturer's recommendation. Both conditions received an application of 39.06 mL m^{-1} linear.

At the time of sowing, the soil was fertilized with goat manure (Table 3) at a proportion of 50 Mg ha $^{-1}$ for treatments that included organic matter application. According to Freitas et al. (2012), this dosage provides optimal performance for the sorghum crop. The manure was incorporated into the surface layer of the soil.

Table 3. Characterization of goat manure used as an organic matter source in the experiment.

D	C	N	P	K^+	Na^+	Ca^{2+}	Mg^{2+}	pH	EC
kg m^{-3}	---------- g kg^{-1} ----------							-	mS cm^{-1}
815.80	119.70	17.90	8.70	4.50	1.10	27.70	10.20	7.87	1.87

D – density; C – carbon; N – nitrogen; pH – hydrogen potential; EC – electrical conductivity.

Sorghum growth and biomass

At the end of each cycle, biometric measurements were taken to determine plant height, stem diameter, and the number of tillers. Five representative plants from each useful plot were evaluated to determine average values.

The plants used for biometric measurements were divided into sections (stem, leaves, and panicle) and weighed. The samples were then placed in a forced air circulation oven at 65°C until a constant mass was achieved and then weighed again using an analytical balance to obtain the dry mass of the plant parts. The shoot fresh and dry masses were determined by adding the fresh and dry masses of leaves, stems, and panicles, respectively. The relative biomass was expressed as the ratio between the shoot dry biomass produced by sorghum under different treatments and that obtained with the control (no attenuator applications). The accumulated shoot fresh and dry biomass and the accumulated relative biomass were calculated by summing the values found across the three cycles.

Statistical analysis

Data were subjected to analysis of variance, normality tests, and the Scott-Knott test at a 5% probability level, using the Rstudio statistical software (R Core Team, 2019). Graphs were generated using SigmaPlot (version 14.0) (Systat Software Inc., San Jose, California, USA).

Results

Sorghum growth

When applied in combination with silicon, *T. harzianum*, and organic matter significantly increased ($p < 0.05$) plant growth compared to the control (Figure 3A) in all cycles. On average, silicon alone increased plant height by 7.25% across all cycles, while the Si + OM, Si + T, and Si + T + OM combinations resulted in increases of 42.17, 35.49, and 27.51% compared to the control treatment, respectively. The application of silicon alone did not show a significant difference in plant height compared to the control.

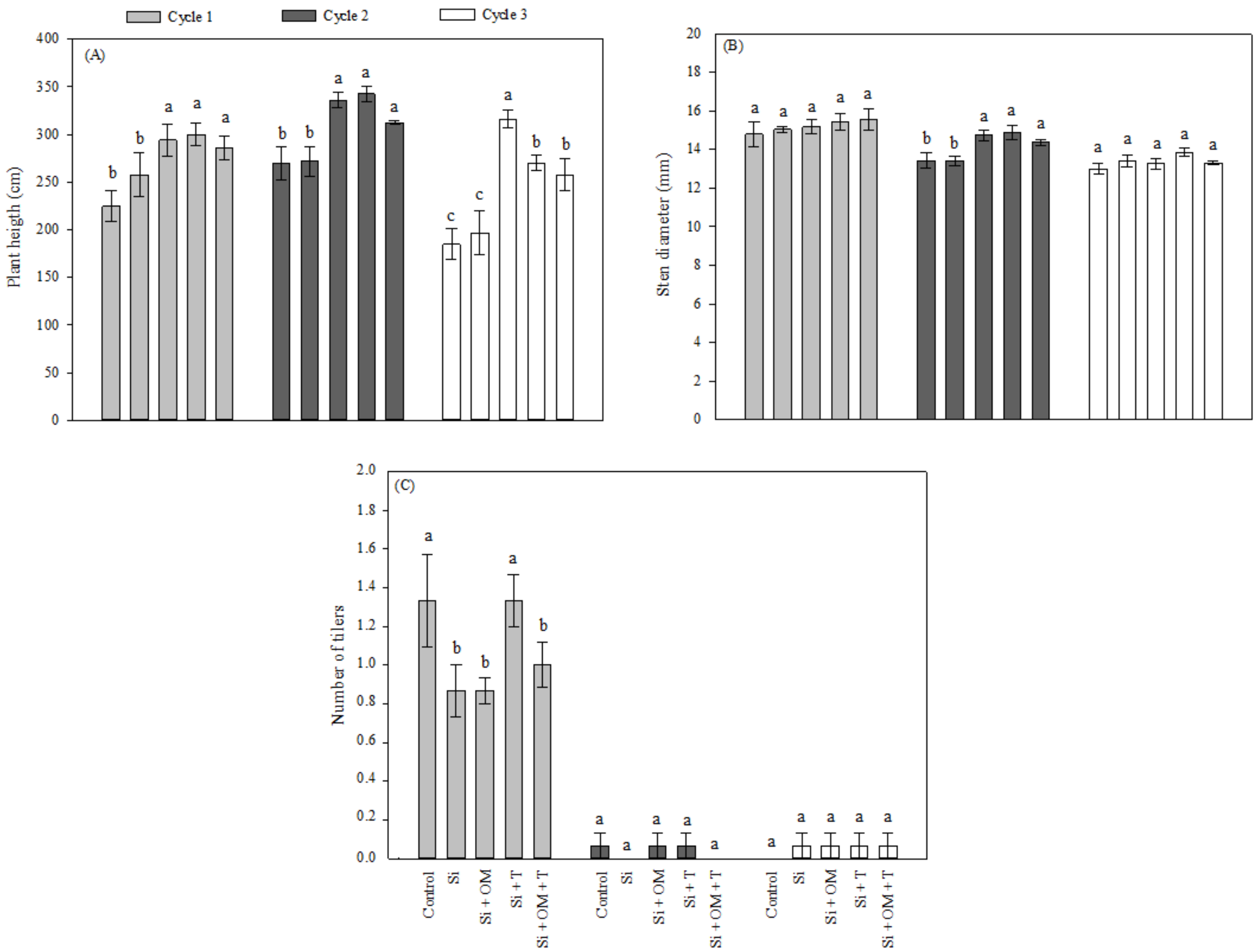

Figure 3. Plant height (A), stem diameter (B), and number of tillers (C) of sorghum plants over three successive cycles under saline stress as a function of the attenuator use.

Plant height averages for the treatments control, silicon alone, Si + OM, Si + T, and Si + T + OM were 224.6, 257.8, 294.3, 299.7, and 286.1 cm, respectively, for the first cycle; 269.75, 271.67, 335.98, 342.47, and 312.0 cm for the second cycle; and 184.89, 196.43, 316.03, 270.08, and 257.72 cm for the third cycle.

Stem diameter showed a significant difference only in the second cycle (Figure 3B). The combinations Si + OM, Si + T, and Si + T + OM increased stem diameter by 1.3, 1.4, and 0.96 mm, respectively, compared to the control. Although these values may not seem substantial, minor variations in stem diameter directly influence the accumulation of stem biomass (Maia Júnior et al., 2018). No significant difference was observed in this variable between the control treatment and silicon alone. As for the number of tillers, significant emission was observed only in the first cycle (Figure 3C), with the highest tillering in the control treatment and the Si + T combination.

Sorghum biomass as influenced by the salt stress attenuators

Significant differences ($p < 0.05$) were observed for fresh biomass, dry biomass, and relative biomass in all cycles (Figures 4, 5, and 6), as well as in the accumulated cycles (Figure 7). In the first cycle, Si + OM and Si + T + OM produced greater shoot fresh and dry masses (Figure 6A and B), due to their higher production of fresh and dry masses of stems and panicles compared to the other treatments (Figure 4A and C, Figure 5A and C). However, there was no significant difference in relative biomass among the attenuator combinations Si + OM, Si + T, and Si + T + OM, which all showed biomass accumulation values 30.63%, 29.99%, and 32.86% higher than the control treatment, respectively (Figure 6C).

In the second cycle, Si + T was the most effective in producing shoot fresh and dry masses (Figure 6A and B), and its relative biomass was 38.02% higher than the control treatment (Figure 6C). In the third cycle, the combinations Si + OM, Si + T, and Si + T + OM were the most effective in producing shoot fresh mass (Figure 6A). The Si + OM combination showed the best performance for shoot dry mass, followed by Si + T and Si + T + OM attenuators (Figure 6B). However, there was no significant difference in relative biomass among the Si + OM, Si + T, and Si + T + OM attenuators, which all showed relative biomass values 95.26, 55.91, and 73.28% higher than the control treatment, respectively.

Silicon alone did not differ statistically from the control for most variables. However, in all cycles, biomass accumulation increased on average by 9.86% compared to the control.

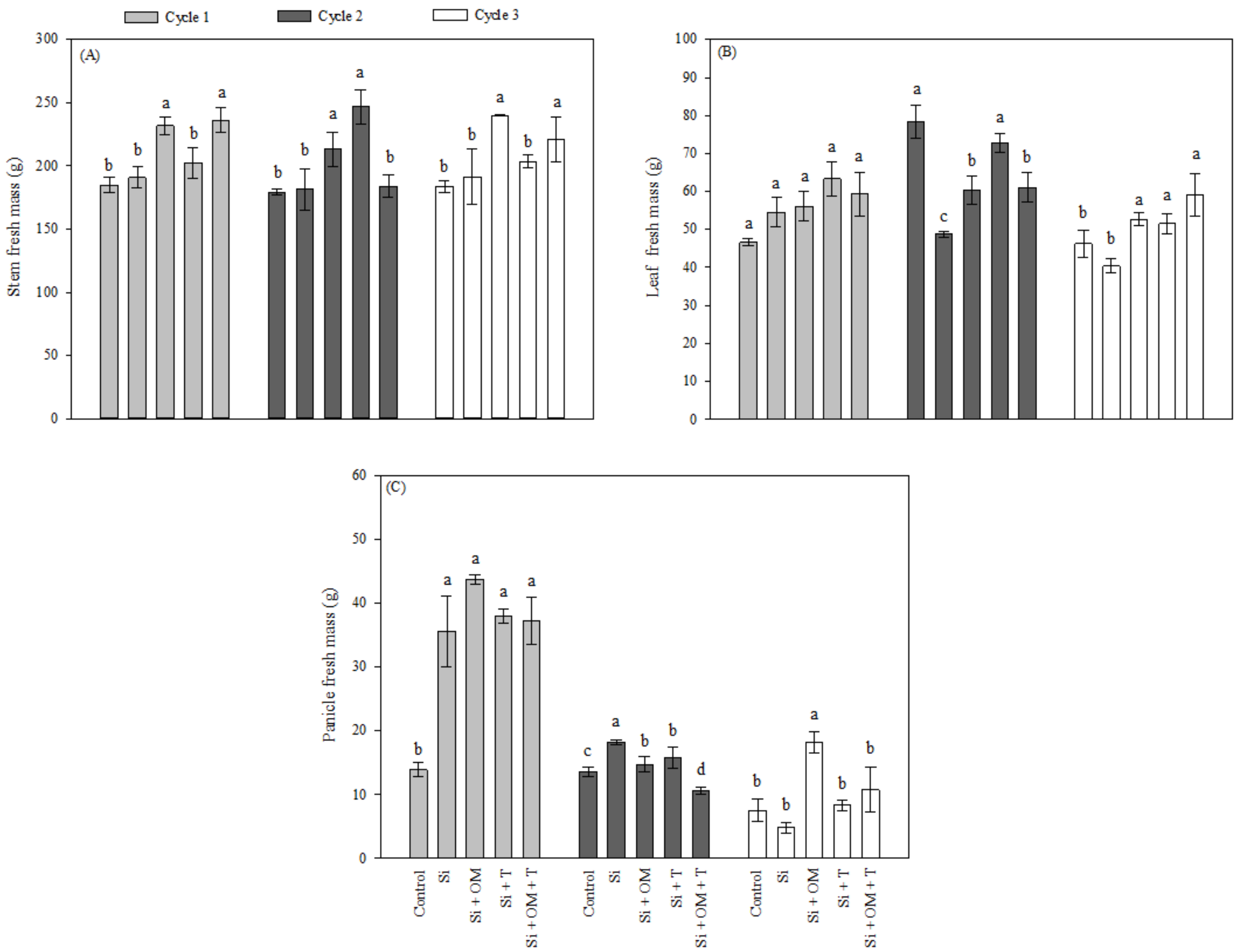

Figure 4. Fresh masses of stems (A), leaves (B), and panicles (C) of sorghum plants under saline stress as a function of the attenuator use.

Significant differences were observed in shoot dry and fresh masses (stems + leaves + panicles) among the attenuators in all cycles (Figure 6A and B). Although relative biomass did not differ significantly in the second cycle, compensation was found when the three cycles were combined (Figures 6C and 7C). The combinations Si + OM, Si + T, and Si + T + OM showed the best performance for these variables. In terms of accumulated shoot dry mass, the combined attenuators Si + OM, Si + T, and Si + T + OM promoted gains of 86.15, 88.67, and 71.26 g, respectively (Figure 7B), which corresponded to gains of 39.42, 40.44, and 31.77% in additional biomass accumulation compared to the control (Figure 7C). While silicon alone increased biomass accumulation by 9.86%, its application in combination with *T. harzianum* or organic matter led to more significant gains.

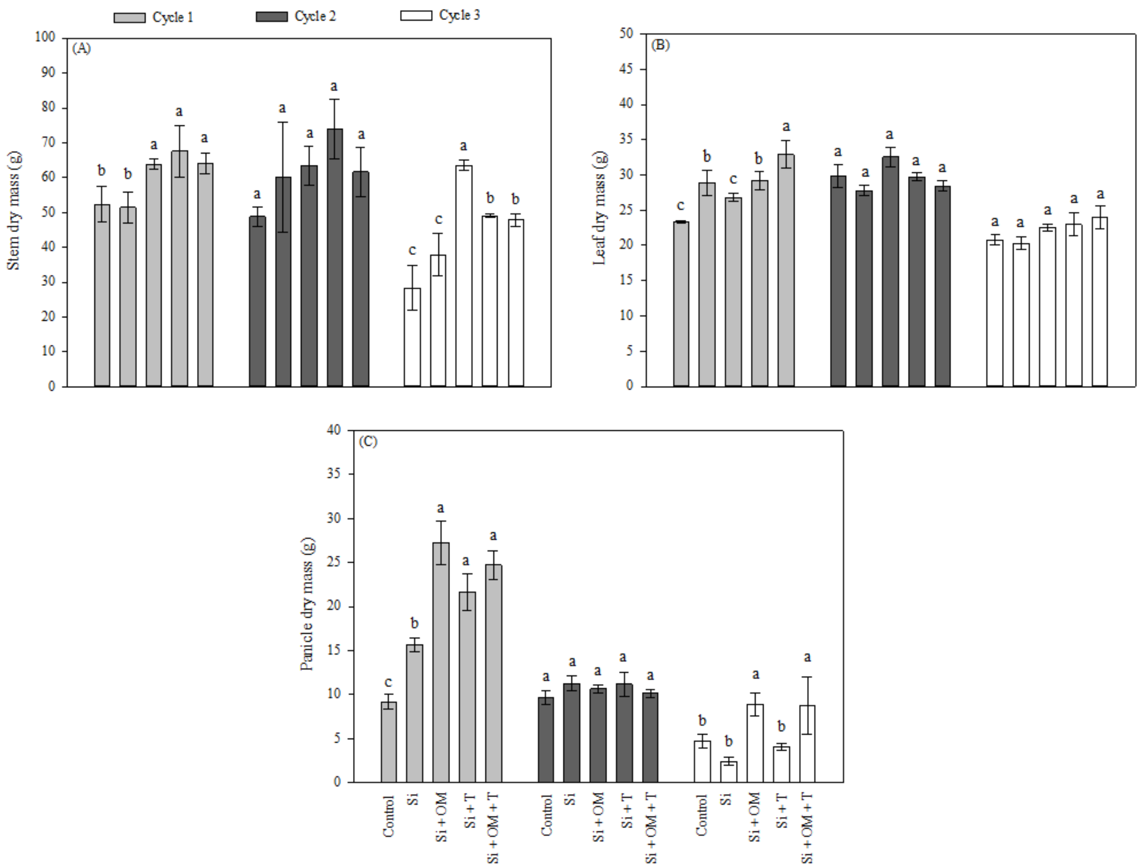

Figure 5. Dry masses of stems (A), leaves (B), and panicles (C) of sorghum plants under saline stress as a function of the attenuator use.

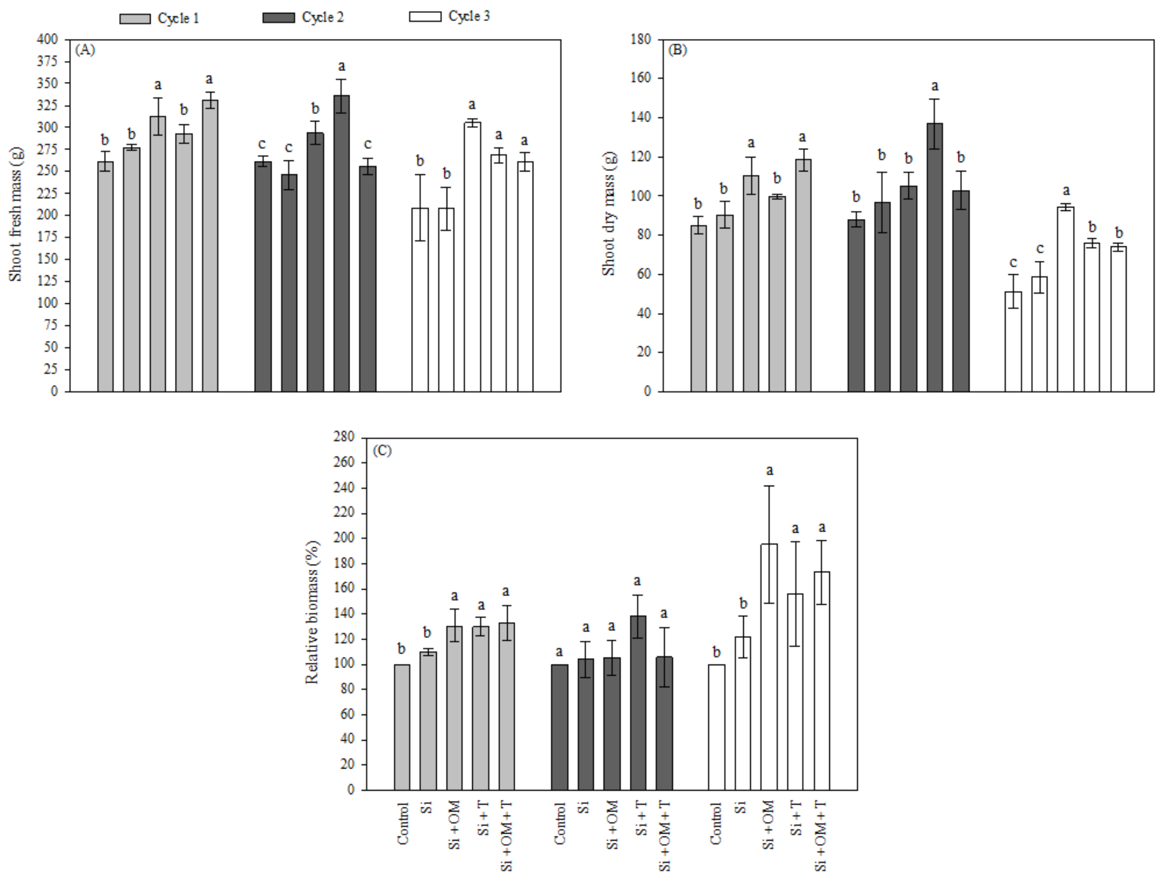

Figure 6. Shoot fresh (A), dry (B), and relative (C) biomasses of sorghum plants under saline stress as a function of the attenuator use.

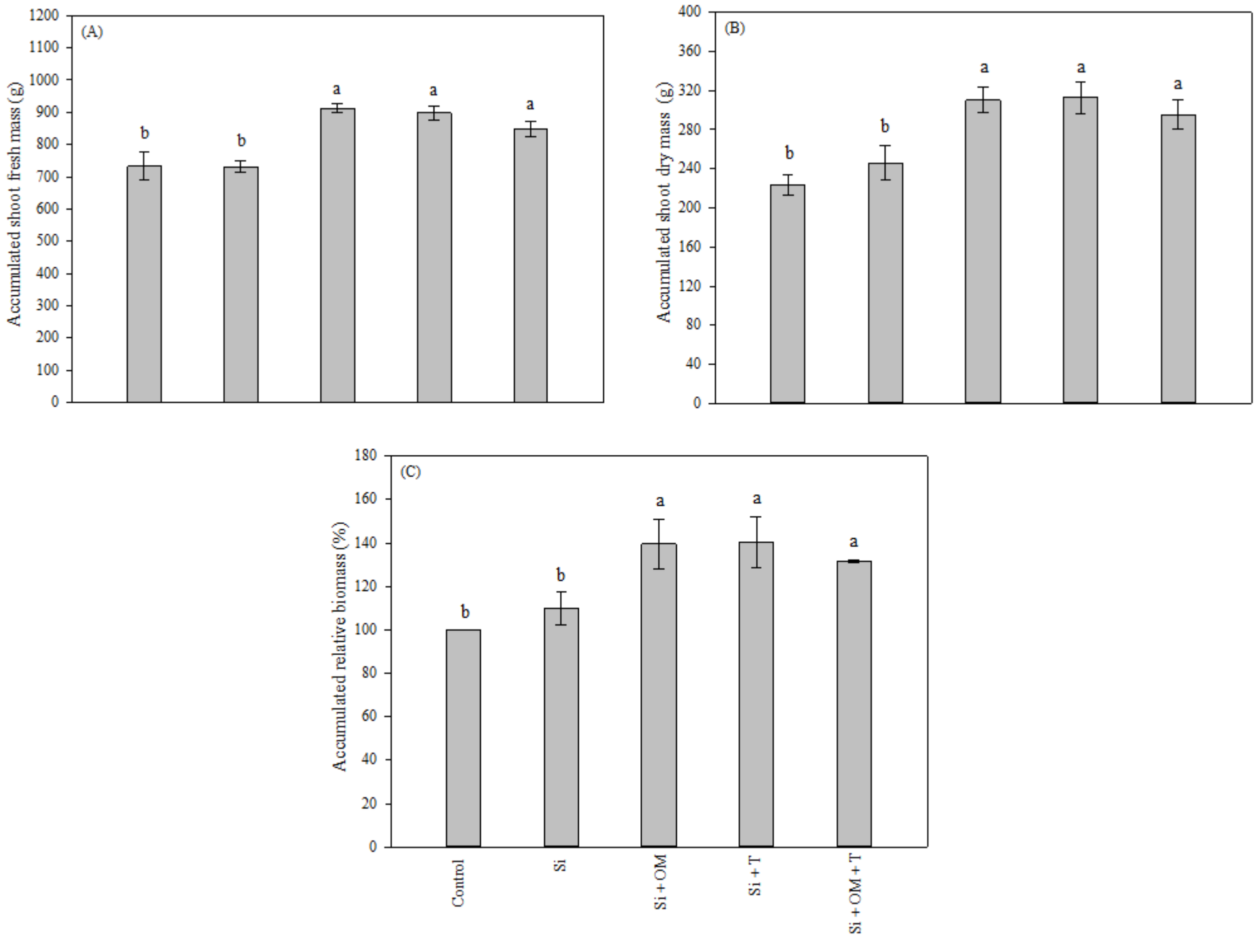

Figure 7. Accumulated shoot fresh (A), dry (B), and relative (C) biomasses of sorghum plants under saline stress as a function of the attenuator use.

Discussion

Our results suggest that combining Trichoderma, organic matter, and silicon attenuates salt stress and its impact on the growth and biomass accumulation of sorghum plants. Control plants displayed lower growth and biomass accumulation, likely due to the osmotic effect of high soil salt concentration, reducing water absorption and affecting cell expansion (Munns & Tester, 2008; Taiz & Zeiger, 2017). This result may also be associated with ionic stress derived from the excessive absorption of ions excessively found in the soil and irrigation water, such as Na^+ and Cl^- (Tables 1 and 2).

The ~10% growth and biomass gain with silicon are due to its positive effects on plant tissue. According to Pinheiro et al. (2022), sorghum can absorb and accumulate Si in abundance, improving its tolerance to salt stress. The greater the Si accumulation in tissue, the more benefits can be observed in reducing the damage caused by salt stress in plants, as reported by Migliorini et al. (2021). However, Abdolzadeh, Zarooshan, Sadeghipour, and Mehrabanjoubani (2022) demonstrated that more available forms of Si, such as nano silicon, are more effective in mitigating salt stress in sorghum, which could explain the low biomass production observed in the treatment where silicon was applied alone compared to the control.

One of the primary benefits of Si in mitigating salinity in sorghum plants is its ability to reduce Na^+ absorption and increase K^+ concentration in plant tissue, thus balancing the Na^+/K^+ ratio (Yin et al., 2016; Dhiman et al., 2021; Hurtado et al., 2021). In this study, potassium silicate was used as a source of Si, which also serves as a source of potassium, further improving the Na^+/K^+ ratio. Silicon enhances plant tolerance to abiotic stresses, such as soil salinity, mainly by regulating photosynthesis, activating antioxidant enzymes, and forming a silica barrier in roots to reduce the passage of salts to the shoot (Xie, Song, Xu, Shao, & Song, 2014; Coskun et al., 2016; Ahmad et al., 2021; Gupta et al., 2021a; Kaloterakis et al., 2021). Additionally, silicon increases the production of osmoregulators, adjusting the osmotic potential of roots to the soil and improving the water status of plants, which is a critical characteristic that grants tolerance to saline stress in plants (Abdelaal, Mazrou, & Hafez, 2020). However, in this study, the application of Si alone was not enough to mitigate the damage caused by salts in plants. Still, its effectiveness was enhanced when combined with *Trichoderma* or organic matter.

The interaction of *Trichoderma* and silicon increased growth and biomass production by 35.49% and 40.44%, respectively, compared to the control treatment. Studies in the literature show that *Trichoderma* can mitigate the damage caused by salinity and enhance growth and biomass production in other cultivated species. For example, using *Trichoderma* improved germination and plant growth in wheat due to metabolic changes (Rawat, Singh, Shukla, & Kumar, 2011). *Trichoderma* alleviated saline stress in peanuts by promoting greater nutrient absorption and improved enzymatic activity (Yusnawan et al., 2021). Although there are few reports on the efficiency of *Trichoderma* in relieving salt stress in sorghum, they suggest that this benefit may be attributed to improvements in enzymatic and photosynthetic activities (Anam, Reddy, & Ahn, 2019).

Trichoderma is known to produce phytohormones, including cytokinins and gibberellins, which promote plant growth under stressful conditions (Benìtez, Rincón, Limón, & Codón, 2004). It also increases root growth to enhance water and nutrient uptake and releases compounds that help plants respond more efficiently to salt stress (Harman, Howell, Viterbo, & Che, 2004; Kumar, Manigundan, & Amaresan, 2017; Rawat et al., 2011). Additionally, *Trichoderma* can solubilize phosphate and potassium, making these elements more available to plants (Ikram et al., 2019). In the second cycle, Si + T was more effective in increasing biomass production than the other attenuating factors (Figure 6A and B), likely due to the higher soil moisture resulting from rain (Figure 2), which affects the effectiveness of *Trichoderma* (Milanesi et al., 2013). Some studies have suggested that combining *Trichoderma* with organic compounds can improve its efficiency (Kong, Ling, Iqbal, Zhou, & Meng, 2023), but in this study, there was no significant difference between the Si + T and Si + T + OM treatments.

The use of Si + OM resulted in a 42.17% increase in growth and a 39.42% increase in biomass production in sorghum plants. These improvements were attributed to better plant nutrition and benefits from organic matter in mitigating soil salinity. Organic matter has several positive effects, such as water retention, soil structure improvement, salts leaching, osmotic potential reduction in the soil solution, and release of Ca^{2+} and Mg^{2+} ions, which replace Na^{+} in the exchange complex (Yousaf et al., 2021). Many studies in the literature report the effectiveness of organic matter in reducing the damage caused by salts in plants (Yarami & Sepaskhah, 2015; Naveed et al., 2020; 2021; Oliveira et al., 2022). Our findings corroborate those of Sousa et al. (2018) and Sousa, Lacerda, Aguiar, and Praxedes (2017), who found increased growth of sorghum plants under saline stress associated with improvements in soil chemistry and increased soil fertility, promoted by the addition of organic matter.

The number of tillers (Figure 3C) was the highest in the control treatment, which may be associated with the higher concentration of carbohydrates in the stems of the plants, a characteristic that induces plants to increase the degree of tillering (Magalhaes, Duraes, & Rodrigues, 2003). Similarly, *Trichoderma* induces plants to concentrate more carbohydrates in the stems, thereby increasing the number of tillers (Musa, Bahrun, & Kardina, 2021).

To effectively attenuate salt stress in sorghum plants, the application of silicon alone should be combined with other attenuators such as *Trichoderma* and organic matter, as its effectiveness was enhanced by their use. In this study, the combined application of *Trichoderma* and organic matter with silicon was found to be effective in attenuating salinity, without requiring a mixture of both. While the organic matter is an affordable and readily available attenuator suitable for small areas, its use on a large scale is not feasible due to the exorbitant amount required. In contrast, the management of *Trichoderma* in large areas is easier and more cost-effective (Sivila & Alvarez, 2013).

Conclusion

Our study found that applying a combination of silicon, *Trichoderma harzianum*, and organic matter promotes significant growth in forage sorghum by mitigating saline stress. The increase in sorghum growth and biomass production with these combined attenuators supports the recommendation for their application to enhance sorghum cultivation under salt-stress conditions, especially in semiarid environments. We suggest further research to explore other *Trichoderma* species and sources of silicon and organic matter to expand the range of cultivation possibilities and mitigate the harmful effects of salinity on forage sorghum and other crops.

Acknowledgements

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