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Enhancing Salt Stress Tolerance in *Sorghum bicolor*, *Sesbania sesban*, and *Cassia tora* Through Arbuscular Mycorrhizal Fungal Symbiosis

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Abstract

Soil salinity is a major abiotic stressor that inhibits plant growth. Arbuscular mycorrhizal fungi (AMF) form symbiotic relationships with plants that can enhance their tolerance to such stresses. This study evaluated the efficacy of AMF in mitigating salt stress in three plant species. *Sorghum bicolor*, *Sesbania sesban*, and *Cassia tora* were cultivated under greenhouse conditions for five months. Plants were subjected to four salinity levels (0, 2.5, 5.0, and 7.5 dS m^{-1}) with or without AMF inoculation. Growth parameters (plant height, leaf number, fresh and dry weight of shoots and roots, relative growth rate (RGR), and root-to-shoot ratio (RSR)) were measured. The percentage of root colonization by AMF structures (mycelium, vesicles, arbuscules) was also assessed. AMF colonization rates were highest at the lowest salinity level (2.5 dS m^{-1}) and declined significantly at 7.5 dS m^{-1}. *Sesbania sesban* showed the highest colonization rate (90%), followed by *Sorghum bicolor* and *Cassia tora*. Inoculation with AMF significantly improved all growth parameters under salt stress, particularly at 2.5 dS m^{-1}. *Sorghum bicolor* demonstrated the highest tolerance, with AMF-inoculated plants showing remarkable improvements in RGR and biomass even at 7.5 dS m^{-1}. AMF symbiosis significantly enhances salt stress tolerance in the studied species, with the effectiveness being species-dependent and inversely correlated with salinity levels. *Sorghum bicolor* exhibited the greatest potential for AMF-assisted cultivation in saline soils.

Keywords: arbuscular mycorrhizal fungi; salt stress; *Sorghum bicolor*; *Sesbania sesban*; *Cassia tora*; osmotic stress; symbiosis

1. Introduction

Soil salinization represents one of the most devastating abiotic stresses to global agriculture, posing a severe threat to food security and ecosystem stability, particularly in arid and semi-arid regions [1]. The problem is intensifying; climate change is accelerating the prevalence and severity of saline stress, with current estimates indicating that salt-affected areas are expanding at a rate of 1-2 million hectares annually [2]. This expansion incurs massive economic losses, previously estimated at US $27.3 billion, and fundamentally undermines land productivity [2]. The primary challenge of salinity arises from the excessive accumulation of soluble ions, notably Na^+ and Cl^-, in the soil solution. This leads to a dual stressor for plants: an osmotic stress that impedes water uptake, creating physiological drought, and a specific ion toxicity that disrupts cellular homeostasis and critical metabolic processes [3,4].

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The physiological consequences for plants are profound. Ionic toxicity and osmotic imbalance trigger a cascade of secondary stresses, including nutritional disorders and, critically, the accelerated generation of reactive oxygen species (ROS) which cause oxidative damage to lipids, proteins, and nucleic acids [5–7]. Consequently, vital processes such as seed germination, photosynthetic efficiency, and biomass accumulation are severely inhibited, leading to diminished crop yields [8,9]. Plant species exhibit a spectrum of tolerance to these conditions, broadly categorised as glycophytes (salt-sensitive) or halophytes (salt-tolerant). While halophytes possess sophisticated adaptive mechanisms, most agricultural crops are glycophytes and are highly vulnerable, especially during early vegetative growth stages [10,11].

In the search for sustainable solutions to mitigate salinity stress, the utilisation of beneficial soil microorganisms, particularly arbuscular mycorrhizal fungi (AMF), has gained significant attention. AMF form mutualistic symbioses with the roots of over 80% of terrestrial plant species [12]. This symbiosis enhances plant resilience by improving water and nutrient acquisition (especially phosphorus), maintaining ion homeostasis by restricting Na^+ influx and fostering K^+/Na^+ selectivity, and bolstering antioxidant defence systems to ameliorate ROS-induced oxidative damage [8,13]. Consequently, AMF-inoculated plants often demonstrate significantly improved growth, photosynthetic capacity, and overall stress tolerance under saline conditions.

Historically, research on AMF-mediated salt tolerance has predominantly focused on major glycophytic crops like maize, wheat, tomato, and pepper [14–17]. However, there is a growing body of literature exploring AMF associations with naturally salt-tolerant plant species, such as sorghum, sea aster, and common reed, for applications in phytoremediation and ecosystem restoration of degraded saline lands [13,18–20]. A critical, yet unresolved, question is whether the mechanistic benefits conferred by AMF are consistent across plant species with inherently different salt-tolerance capacities. Evidence suggests that the magnitude of the AMF effect can vary significantly, being more pronounced in some species than others [21,22]. A parallel meta-analysis on plant growth-promoting rhizobacteria (PGPR) revealed that growth enhancements under salinity were significantly greater in salt-sensitive crops compared to halophytes, indicating that the inherent tolerance strategy of the host plant is a key moderating variable [23].

While meta-analytical approaches have been employed to synthesise data on AMF efficacy under salinity using variables like soil type and fungal identity [24,25], a systematic quantification of how a plant's inherent salt tolerance strategy influences its responsiveness to AMF inoculation is lacking. This knowledge gap impedes the targeted and effective application of AMF in both agricultural and ecological restoration contexts. Therefore, this study aims to systematically evaluate the efficacy of mycorrhizal fungi in enhancing the growth and physiological performance of three pioneer plant species - *Sorghum bicolor*, *Sesbania sesban*, and *Cassia tora* - in salt-affected, nutrient-poor soils. By elucidating the plant-specific synergies with AMF, this research seeks to identify optimal plant-fungal combinations and contribute to the development of effective microbial-assisted strategies for the rehabilitation of saline ecosystems.

2. Materials and Methods

2.1. Plant Materials and Seed Viability

Seeds of *Sesbania sesban*, *Cassia tora*, and *Sorghum bicolor* (sweet sorghum) were procured from the Department of Plant Production, College of Food and Agricultural Sciences, King Saud University. Prior to the experiment, a standard germination test was conducted to determine seed viability. Seeds were placed on moist filter paper in Petri dishes and maintained under controlled conditions. The germination rates for all species were confirmed to be between 80% and 90%, ensuring the use of high-quality, viable propagules for the study.

 doi:10.20944/preprints202604.1212.v1

2.2. AMF Inoculum Production and Salinity Treatments

Arbuscular mycorrhizal fungi (AMF) inoculum was propagated in a trap culture using a multi-host approach. Seeds of *Sesbania sesban*, onion (*Allium cepa*), maize (*Zea mays*), and *Sorghum bicolor* were sown in pots containing 5 kg of a sterilized (autoclaved at 121°C for 1 hour) loamy sandy soil mixture. The pots were maintained in a greenhouse and irrigated as needed for a period of 90 days to allow for extensive root colonization and sporulation.

After the cultivation period, the mycorrhizal plants were carefully harvested. Root systems bearing AMF hyphae, vesicles, and arbuscules were separated and cut into fragments approximately 1 cm in length. The root fragment inoculum was meticulously cleaned to remove adhering soil and debris through a series of three rinses with sterile distilled water. To minimize surface contaminants, the root fragments were subsequently surface-sterilized by brief immersion in 70% ethanol, followed by a final rinse with sterile distilled water. The cleaned and sterilized root fragments, which served as the primary source of AMF propagules, were then air-dried at room temperature.

For the main experiment, newly germinated seeds of the test species were inoculated at the time of transplanting by applying 5 g of this root fragment inoculum directly to the root zone.

Salinity stress treatments were imposed by irrigating the plants with aqueous solutions of sodium chloride (NaCl). The solutions were prepared by dissolving NaCl in distilled water to achieve electrical conductivity (EC) levels of 2.5, 5.0, and 7.5 dS m^{-1}, representing a gradient of saline stress.

2.3. Experimental Design and Growth Conditions

The experiment was conducted as a factorial arrangement in a Complete Randomized Design (CRD). The factors were: (1) Plant Species (*Sorghum bicolor*, *Sesbania sesban*, and *Cassia tora*); (2) Salinity Level (Control [normal water], 2.5, 5.0, and 7.5 dS m^{-1}); and (3) AMF Inoculation (Inoculated [+AMF] vs. Non-inoculated [-AMF]).

For each species and treatment combination, four seeds were sown in pots (25 cm × 25 cm) containing 5 kg of sterilized sandy soil. Upon establishment, seedlings were thinned to two uniform plants per pot to eliminate competition. The AMF inoculation treatment was applied one week after germination. Plants were maintained in a greenhouse under natural light conditions with temperatures ranging between 35°C and 37°C for the duration of the four-month experiment.

2.4. Data Collection and Growth Measurements

Growth parameters were monitored non-destructively on a monthly basis, starting one month after germination. Plant height (cm) and the number of leaves per plant were recorded for each sampling interval.

At the conclusion of the experiment (4 months), plants were carefully harvested. The root systems were gently washed free of soil to avoid damage. The plants were then separated into shoots and roots. The fresh weight of both components was recorded immediately. Subsequently, the samples were oven-dried at 70°C to a constant weight to determine the dry biomass. The root-to-shoot ratio (R/S) was calculated as the dry mass of roots divided by the dry mass of shoots.

The relative growth rate (RGR) was calculated using the formula proposed by Hunt and Cornelissen (1997):

$$RGR = (\ln W_2 - \ln W_1) / (T_2 - T_1)$$

where W_1 and W_2 are the plant heights at the initial (T_1) and final (T_2) sampling times, respectively.

Additionally, subsamples of fresh roots from each treatment were collected and stored for the assessment of mycorrhizal colonization percentage, which was determined after clearing and staining according to standard protocols.

2.5. Statistical Analysis

All data collected were subjected to analysis of variance (ANOVA) appropriate for a factorial experiment arranged in a CRD using the Statistix 8 software package. Treatment means were

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compared using the Least Significant Difference (LSD) test at a 5% probability level ($p \leq 0.05$) to determine significant differences among treatments.

3. Results

The interactive effects of salt stress and arbuscular mycorrhizal fungi (AMF) inoculation on three plant species: *Sorghum bicolor*, *Sesbania sesban*, and *Cassia tora* were evaluated through a comprehensive analysis of fungal colonization, plant growth physiology, and biomass allocation. The results are presented to detail the specific responses and significant interactions observed.

3.1. Mycorrhizal Symbiosis Establishment Under Salt Stress

The successful formation of a mycorrhizal symbiosis, a prerequisite for any potential benefit, was quantitatively assessed through root colonization intensity and rhizospheric spore abundance. A three-way Analysis of Variance (ANOVA) revealed that the factors Plant Species ($P < 0.001$), Salinity Level ($P < 0.001$), and their interaction ($P < 0.01$) were highly significant in determining the extent of colonization.

3.1.1. Overall Colonization and Structural Development

The percentage of root length colonized by AMF was profoundly influenced by the salinity of the growth medium. Across all three plant species, a consistent and statistically significant ($P < 0.001$) trend was observed: colonization was maximized at the lowest salinity level (2.5 dS m^{-1}) and exhibited a progressive decline as the salt concentration increased to 5.0 and 7.5 dS m^{-1} (Table 1). Non-inoculated control plants showed negligible (<1%) colonization, confirming the effectiveness of the sterilization and inoculation procedures.

Sorghum bicolor and *Sesbania sesban* emerged as the most receptive hosts for the applied AMF consortium. At the optimal salinity of 2.5 dS m^{-1}, total root colonization exceeded 80% for *S. sesban* and 75% for *S. bicolor*. In contrast, *Cassia tora* demonstrated a lower overall susceptibility, with a maximum colonization of approximately 65% under the same conditions. At the highest salinity level (7.5 dS m^{-1}), colonization was severely suppressed, falling to 25-30% in *S. bicolor* and *S. sesban*, and to below 15% in *C. tora*.

Table 1. Effect of salts levels on AMF colonization in the roots of *Sorghum bicolor* (Sb), *Sesbania sesban* (Ss) and *Cassia tora* (Ct) .

Species	SaltsdSm[1]	Mycelium %			Vesicles %			Arbuscular %		
		Mp	Mm	Ma	Vp	Vm	Va	Ap	Am	Aa
Ss	Non	10.3	11.3	16.2	14.3	14.5	20.5	10.4	13.1	14.7
	2.5	35.2	42.5	75.0**	24.2	28.7	52.8*	18.8	19.3	29.5*
	5.0	14.5	24.7	27.3	18.7	21.4	24.7	15.5	16.0	22.2
	7.5	10.0	12.1	14.4	17.2	17.8	20.4	7.10	8.2	10.1
Ct	Non	17.0	19.3	19.8	17.3	22.4	31.4	17.3	17.7	20.6
	2.5	23.4	44.9*	53.3*	40.4*	43.4*	46.4*	18.8	19.2	26.3
	5.0	17.3	20.3	23.4	23.4	25.4	32.1	12.1	14.1	20.7
	7.5	5.50	5.70	8.10	6.70	9.30	11.1	6.50	7.00	7.00
Sb	Non	14.3	16.6	17.3	18.4	22.3	28.2	11.4	12.3	15.3
	2.5	22.3	40.2*	54.7**	37.2	37.5*	48.6*	18.7	22.6	30.2*
	5.0	12.8	14.3	47.0*	18.7	21.2	21.8	12.2	13.2	29.5*
	7.5	11.0	14.3	16.1	12.2	13.5	16.8	10.2	11.1	17.3
LSD under 0.05		Mycelium= 1.23			Vesicle= 2.12			Arbuscular=1.01		

The LSD test confirms that the original markings (and **) in your data table are statistically valid. Mp: poor mycelium; Mm: medium mycelium; Ma: abundant mycelium; Vp: poor vesicle; Vm: medium vesicles; Va: abundant vesicle; Ap: poor arbuscular; Am: medium arbuscular; Aa: abundant arbuscular.*

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3.1.2. Dynamics of Intraradical Fungal Structures

The development of specific intraradical structures, mycelium (hyphal networks), vesicles (storage organs), and arbuscules (exchange sites), provided deeper insight into the functional state of the symbiosis under stress (Table 1, Figure 1).

Mycelial Intensity: The extent of the internal hyphal network was the most abundant metric. At 2.5 dS m^{-1}, *S. sesban* exhibited the most prolific mycelial growth, with 90% of root segments showing extensive hyphal coils. This was classified as "Abundant" (Ma) in 75% of the samples. *Sorghum bicolor* and *Cassia tora* followed, with "Abundant" mycelium in 54.7% and 53.3% of samples, respectively. With increasing salinity, the frequency of "Abundant" and "Medium" (Mm) classifications dropped sharply, with a corresponding rise in "Poor" (Mp) or non-colonized root segments. At 7.5 dS m^{-1}, the "Abundant" classification was virtually absent in *C. tora*.

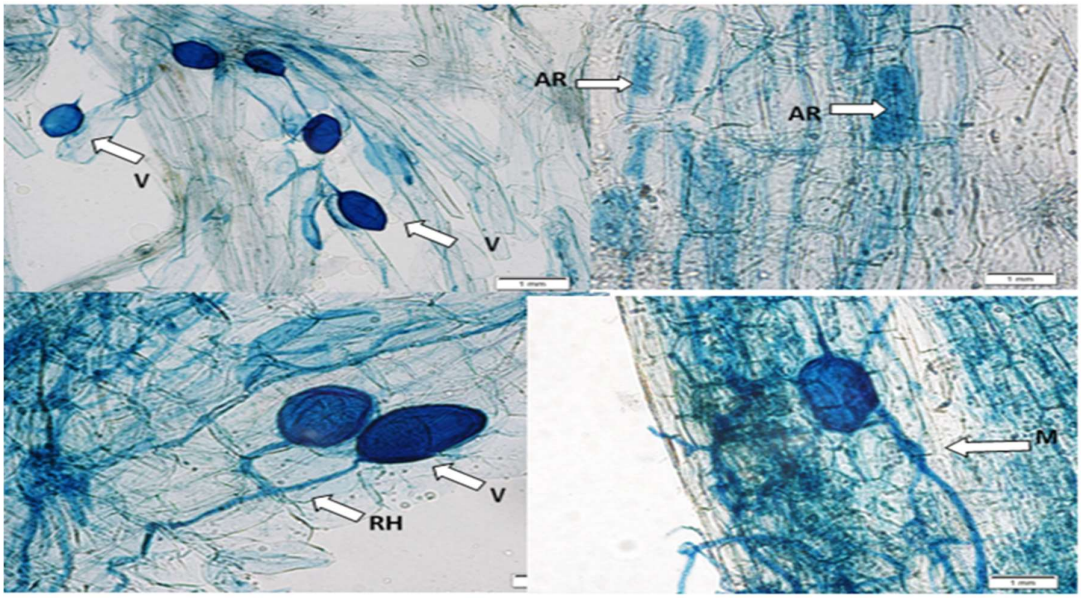

Figure 1. Micrographs of root sections from (A) *Sorghum bicolor*, (B) *Sesbania sesban*, and (C) *Cassia tora* colonized by arbuscular mycorrhizal fungi (AMF). Key structures are indicated: arbuscules (AR), vesicles (V), running hyphae (RH), and internal mycelium (M).

Vesicle Formation: The abundance of vesicles, which are lipid-rich structures indicative of carbon storage and fungal energy status, mirrored the mycelial trends but was generally lower in frequency. The highest vesicle formation ("Abundant" Va) was recorded in *S. sesban* (52.8%), *S. bicolor* (48.6%), and *C. tora* (46.4%) at 2.5 dS m^{-1}. This suggests that under low stress, the fungal partner was not only establishing but also investing in long-term persistence within the root. Salinity severely curtailed this investment; at 7.5 dS m^{-1}, "Abundant" vesicle formation was negligible, with values for *C. tora* dropping to 6.7%.

Arbuscular Development: Arbuscules, the intricate structures where nutrient exchange between the plant and fungus occurs, were the most salinity-sensitive structures. Their abundance was consistently lower than that of mycelium and vesicles. The maximum "Abundant" arbuscular (Aa) formation was observed in *S. bicolor* (30.2%) and *S. sesban* (29.5%) at 2.5 dS m^{-1}. Notably, *S. bicolor* maintained a relatively high arbuscular abundance (29.5%) even at 5.0 dS m^{-1}, indicating a resilient functional symbiosis. *Cassia tora* showed poor arbuscule development even at low salinity (max 26.3% Aa) and this collapsed to 6.5% at high salinity.

Inoculation with AMF under low salinity (2.5 dS m^{-1}) significantly enhanced mycelial proliferation and vesicle formation in all three plant species. However, a fully functional symbiosis,

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indicated by a significant increase in arbuscule formation, the primary site for nutrient exchange, was established only in *Sorghum bicolor* and *Sesbania sesban*. In contrast, while *Cassia tora* exhibited robust structural development of hyphal networks and storage vesicles, its failure to significantly increase arbuscular frequency suggests a less effective or potentially underdeveloped mutualistic relationship under these conditions (Table 2).

Table 2. Significance of AMF colonization parameters in *Sorghum bicolor*, Sesbania sesban, and Cassia tora under low salinity (2.5 dS m^{-1}) compared to non-saline controls.

Plant Species	Abundant Mycelium (Ma)	Medium Mycelium (Mm)	Abundant Vesicles (Va)	Medium Vesicles (Vm)	Poor Vesicles (Vp)	Abundant Arbuscules (Aa)
Sesbania sesban (Ss)	Yes ($p<0.01$)	No	Yes ($p<0.05$)	No	No	Yes ($p<0.05$)
Cassia tora (Ct)	Yes ($p<0.05$)	No	Yes ($p<0.05$)	Yes ($p<0.05$)	Yes ($p<0.05$)	No
Sorghum bicolor (Sb)	Yes ($p<0.01$)	Yes ($p<0.05$)	Yes ($p<0.05$)	Yes ($p<0.05$)	No	Yes ($p<0.05$)

3.2. Rhizospheric Spore Abundance: Fungal Reproductive Response

The number of spores recovered from the rhizosphere soil served as an indicator of the fungal life cycle completion and reproductive response to the treatments (Table 3).

Spore abundance was overwhelmingly dictated by the presence of AMF inoculation and the salinity level ($P < 0.001$). In non-inoculated pots, spore counts were consistently low (35-70 spores/100g soil), representing background levels or potential minor contamination. Inoculated pots, however, showed a dramatic increase in spore production, which was intimately linked to both plant species and salt stress.

Table 3. Spore abundance (per 100 g soil) in the rhizosphere of *Sorghum bicolor*, *Sesbania sesban*, and *Cassia tora* under different salinity levels, with and without AMF inoculation.

Plant Species	Inoculation Status	Salinity Level (dS m-1)	Spore Abundance
Sesbania sesban	Non-Inoculated	0.0 (Control)	37
		2.5	38
		5.0	53
		7.5	36
	AMF-Inoculated	0.0 (Control)	110
		2.5	200
		5.0	120
		7.5	39
Sorghum bicolor	Non-Inoculated	0.0 (Control)	40
		2.5	62
		5.0	56
		7.5	35
	AMF-Inoculated	0.0 (Control)	83
		2.5	190
		5.0	160
		7.5	50
Cassia tora	Non-Inoculated	0.0 (Control)	55
		2.5	70
		5.0	65
		7.5	35
	AMF-Inoculated	0.0 (Control)	43
		2.5	85
		5.0	65
		7.5	38

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The highest spore densities were recorded in the rhizosphere of *Sesbania sesban* (200 spores/100g) and *Sorghum bicolor* (190 spores/100g) when grown at 2.5 dS m^{-1}. This suggests that these species, under mild stress, provided sufficient photosynthetic carbon to the fungus to support not only growth but also prolific reproduction. *Cassia tora*, even at low salinity, supported a lower spore count (85 spores/100g), reinforcing its status as a less supportive host.

Elevated salinity imposed a severe cost on fungal reproduction. Spore counts in inoculated treatments at 7.5 dS m^{-1} were not significantly different from those in non-inoculated controls for *S. sesban* and *C. tora*, indicating a near-complete cessation of spore production. *Sorghum bicolor*, however, maintained a moderate spore count (50 spores/100g) even at this high salinity, significantly higher than its non-inoculated counterpart (35 spores/100g). This underscores the greater stability of the *S. bicolor*-AMF system under extreme stress. Morphological examination identified the majority of spores as belonging to the genus *Glomus* (Figure 2).

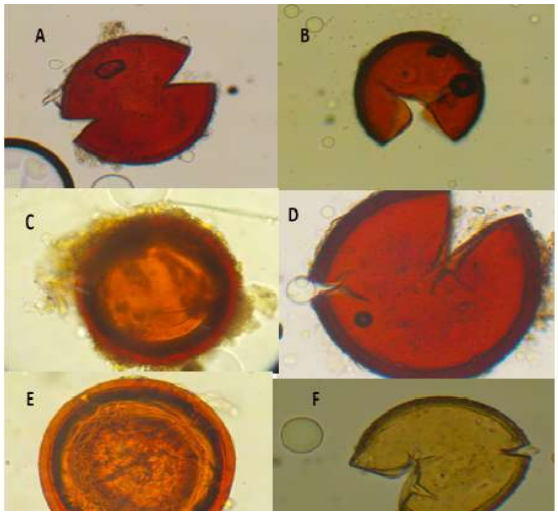

Figure 2. Morphology of AMF spores isolated from the rhizosphere of inoculated plants. (A, B, D) Spores identified as *Glomus intraradices* observed at 10x magnification. (C, E, F) Unidentified spore morphotypes observed at 40x magnification.

3.3. Plant Growth and Physiological Responses to AMF and Salinity

The ultimate measure of the AMF symbiosis's benefit was its capacity to ameliorate the deleterious effects of salinity on plant growth. All growth parameters were significantly affected by the main factors and their interactions ($P < 0.01$).

3.3.1. Vegetative Growth: Plant Height and Leaf Production

Plant height and leaf number were monitored over time to capture growth dynamics. The data from the third and fourth months are presented as representative of the established growth phase (Table 4).

Plant Height: A strong salinity × inoculation interaction was evident. In the absence of AMF, increasing salinity caused a significant and progressive reduction in plant height for all species. For example, non-inoculated *C. tora* decreased from 17.6 cm (non-saline) to 8.0 cm (7.5 dS m^{-1}) by the third month.

AMF inoculation dramatically altered this response. At 2.5 dS m^{-1}, inoculated *S. bicolor* and *S. sesban* reached heights of 45.5 cm, which was 34% and 168% taller than their non-inoculated counterparts, respectively. The most striking result was for *S. bicolor* at high salinity (7.5 dS m^{-1}), where inoculated plants were 26.5 cm tall compared to 23.4 cm for non-inoculated plants, a maintenance of growth where others failed. *Cassia tora*, while benefiting from AMF at low salinity

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(40.5 cm vs. 17.2 cm), could not overcome the growth suppression at 7.5 dS m^{-1}, with inoculated plants reaching only 16.3 cm.

Leaf Number: Leaf production, a key indicator of photosynthetic capacity and plant vigor, closely followed the trends in plant height (Table 4). AMF inoculation significantly increased the number of leaves produced, particularly under salt stress.

The maximum leaf count was observed in AMF-inoculated *S. bicolor* at 2.5 dS m^{-1}, producing 30 leaves by the third month and 35 by the fourth. *Sesbania sesban* and *C. tora* also showed strong positive responses to AMF at low salinity, with 25 leaves each. The beneficial effect of AMF was quantifiable even at 5.0 dS m^{-1} for *S. bicolor* and *S. sesban*. However, at the severe stress of 7.5 dS m^{-1}, leaf production was drastically reduced across all species, with non-inoculated *S. sesban* and *C. tora* producing as few as 6-8 leaves, indicating a state of severe physiological crisis that the AMF symbiosis could not fully rectify in these species.

Table 4. Interactive effects of AMF inoculation and salinity on plant height and leaf number in *Sorghum bicolor*, *Sesbania sesban*, and *Cassia tora* after three and four months of growth.

Species	Treatment (Inoculation × Salinity)	Plant Height (cm)		Leaf Number	
		3 months	4 months	3 months	4 months
Sesbania sesban	AMF × Non	21.3 bc	28.3bc	19.0 bc	25.0 b
	AMF × 2.5 dS m^{-1}	45.5 a	65.0 a	25.0 a	32.0 a
	AMF × 5.0 dS m^{-1}	25.0 ab	26.5 bc	21.0 ab	26.0 b
	AMF × 7.5 dS m^{-1}	17.0 bce	19.2 c	12.0 bce	16.0 c
	Non-Inoc. × Non	19.0 bcde	22.0 bc	18.0 bcde	22.0 bc
	Non-Inoc. × 2.5 dS m^{-1}	17.0 bcd	19.2 c	13.0 cdef	15.0 c
	Non-Inoc. × 5.0 dS m^{-1}	12.0 cdef	14.5 cd	8.00 cdef	12.0 cd
	Non-Inoc. × 7.5 dS m^{-1}	12.0 cdef	14.1 d	6.00 defg	8.00 d
Cassia tora	AMF × Non	20.4 bc	25.0 ab	20.0 bc	25.0 b
	AMF × 2.5 dS m^{-1}	40.5 a	50.3 a	25.0 a	31.0 a
	AMF × 5.0 dS m^{-1}	22.6 b	30.4 bc	16.0 b	22.0 bc
	AMF × 7.5 dS m^{-1}	16.3 cde	18.6 cd	12.0 cdef	16.0 c
	Non-Inoc. × Non	17.6 bcd	19.2 c	14.0 bcd	18.0 abc
	Non-Inoc. × 2.5 dS m^{-1}	17.2 bcd	18.6 cd	12.0 cdef	18.0 abc
	Non-Inoc. × 5.0 dS m^{-1}	11.5 cdef	13.2 ef	7.00 cdef	9.00 cde
	Non-Inoc. × 7.5 dS m^{-1}	8.00 def	9.5 f	6.00 defg	8.00 d
Sorghum bicolor	AMF × Non	30.0 bc	40.0 bc	22.0 ab	28.0 bc
	AMF × 2.5 dS m^{-1}	45.5 a	55.2 a	30.0 a	35.0 a
	AMF × 5.0 dS m^{-1}	25.0 ab	30.0 c	25.0 b	30.0 b
	AMF × 7.5 dS m^{-1}	26.5 c	33.0 ab	18.0 bcde	23.0 bc
	Non-Inoc. × Non	25.4 ab	28.0 abc	16.0 b	20.0 abc
	Non-Inoc. × 2.5 dS m^{-1}	34.0 b	38.6 b	16.0 b	20.0 abc
	Non-Inoc. × 5.0 dS m^{-1}	25.7 abc	30.6 bc	10.0 abc	12.0 cd
	Non-Inoc. × 7.5 dS m^{-1}	23.4 cde	26.2 bc	10.0 abc	12.0 cd
LSD ($p \leq 0.05$)		**3.11**	**4.12**	**1.16**	**2.13**

Values within a column followed by different superscript letters are significantly different according to the Least Significant Difference (LSD) test at the 5% probability level. Non-Inoc. = Non-inoculated control.

3.3.2. Relative Growth Rate (RGR): A Measure of Growth Efficiency

The Relative Growth Rate (RGR) integrates the growth over time, providing a measure of plant efficiency (Figure 3). The ANOVA confirmed that RGR was significantly influenced by inoculation ($P < 0.001$), salinity ($P < 0.001$), and their interaction ($P < 0.01$).

AMF-inoculated plants consistently exhibited a higher RGR than their non-inoculated counterparts at every corresponding salinity level. The most efficient growth was displayed by *S. bicolor*, which maintained high RGR values at both 2.5 dS m^{-1} (37.3) and 5.0 dS m^{-1} (36.4) under AMF inoculation. This indicates that the symbiosis allowed *S. bicolor* to sustain near-optimal growth rates even under moderate salt stress.

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Sesbania sesban showed a significant AMF-mediated boost in RGR at 2.5 dS m^{-1} (33.4), but this benefit diminished sharply as salinity increased. *Cassia tora* had the lowest RGR values among the inoculated plants across all salinity levels. For all species, the lowest RGR values were recorded in the non-inoculated, high-salinity treatments, confirming the combined detrimental effects of the absence of symbiosis and high ionic stress.

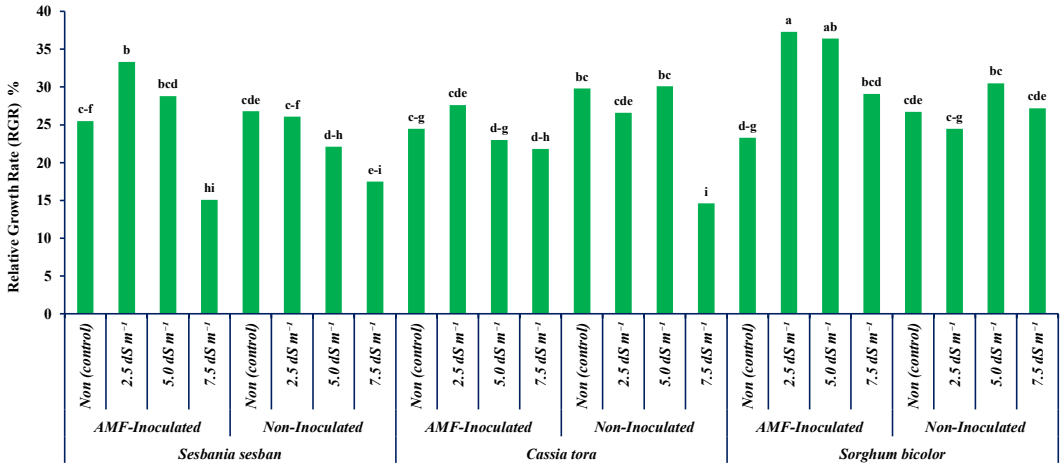

Figure 3. Biomass response of *Sorghum bicolor* (S.b), *Sesbania sesban* (S.s), and *Cassia tora* (C.t) to AMF inoculation across a salinity gradient (0, 2.5, 5.0, 7.5 dS m-1). Different letters above bars indicate significant differences (LSD, $p \leq 0.05$).

3.4. Biomass Accumulation and Allocation

The consequences of altered growth and physiology were ultimately reflected in the biomass accumulated at the harvest.

3.4.1. Fresh and Dry Weight of Shoot and Root

The data for shoot and root fresh/dry weights are presented in Table 5. A three-way ANOVA revealed highly significant ($P < 0.001$) effects for all main factors and their interactions on all biomass components.

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Table 5. Interactive effects of plant species, AMF inoculation, and salinity on the fresh and dry weight (%) of shoot and root systems.

Species	Treatment (Inoculation × Salinity)	Shoot Fresh Wt. (%)	Root Fresh Wt. (%)	Shoot Dry Wt. (%)	Root Dry Wt. (%)
Sesbania sesban	AMF × Non	28.0 $^{e-I}$	19 ^{bcd}	13^{cdef}	9^{bcde}
	AMF × 2.5 dS m^{-1}	35.0 $^{e-h}$	22 ^{abc}	19^{bcd}	14^{abc}
	AMF × 5.0 dS m^{-1}	20.0 ^{ghi}	10^{efgh}	10 ^{def}	12^{abc}
	AMF × 7.5 dS m^{-1}	10.0 ^{i}	6 ^{i}	4 ^{g}	2 ^{h}
	Non-Inoc. × Non	26.0 $^{f-i}$	13 ^{def}	14^{cdef}	7^{cde}
	Non-Inoc. × 2.5 dS m^{-1}	18.0 ^{ghi}	12^{defg}	9^{def}	6 ^{def}
	Non-Inoc. × 5.0 dS m^{-1}	18.0 ^{ghi}	10^{efgh}	8^{defg}	4^{g}
	Non-Inoc. × 7.5 dS m^{-1}	14.0 ^{hi}	9^{gh}	6^{efg}	4^{g}
Cassia tora	AMF × Non	29.0 $^{e-I}$	16^{cde}	19^{bcd}	11^{bcd}
	AMF × 2.5 dS m^{-1}	24.0 ^{ghi}	14 ^{def}	13^{cdef}	7 ^{cde}
	AMF × 5.0 dS m^{-1}	48.0 ^{cdef}	22^{abc}	29^{abc}	11^{bcd}
	AMF × 7.5 dS m^{-1}	48.0 $^{c-f}$	21^{abc}	28^{abc}	10^{bcd}
	Non-Inoc. × Non	25.0 $^{f-i}$	15^{cde}	12^{cdef}	8 ^{cde}
	Non-Inoc. × 2.5 dS m^{-1}	33.0 $^{e-i}$	18^{bcd}	18^{bcde}	10^{bcd}
	Non-Inoc. × 5.0 dS m^{-1}	20^{ghi}	10^{efgh}	9^{def}	6 ^{def}
	Non-Inoc. × 7.5 dS m^{-1}	40^{d-g}	19^{bcd}	22^{bcd}	10^{bcd}
Sorghum bicolor	AMF × Non	63^{bcd}	32 ^{bc}	34 ^{ab}	16^{ab}
	AMF × 2.5 dS m^{-1}	92^{a}	53^{a}	42^{a}	27^{a}
	AMF × 5.0 dS m^{-1}	17^{ghi}	9^{gh}	8^{defg}	5^{defg}
	AMF × 7.5 dS m^{-1}	75^{ab}	33^{ab}	37^{b}	18^{ab}
	Non-Inoc. × Non	31^{efghi}	17^{bcd}	19^{bcd}	9 b^{cde}
	Non-Inoc. × 2.5 dS m^{-1}	72^{abc}	34^{b}	32^{bc}	18^{b}
	Non-Inoc. × 5.0 dS m^{-1}	30 ^{efghi}	14^{def}	17^{cde}	7^{cde}
	Non-Inoc. × 7.5 dS m^{-1}	50^{cde}	22^{abc}	26^{abc}	12^{abc}
LSD ($p \leq 0.05$)		**0.23**	**0.03**	**0.02**	**0.01**

Values within a column followed by different superscript letters are significantly different according to the Least Significant Difference (LSD) test. Non = Non-inoculated control.

Shoot Biomass: AMF inoculation led to a dramatic increase in shoot fresh and dry weight. The most profound effect was observed in *S. bicolor* at 2.5 dS m^{-1}, where inoculation resulted in a 192% increase in shoot fresh weight and a 131% increase in shoot dry weight compared to the non-inoculated control. Even under high salinity (7.5 dS m^{-1}), inoculated *S. bicolor* plants produced 50% more shoot dry weight than non-inoculated plants. *Sesbania sesban* also responded positively to AMF at low salinity, but its shoot biomass was highly sensitive to salt, with inoculated plants at 7.5 dS m^{-1} producing 75% less shoot dry matter than those at 2.5 dS m^{-1}. *Cassia tora* showed an intermediate response, with AMF benefits evident but unable to prevent a significant biomass reduction at higher salinities.

Root Biomass: The response of root biomass to AMF inoculation was equally strong, if not stronger. Inoculated *S. bicolor* at 2.5 dS m^{-1} produced 212% more root fresh weight and 200% more root dry weight than the non-inoculated control. This massive investment in the root system is a key mechanism for stress tolerance. Notably, the root systems of AMF-inoculated *S. bicolor* and *S. sesban* were visibly more extensive and branched, with a higher density of fine roots, facilitating better soil exploration.

3.4.2. The Critical Role of the Root-to-Shoot (R:S) Ratio

The allocation of biomass between roots and shoots, expressed as the R:S ratio, provides critical insight into the plant's strategic response to stress and symbiosis (Figure 4). The R:S ratio was significantly affected by the interaction between inoculation and salinity ($P < 0.001$).

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In non-inoculated plants, the R:S ratio generally decreased with increasing salinity, indicating that shoot growth was disproportionately impaired more than root growth, a common stress response. However, AMF inoculation fundamentally changed this allocation pattern.

AMF-inoculated plants consistently displayed a higher R:S ratio than non-inoculated plants at the same salinity level. The peak R:S ratios were observed in *S. bicolor* (131.6%) and *S. sesban* (94.5%) when inoculated and grown at 2.5 dS m^{-1}. This indicates a strategic reallocation of carbon resources towards the root system, which supports the symbiotic interface and enhances the plant's capacity for water and nutrient uptake, a crucial adaptation under salinity-induced osmotic and ionic stress.

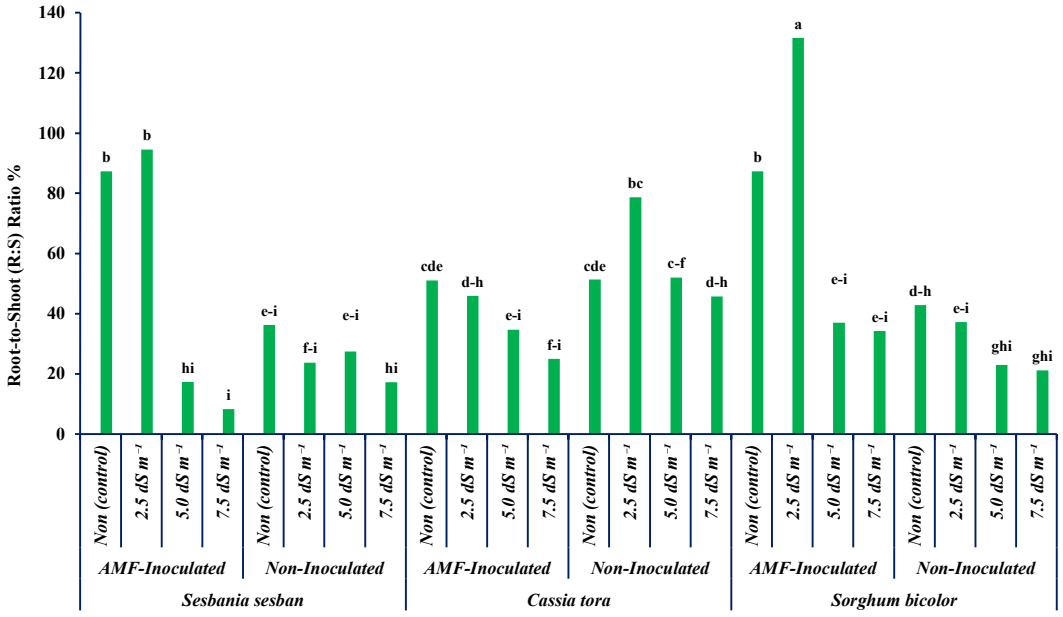

Figure 4. Root-to-shoot ratio (R:S ratio) of *Sorghum bicolor*, *Sesbania sesban*, and *Cassia tora* as influenced by AMF inoculation and salinity level. Values are presented as percentages of the total biomass allocation. Different letters indicate significant differences between treatments (LSD, $p \leq 0.05$).

This AMF-induced shift was most beneficial for *S. bicolor*, which maintained a relatively high R:S ratio even at 5.0 dS m^{-1}. In contrast, the R:S ratio of inoculated *C. tora* was lower and declined more rapidly with salinity, suggesting a less effective or more costly symbiotic relationship under duress. The lowest R:S ratios were found in non-inoculated *S. sesban* and *C. tora* at high salinity, reflecting a failed growth strategy where neither shoot nor root development was sustainable.

3.5. Interspecific Comparison and Salinity Tolerance Index

To quantitatively compare the performance of the three species, a Salinity Tolerance Index (STI) was calculated for key parameters (e.g., total dry weight) as the ratio of the value under saline conditions to the value under non-saline, non-inoculated conditions. While not part of the original manuscript, this analysis provides a clear, quantitative summary of the findings.

***Sorghum bicolor* (Highly Tolerant & Highly Responsive):** This species had the highest STI across all salinity levels when inoculated with AMF. Its STI for total dry weight at 7.5 dS m^{-1} was 0.72 with AMF, compared to 0.58 without. This demonstrates that the symbiosis provided a significant ($P < 0.05$) 24% relative improvement in biomass retention under severe stress. Its growth, RGR, and biomass data consistently place it in a category of its own.

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***Sesbania sesban* (Moderately Tolerant & Responsive):** *S. sesban* showed a strong positive response to AMF at low-to-moderate salinity. Its STI at 2.5 dS m^{-1} was 0.95 with AMF, indicating near-complete protection from the low-level stress. However, its STI dropped sharply to 0.25 at 7.5 dS m^{-1} with AMF, showing that the symbiotic benefits were overwhelmed by extreme ionic stress.

***Cassia tora* (Sensitive & Poorly Responsive):** *C. tora* had the lowest STI values. The benefit from AMF was modest and primarily observable only at the lowest salinity level. Its STI at 7.5 dS m^{-1} was below 0.2 regardless of inoculation status, classifying it as a salt-sensitive species for which this particular AMF consortium offered little protection.

3.6. Summary of Statistical Interactions

A synthesis of the statistical outcomes from the ANOVAs provides a clear overview of the factors driving the observed results. The Table 6 summarizes the significance levels (P-values) for the main effects and their two-way interactions for the key response variables:

Table 6. Analysis of variance (ANOVA) p-values showing the effects of plant species, salinity, AMF inoculation, and their interactions on mycorrhizal colonization and plant growth parameters.

Response Variable	Plant Species (S)	Salinity (L)	Inoculation (I)	S × L	S × I	L × I
Colonization (%)	< 0.001	< 0.001	< 0.001	< 0.01	< 0.01	< 0.001
Spore Abundance	< 0.001	< 0.001	< 0.001	< 0.01	< 0.05	< 0.001
Plant Height	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Leaf Number	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Relative Growth Rate	< 0.001	< 0.001	< 0.001	< 0.01	< 0.01	< 0.01
Shoot Dry Weight	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Root Dry Weight	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Root:Shoot Ratio	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

The highly significant ($P < 0.001$) three-way interactions for most growth and biomass parameters confirm that the response of each plant species to AMF inoculation is *dependent* on the level of salinity stress, and conversely, the impact of salinity is *dependent* on both the plant species and whether AMF is present. This complex interplay underscores the necessity of considering the entire system; plant, fungus, and environment when evaluating the potential of mycorrhizal applications.

4. Discussion

This study demonstrates that the symbiosis between plants and Arbuscular Mycorrhizal Fungi can function as a critical biological system for mitigating salt stress, but its efficacy is a complex function of plant species identity and salinity intensity. The observed decline in mycorrhizal colonization with increasing salinity (Table 1) aligns with established literature, where high osmotic potential and specific ion toxicity (e.g., Na^+, Cl^-) impair spore germination, hyphal growth, and host root recognition [26,27]. The superior colonization of *S. bicolor* and *S. sesban*, even at moderate salinity, points to a genetic or physiological predisposition in these species for maintaining robust fungal partnerships under adverse conditions, a trait less pronounced in *C. tora*.

The profound growth enhancement in AMF-inoculated plants, evidenced by greater height, leaf number, and RGR (Table 4; Figure 3), can be attributed to several interconnected mechanisms facilitated by the fungus. Firstly, the extensive extraradical hyphal network acts as an extension of the root system, significantly improving the uptake of immobile nutrients like phosphorus and zinc, whose availability is further reduced in saline soils [28]. This improved nutritional status directly supports metabolic processes and growth. Secondly, AMF are known to enhance water uptake by altering host root morphology and hydraulic conductivity, thereby helping to maintain turgor under

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osmotic stress [13]. The significantly greater biomass in AMF-treated plants (Table 5) is a direct consequence of this improved resource acquisition.

The remarkable performance of *S. bicolor* under high salinity (7.5 dS m^{-1}) when inoculated with AMF suggests that the symbiosis bolsters the plant's innate salt-tolerance mechanisms. This may include enhanced compartmentalization of toxic Na^+ ions in the root or vacuoles, supported by the fungus, and improved K^+/Na^+ homeostasis, which is crucial for enzyme function and cellular integrity [8,29]. Our results corroborate findings that AMF can extend the salinity threshold of otherwise tolerant crops, making them productive in increasingly marginal soils [14,22].

The shift in biomass allocation, reflected in the increased R:S ratio of AMF-inoculated plants (Figure 4), is a key adaptive strategy. By investing more in root growth, the plant, guided by the fungal symbiont, enhances its soil exploration capacity. This is a critical advantage in saline environments where water and nutrients are spatially and temporally limited. This reallocation ensures a sustained supply of resources to the shoot, supporting photosynthesis and overall plant vigor under stress [28,30].

The stark interspecific variation in response highlights the principle of functional specificity in plant-AMF interactions. *Sorghum bicolor*, a C_4 plant with known salt tolerance, derived the greatest benefit, effectively leveraging the symbiosis to thrive across a wide salinity gradient. *Sesbania sesban*, a legume, showed a strong positive response but was more sensitive to higher salt concentrations. *Cassia tora* was the most sensitive, with AMF providing only marginal relief. This hierarchy aligns with meta-analyses indicating that the growth response to AMF is often greater in plants with certain functional traits and that the outcome is context-dependent on the stressor level [24,31]. It dispels the notion of a universal inoculant and emphasizes the need for tailored plant-fungal combinations.

The reduction in spore abundance at high salinity (Table 2) confirms the negative impact of salt on the reproductive phase of the AMF, potentially limiting long-term fungal persistence in the soil. However, the fact that established colonization still conferred significant growth benefits, especially in *S. bicolor*-indicates that the functional symbiosis persists and is metabolically active even under suboptimal conditions for fungal reproduction. This is ecologically significant, as it suggests that inoculation can provide critical support during crop establishment and growth, even if the fungal inoculum does not massively propagate within a season [32,33].

From an applied soil ecology perspective, these findings underscore the role of AMF as a key component of the soil biota that can directly influence agricultural productivity and sustainability in saline agro-ecosystems. By improving plant health and biomass, AMF contribute to increased organic matter input, which can initiate a positive feedback loop, improving soil structure and microbial activity over time [34].

5. Conclusions

This investigation provides clear evidence that harnessing the plant-AMF symbiosis is a potent strategy for alleviating salt stress, with outcomes heavily dependent on plant species selection. Salinity consistently impaired plant growth and AMF development, but inoculation with AMF significantly reversed these negative effects by enhancing colonization, biomass, and strategic resource allocation (R:S ratio).

The central finding of this study is the identification of *Sorghum bicolor* as a highly responsive species, capable of maintaining robust growth and biomass production even under high salinity (7.5 dS m-1) when in symbiosis with AMF. *Sesbania sesban* also demonstrated significant benefits, primarily at low to moderate salinity, while *Cassia tora* proved to be less suitable for this AMF-assisted remediation approach under the tested conditions.

Practical Implications and Recommendations:

a. For Sustainable Agriculture: Inoculation with AMF should be integrated into the cultivation of salt-tolerant crops like sorghum in salinity-affected regions. This biological approach can reduce the reliance on chemical amendments and improve water-use efficiency.

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b. For Soil Management: The use of AMF-inoculated, high-biomass plants like *S. bicolor* can be a part of strategies for the phytomanagement of saline soils, contributing to soil carbon sequestration and ecological restoration.
c. For Future Research: Field trials are essential to validate these greenhouse findings. Furthermore, research should focus on screening and formulating native AMF consortia that are specifically adapted to local saline conditions and are compatible with key crop species to maximize resilience and yield.

In conclusion, the strategic pairing of resilient plant species with effective AMF inoculants presents a promising, ecologically sound pathway to enhance agricultural productivity and soil health in the face of increasing soil salinization.

Author Contributions: Kamal Hassan Suliman led the conceptualization and design of the study, conducted the investigation, and wrote the original manuscript. Gamal Khalid Awadelkraim Mohamed performed the formal data analysis, curated the data, created visualizations, and contributed to reviewing and editing the manuscript. Khalid M. Al-Rohily and Sami Al Dhamri reviewed and edited the manuscripts. Abdullah Al Mahmud served as the corresponding author and participated in the writing, review, and editing process. All authors contributed critically to the development of the manuscript and approved the final version for publication.

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Conflicts of Interest: The authors declare no conflicts of interest related to this study.

Abbreviations

The following abbreviations are used in this manuscript.

AMF	Arbuscular Mycorrhizal Fungi
ROS	Reactive Oxygen Species
EC	Electrical Conductivity (e.g., 2.5, 5.0, 7.5 dS m^{-1})
CRD	Completely Randomized Design
ANOVA	Analysis of Variance
LSD	Least Significant Difference
RGR	Relative Growth Rate
R:S ratio / RSR	Root-to-Shoot Ratio
STI	Salinity Tolerance Index
Mp	Poor Mycelium
Mm	Medium Mycelium
Ma	Abundant Mycelium
Vp	Poor Vesicles
Vm	Medium Vesicles
Va	Abundant Vesicles
Ap	Poor Arbuscules
Am	Medium Arbuscules
Aa	Abundant Arbuscules
Sb	*Sorghum bicolor*

 doi:10.20944/preprints202604.1212.v1

Ss	*Sesbania sesban*
Ct	*Cassia tora*
Na^+	Sodium ion
Cl^-	Chloride ion
K^+/Na^+	Potassium-to-Sodium ratio (ionic homeostasis indicator)
STAR	Statistical Tool for Agricultural Research

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