Evaluation of Integrated Application of Arbuscular Mycorrhizal Fungi and Silicon to Improve Growth and Yield of Sunflower under Salt Stress

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Dedicated

To

My Beloved Parents

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ABSTRACT

Salinity is the most devastating abiotic stress that exacerbates its detrimental impacts on agricultural production. Judicious application of silicon fertilizer and arbuscular mycorrhizal fungus (AMF) may have potential to mitigate the adverse impact of salt stress on sunflower. Therefore, the current study aimed to evaluate the integrated application of silicon fertilizer and AMF to enhance growth, physiological responses, yield performance and yield attributes of sunflower under salt stress. Pot experiment was conducted at Professor Dr. Purnendu Gain field laboratory where factorial experiment consists of three factors, namely; Si dose (non-treated control, 150 kg ha^{-1}, 300 kg ha^{-1} and 450 kg ha^{-1} designated as Si_0, Si_{150}, Si_{300}, Si_{450} respectively); water salinity levels (control- 0.54, 4, 8 and 12 dS m^{-1}) and two levels of AMF inoculation (with AMF [+AMF] and without AMF [–AMF]). Factorial experiment was laid out where treatment combinations were assigned completely randomized design (CRD) with three replications. All of the evaluated growth, physiological traits and seed yield of sunflower significantly declined by various levels of water salinity but silicon treated plants upgraded leaf relative water content, leaf greenness and membrane stability index 45%, 17 % and 60 %, respectively, with lower electrolyte leakage (15 %) at 12 dS m^{-1} water salinity compared to non-treated plants. In addition, the maximum proline concentration was observed with Si_{450} which was 19% and 28% higher than the control (0.54 dS m^{-1}) for AMF-inoculated plants and non-inoculated plants at water salinity 12 dS m^{-1}. AMF inoculated plant increased the head weight and seed number $plant^{-1}$ by 23% and 41% respectively compare to non-inoculated plant. At 12 dS m^{-1} salinity level, plants treated with Si_{450} had promoted seed yield by 56% and 50% in AMF inoculated and non-inoculated plant than Si_0. Taken together, it might be inferred that integrated application of Si and AMF had boosted up morpho-physiological traits, yield attributes and seed yield of sunflower and to ameliorate the deleterious effects of salt stress.

Keywords

Sunflower, salt stress, silicon, AMF, electrolyte leakage and proline content.

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CHAPTER I

INTRODUCTION

Soil salinity is the major global constraint on agricultural productivity which is aggravating by climate change, weathering process, seawater intrusion and agricultural malpractices. Salt stress inhibits the majority of plant physiological and biochemical activities, including transpiration, stomatal conductance, and absorption of carbon; all of which together reduce crop growth and yield potential (Rabbi et al., 2021; Das et al., 2021a). Continuous accumulation of salt is declining soil fertility and productivity over period that necessitating either development of salt-tolerant cultivars or improvement plant tolerance against salt stress (Roy et al., 2017; Alam et al., 2021). Therefore, the sustainable and prudent management of nutrients is currently receiving a lot of attention in order to maintain soil fertility, health, and productivity. Applying arbuscular mycorrhizal fungi (AMF) conjugated silicon (Si) might be a potential way to enhance sunflower growth, physiological characteristics, yield, and salt tolerance. Sunflower (*Helianthus annuus* L.) is the most popular oil crop consisted of protein, fat, fatty acid, and phytochemicals (Agubosi et al., 2022). Sunflower is a promising and popular oil crop, that has the ability to overcome the yield gap and support Bangladesh's domestic edible oil production. To improve sunflower growth and yield in Bangladesh's coastal saline soil, salt-tolerant sunflower cultivars, genotype identification, or improved resistance against salt stress are essential.

Silicon (Si) is a beneficial element and second-most copious element in the soil after oxygen and its average content in soil is nearly 29% (Sommer et al., 2006). Plants absorb Si in the form of monocyclic acid (MSA) [Si $(OH)_4$], whereas Si is predominantly available in the form of silicon dioxide (SiO_2) in the soil, which plants cannot uptake (Zargar et al., 2019). Silicon might play a role in a plant's ability to withstand a variety of stresses results from improved root hydraulic conductivity, better uptake of water and nutrients, increased photosynthetic rate, gaseous exchange, increased water use efficiency, improved leaf water status, and intensified antioxidant system activation (Tripathi et al., 2015; Zargar et al., 2019). Si-supplemented plants have been found to have better leaf-water interactions when exposed to salt stress due to increased water uptake, decreased stomatal conductance, and enhanced osmotic adjustment (Rizwan et al., 2016; Chen et al., 2018). Through the improvement of root hydraulic conductivity, water use efficiency, nutrient acquisition, and photochemical efficiency, Si lessens the effects of salt stress on plants. (Tripathi et al., 2015). Moreover, Si availability in the rhizosphere is mostly influenced by soil texture, soil temperature, soil reaction, root exudation and nutrient recycling through chemical weathering (Ahmed et al., 2011). Elevated bioavailability of Si in soil forwards nutrient uptake, root length, root density and root dry matter production towards vigorous root growth and tissue mechanical strength for upgrading tolerance against abiotic stresses (Raven, 2023).

Arbuscular mycorrhizal fungi (AMF) are perceived to be a promising option for sustainable agriculture practices. (Thirkell et al., 2021). It is typically referred to as bio-fertilizers which strengthens plants' resistance to environmental challenges while also promoting growth of plants and increasing the yield of crops. AMF have demonstrated potential in reducing the detrimental effects of salt stress and enhancing stress tolerance capacity (Mohammadi et al., 2019). AMF improves root growth by promoting root colonization, root length density and root hair to penetrate deeper soil layers in pursuit of moisture and nutrients (Pal et al., 2024). AMF form a symbiotic relationship with plants in which the plant provides supply of carbon to the fungi which use their hyphae to acquire immobile nutrients such as macro (N, P, K etc) and micronutrients (Mn, B, Zn etc) that are outside the root zone of the plant (Ebbisa et al., 2022). It was revealed that AMF is the most prevalent kind of beneficial microbial community in the soil that lessens the negative impacts of chemical fertilizers used in conventional farming (De Souza et al., 2018). AMF triggers the synthesis and accumulation of several osmolytes and proteins in response to salt stress, which shield plant cells from oxidative damage (Zong et al., 2023). AMF release enzymes that promote effective plant-soil interactions and increase the formation of secondary metabolites (Scavo et al., 2019). These interactions cause mineralization in the surrounding root zone and provide access to available phosphorus (P). Furthermore, the mutualistic exchange of nutrients, water, and other materials between AMF and plant roots is enabled by their symbiotic relationship (Pal et al 2024). Moroever mycorrhizal colonization has been shown to enhance sunflower growth, development, and morphological traits (Parça & Unay, 2021).

In the light of aforenamed information, it became apparent that a large body of published literature assessed the individual beneficial effect of AMF and Si on a variety of crops, but their combined effects on the growth, physiological characteristics, and yield of sunflower under salt stress were little bit explored. Hence, we hypothesized that integrated application of AMF and Si fertilizer would improve growth, physiological traits and yield of sunflower under salt stressed conditions. Therefore, the present study was aiming to investigate the ameliorative role of AMF and Si fertilizer on morpho-physiological characteristics and seed yield of sunflower growing under salt stress.

Objective

To promote growth, physiological traits and seed yield of sunflower through integrated application of AMF and silicon fertilization under salt stress

CHAPTER II

REVIEW OF LITERATURE

This chapter reviews the body of research on how salt stress affects plant growth and development, as well as potential of arbuscular mycorrhizal fungi and Si fertilizer to reduce salt stress of sunflowers. This review covers existing domestic and international research findings that are relevant to the current topic.

2.1 Effect of salt stress in plant growth and development

Soil or water salinization is one of the major environmental problems for agriculture worldwide. Salinity is a prevalent concern in arid and semi-arid regions on account of an excess of inorganic salts that have accumulated naturally due to prevailing climatic conditions, increased rates of evapotranspiration, and a lack of leaching water. The deposition of an excess amount of soluble salt in cultivable land directly affects crop yields. Excessive salt absorption by plants may have detrimental effects on their viability by impeding many physiological and metabolic functions (Egamberdieva et al., 2019). Accumulation of Na^+ and Cl^- ions in the tissues of plants exposed to soils rich in NaCl is one of the most detrimental effects of salt. A high concentration of Na^+ prevents plants from absorbing K^+, a macronutrient that is necessary for their growth and development. This might have catastrophic consequences for the production of the plants (Wu et al., 2015). The effects of saline soil-induced physiological and metabolic abnormalities on plant growth, development, productivity, and quality. Salinity negatively impacts seed germination, survival rate, morphological traits, development, yield, and other constituent parts of the plant. Plants that experience stress caused by salt generally have lower rates of photosynthesis and respiration. The effect of salinity increased the amounts of amino acids, particularly proline, and decreased the amounts of protein, fatty acids, and total carbohydrate content (Jouyban, 2016).

Shahid et al., (2020) investigate that, the production of reactive oxygen species, ionic imbalance, osmotic damage, and decreased relative water content are strongly associated with salinity-induced cellular damage. Prolonged antioxidant activity and osmotic adaptation through the synthesis of inorganic and organic osmolytes are crucial and efficient ways for crop plants to cope with salinity. In addition, polyamines enhance the plant body's ability to withstand salt stress by controlling multiple physiological processes, such as somatic advancement, cellular pH maintenance, and ionic homeostasis. Salinity has a detrimental effect on plant growth and development, resulting in a crucial environmental stressor that limits agricultural productivity. Most crop plants are susceptible to salt stress during germination and vegetative stage. The growth of roots and shoots is inhibited by salinity, although this growth suppression can be somewhat combat by additional application of AMF. (Läuchli & Grattan, 2017).

2.2 Effect of Si on plant morphology and physiology

Silicon (Si) is the second most abundant element in the earth's crust following oxygen, when considered either on the basis of mass or number of atoms. Water-soluble Si is also found in the soil, even though the majority of Si is found there as insoluble oxides or silicates. According to the conventional criteria, silicon is a non-essential element in plant nutrition. Si has been shown to have a number of positive effects on plants, including delaying the senescence process, increasing water use efficiency, reducing the effects of abiotic stresses like salt, drought, and nutrient stress, as well as protecting against heavy metal and metalloid toxicities, extreme heat and radiation, freezing, and waterlogging (Guntzer et al., 2012; Savvas & Ntatsi, 2015).

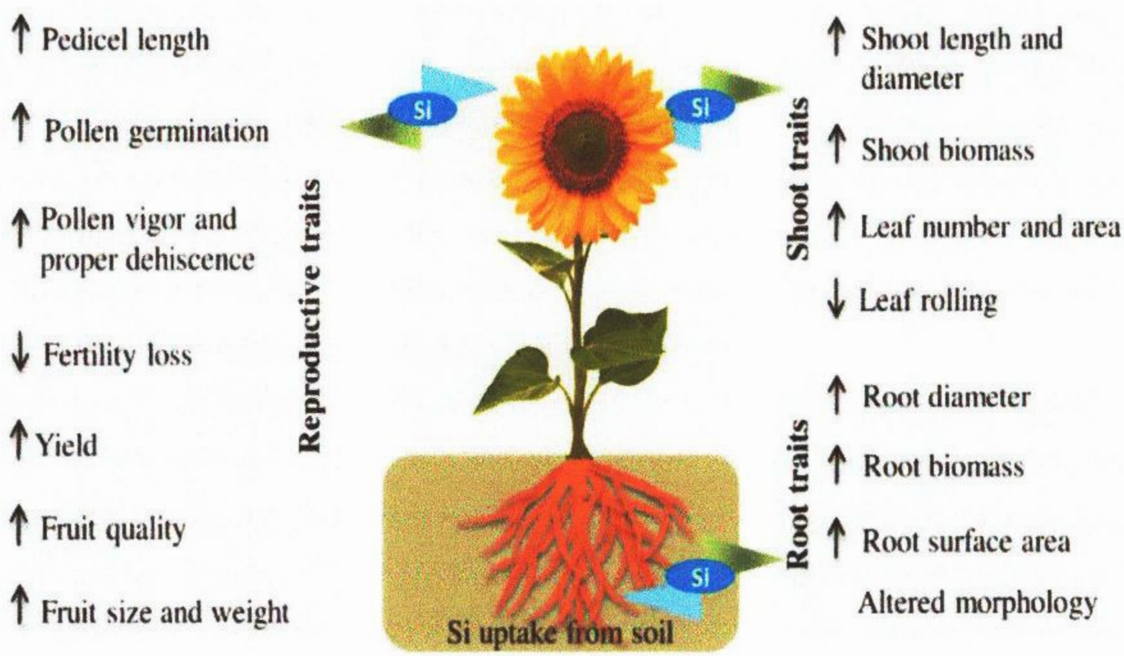

Figure 1. Beneficial effects of silicon (Alka Bishnoi, 2023)

De Melo Peixoto et al. (2022) investigate that, Si has beneficial impacts on plants that are subjected to various stresses. It significantly affects the absorption of CO2, the amount and quality of leaves, the uptake of N, and the overall biomass yield. Plants demonstrated an increase in total leaf area even at the lowest dose of Si applied, without an accompanying reduction in leaf N concentration or net CO2 assimilation rate. The total leaf area of the Si-treated plants was higher, which increased the plant's overall absorption of CO2. Plants treated with different doses of Si exhibited a 24–39% increase in biomass production.

Yavaş & Unay, 2017, conducted an experiment to observe the role of Silicon under different biotic and abiotic stress condition and noticed that, Si might help plants withstand both biotic and abiotic stressors, among which are disease, insects, salt and drought stress. It offers defense against disease infestations because of its buildup in cell walls and intercellular space, especially in small grains. Plants treated with silicon may be less susceptible to environmental stresses while still making good use of nutrients such as nitrogen and phosphorus. Moreover, silicon might mitigate the deleterious effects of heavy metals in soil. The positive effects of silicon on plant growth, development, yield and disease resistance are observed in both monocots and dicots. The use of silicon promotes the accumulation of macro- and micronutrients, which improves sunflower's nutritional value (Savić & Marjanovic-Jeromela, 2013).

2.3 Use of arbuscular mycorrhizal fungi on plant growth

Arbuscular mycorrhizal fungi (AMF) are a type of beneficial fungi that form a symbiotic relationship with the roots of the majority of the crop plants. This symbiosis between AMF and plants is known as arbuscular mycorrhiza. The name "arbuscular" refers to the tree-like structures called arbuscules formed by these fungi inside the plant roots. Begum et al., (2019) stated that, arbuscular mycorrhizal fungi (AMF) have the capability to enhance crop productivity. AMF commonly referred to as biofertilizers. Furthermore, It is commonly thought that AMF inoculation confers resistance to host plants against a range of harsh environmental factors, such as heat, salinity, drought, metals, and extremely high or low temperatures. AMF might assist in the upregulation of tolerance mechanisms in host plants and prevent the downregulation of significant metabolic pathways. The soil's bacteria expand the root region where nutrients can be absorbed and also enhancing the nutrient absorbing ability of plant. AMF helps plants overcome biotic and abiotic stressors including heavy metals, diseases, infections, salt, drought, and extreme temperatures. In terms of abiotic stresses, AMF's response mechanisms are commonly linked to improved hydromineral feeding, ion selectivity, gene regulation, osmolyte production, and the synthesis of antioxidants and phytohormones (Diagne et al.,2020). They investigate that, the majority of terrestrial plants have beneficial interactions with AMF. These soil-dwelling microbes improve the plant's ability to absorb nutrients by increasing the root's capacity to accomplish so. The plant provides the symbiotic fungi with carbohydrates to support their life cycle in return. Additionally, AMF helps plants resist a range of environmental stresses, including heavy metals, diseases, drought, salt, and extreme temperatures.

2.4 Arbuscular mycorrhizal fungi and salt stress alleviation

Salinity is a destructive factor and one of various abiotic stresses which negatively affect crop productivity and reduce the marketable yield of crop. Plants grown in non-saline water (0 mM NaCl), with or without treatments, exhibited considerably more vigorous vegetative growth and the highest fruit yield when compared to those cultivated under salt stress. Arbuscular mycorrhiza-inoculated plants showed improved vegetative development and shoot biomass (total fresh and dry weight per plant (Hegazi et al., (2015). Plants treated with mycorrhiza exhibited the highest leaf relative water content (LRWC) and the lowest membrane permeability (MP) values, respectively (Hegazi et al., 2015). Plant growth parameters, photosynthetic pigments, gas exchange rate, nutritional levels (apart from Na^{+}), and antioxidant enzymes were all markedly increased by AMF. The aforementioned benefits were correlated with the degree of mycorrhizal infection in plant roots. In contrast to non-salinized soil, the AMF in salinized soil appeared to have a significant impact on antioxidant enzymes, photosynthetic pigments, plant development, and nutrition. Mycorrhizal colonization might be used to mitigate the detrimental effects of salt, particularly in soils that are frequently irrigated with salt water (Al-Amri 2019).

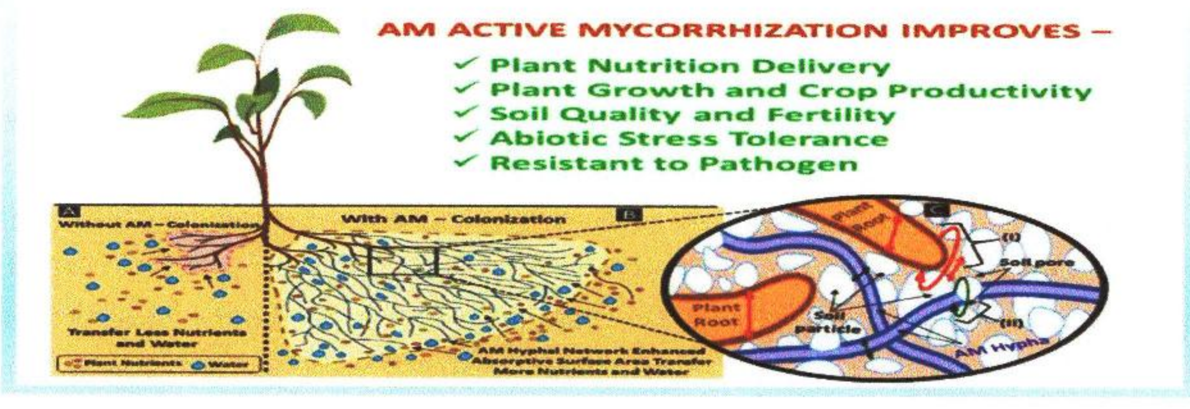

Figure 2. Influence of AMF on plant (Ebbisa, 2022)

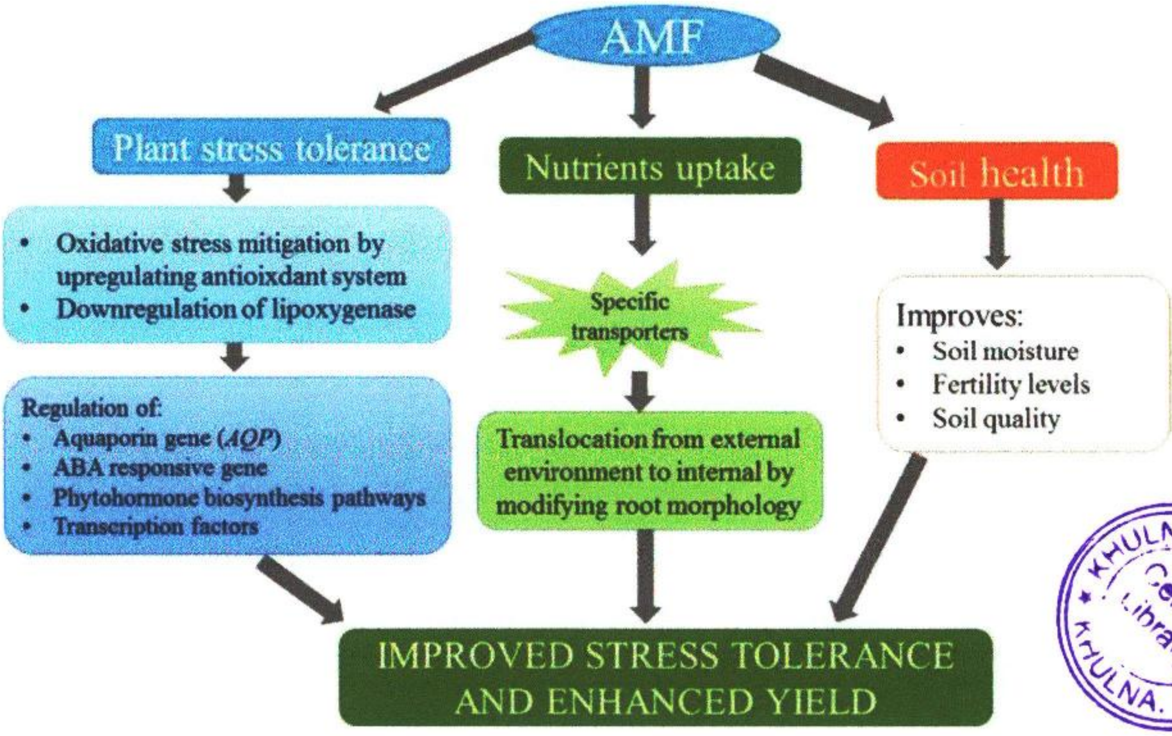

Figure 3. Beneficial role of AMF for improving drought tolerance and crop yield (Source: Begum et al., 2019)

Nguyen (2018) stated that, Plants inoculated with AMF showed better root hydraulic conductivity, altered root morphology, increased stomatal opening, chlorophyll content, higher uptake of magnesium and nitrogen, and, interestingly, decreased Na-transport when exposed to salinity. Along with increases in cytokinin level and photosynthate translocation in mycorrhizal plants under saline stress, enhanced PSI, PSII performance, and carbonic anhydrase concentration were seen in inoculated plants. AM plants under salt stress also demonstrated decreased oxidative stress due to decreased membrane lipid peroxidation with declining malondialdehyde (MDA) levels and enhanced antioxidative defense mechanisms. Remarkably, AM inoculation minimized electrolyte leakage and decreased lipid peroxidation, which enhanced plasma membrane integrity. AM symbiosis plants had lower H_2O_2 and MDA levels than non-mycorrhizal plants. AMF inoculated colonized plants had less membrane damage and accumulated ROS.

2.5 Integrated application of AMF and Si on crop plant

Shah et al., (2024) stated that, integrated application Si and AMF activate the plant defense system by improving the antioxidative enzymes activities, the potassium citrate and glutathione pool, the glyoxalase system, metabolites, and genes encoding these enzymes under Cr stress. When Si and AMF are used in tandem, they effectively mitigate the negative effects of Cr stress and restore growth and physio-biochemical traits. The beneficial effects caused by applying Si and AMF together can therefore be ascribed to metal resistance by mycorrhizae. According to Etesami et al. (2022), it has been demonstrated that symbiosis with arbuscular mycorrhizal fungus (AMF) and adding Si to paddy soils can both enhance rice growth in drought and salinity-stressed conditions. AMF may aid in the accumulation of root Si by absorbing Si through their spores and hyphae. Thus, Si may affect mycorrhizal response; nevertheless, the underlying mechanisms remain mostly elusive. Mycorrhizal inoculation and silicon application can enhance the nutritional and yield attributes of tomato plants even in the face of varying degrees of abiotic stress because it lowers water transpiration loss and increases water use efficiency. Plants may withstand stress better when Si + AMF are combinedly applied. Based on current research, Si and AMF cooperate to support many stress-related traits in plants. Even though the increased amount of water had a positive effect on nutrient uptake, while silicon treatments of 100 and 150 kg ha^{-1} had equally significant effects on the nutrient uptake. The successful outcome is thought to be the result of silicon and robust mycorrhizal colonization interacting. The results provide a useful foundation for the application of AMF and Si fertilizers to enhance tomato yield (CELİK & BAYDAR, 2023). Plants that are subjected to drought stress benefit from the integrated application of arbuscular mycorrhizal fungus (AMF) and silicon (Si). An investigation of the combined effects of Si and AMF in a medicinal plant (*Glycyrrhiza glabra* L.) were carried out by (Haghighi et al.2022) and observed that under stressed conditions, there were significant decreases in root length and dry weight (DW) compared to the control. With proper irrigation levels, Si and AMF may significantly increase root area by 27%. When plants are treated with abiotic stress, the Si and AMF increased the amount of trans ferulic acid (1.11 mg/g DW) and sinapic acid (16.46 mg/g DW) in the roots. The results demonstrated that the interaction between Si and AMF increased 51.51 and 74.07% of the nitrogen and phosphorus concentrations in roots, respectively.

CHAPTER III

MATERIALS AND METHODS

3.1 Experimental site

Pot experiment was conducted at Professor Dr. Purnendu Gain field laboratory of Agrotechnology Discipline, Khulna University, Bangladesh (22°48′ N; 89°32′ E) during the period from December 2023 to April 2024. In addition, postharvest evaluation of harvested plant and head of sunflower was conducted at the Molecular Horticulture Laboratory and Agronomy Laboratory of Agrotechnology Discipline to assess some physio-chemical quality parameters.

3.2 Experimental treatments and design

Table 1: Experimental factor and design

Factor A: Si fertilizer doses	Factor B: Water salinity level	Factor C: AMF inoculation
A_1-Si_0 (0 kg ha^{-1})	B_1: 0.54 dS m^{-1}	C_1: With AMF (+AMF)
A_2-Si_{150} (150 kg ha^{-1})	B_2: 4 dS m^{-1}	
A_3-Si_{300} (300 kg ha^{-1})	B_3: 8 dS m^{-1}	C_2: Without AMF (-AMF)
A_4-Si_{450} (450 kg ha^{-1})	B_4: 12 dS m^{-1}	

Total treatment combinations = Si doses (4) ×Salinity levels (4) × AMF inoculation (2) × Replication (3) = 96

Treatments were arranged in a factorial completely randomized design (CRD) with three replications in which each pot was considered as a specific treatment combination.

3.3 Crop husbandry

Sunflower seed were collected from Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh which were used as experimental materials. Seeds were surface sterilized with 10% H_2O_2 for 10 min, rinsed thoroughly with distilled water and then were soaked in distilled water for 12 h and were sown in well prepared earthen pot which (height: 40 cm; top diameter: 38 cm and bottom diameter: 26 cm) that were filled with 14 kg dry soil. Standard fertilizer dose was applied in all treatment combinations as mentioned in Krishi Projukti Hatboi published from BARI, Gazipur. AMF were collected from Bismillah Traders and the inoculum of AMF (Greenhouse Megastore; Danville, IL, USA) consisted of at least 25 spores (Glomus spp.) per gram of medium.

3.4 Application of AMF and Si doses

The inoculum of AMF (Greenhouse Megastore; Danville, IL, USA) consisted of at least 25 spores (Glomus spp.) per gram of medium and 12 g inoculum was inoculated two days before transplanting in each pot while the control treatment received a similar amount of dead inoculum (autoclaved) in the rhizosphere according to Das et al. (2021a). and the required doses of MSA (0, 150, 300 and 450 kg ha^{-1}) was mixed with the soil in respective pots. A commercial fertilizer in the form of monosilicic acid MSA (20% Si content) was used as a source of Si.

3.5 Imposition of soil salinity as experimental treatments

Commercial sodium chloride (NaCl) was purchased from the local market and running tap water was used to prepare salt solutions in various concentrations. The amount of salt required for a particular salt solution were calculated and adjusted through using a portable EC meter (Lutron CD-4301 Electronic Conductivity Meter, Taiwan) to prepare specific levels of salt solution. The inherent salinity of tap water (control) was 0.54 dS m^{-1}, whereas 2.2 g, 4.3 g, and 6.8 g NaCl was dissolved in 1 L of water for preparing 4 dS m^{-1}, 8 dS m^{-1}, and 12 dS m^{-1} salt-solution respectively (Adhikary et al. 2022). About 500 ml of respective saline solution were applied to each pot (two times per week) starting from the early vegetative stages (4 weeks seeding) to complete flowering stage (65 day after seeding). Then, normal irrigation water was applied as and when required to maintain field capacity of soil for optimum growth and reproductive development of sunflower.

3.6 Data collection

3.6.1 Morphological, physiological and yield attributes

Data were collected on plant height (cm), stem diameter, root biomass (g $plant^{-1}$), shoot biomass (g $plant^{-1}$), leaf greenness (SPAD value), leaf relative water content (LRWC) (%), membrane stability index (%), electrolyte leakage (%), proline concentration (μg g^{-1} fresh weight), head weight, head diameter, seed number and seed yield (g $plant^{-1}$). Plant height was measured from ground level to the topmost leaf just 7 days before harvest. Root and shoot samples from specific treatment combinations were collected, cleaned and were placed in an oven at 72°C for 36 hours. Then the volume of root was measured through the following ways. A beaker of 1L was taken and poured with 500ml water. Then the dried root was dipped into the beaker and observed the increased volume of the water. Then the first volume was subtracted from the increased volume and the result were the volume of dried root. Stem dry weight were measured in the laboratory by balance properly.

3.6.2 Physiological and biochemical traits

SPAD values were noted using a portable chlorophyll meter (SPAD-502 Plus, Konica Minolta, Tokyo, Japan) during flowering period of the plants. Fully expanded leaves were randomly selected, and two SPAD values were measured from each experimental unit followed by average to obtain a representative reading for each experimental unit. LRWC was determined at the initial flowering stage using the method described by Das et al. (2021b). Initially, the topmost second leaves of the plants were collected and were weighed immediately to obtain their fresh weight. After that, each leaf was sliced into 2 cm segments and submerged into distilled water for 12 hours to become fully turgid followed by measuring turgid weight. Then, fully turgid leaf segments were dehydrated in an oven at 72°C for 36 hours. Finally, LRWC was measured using the following formula:

$$\text{Leaf relative water content (\%)} = \frac{FW-DW}{TW-DW} \times 100 \quad \text{.........} \quad (1)$$

For determining electrolyte leakage (EL), to avoid any surface contamination, fresh leaf sample was rinsed with distilled water. Then, 0.5 g fresh leaf disk was sliced into small 15 pieces and put into a test tube with adding 20 ml of distilled water followed by random shaking continuously for 12 hours according to Camejo et al., (2005) and Das et al., (2021c). Then, initial conductivity (EC_1) was measured using a conductivity meter (Eutech CON 150, Thermo Scientific Eutech Instrument, Singapore) and followed by heating the sample for 15 min. Subsequently, conductivity (EC_2) was measured and electrolyte leakage was calculated as the ratio between EC_1 and EC_2 that was expressed in percentage as equation 2.

$$EL\ (\%) = \frac{EC_1}{EC_2} \times 100 \quad \text{.........} \quad (2)$$

Where, EC_1 is the initial conductivity and EC_2 is the final conductivity after boiling (60°C) the solution. Similarly, membrane stability index was computed by following the method prescribed by Hayat et al. (2008). Where EC_1 is the initial electrical conductivity at 40 °C and EC_2 is the final electrical conductivity at 100 °C.

$$\text{Membrane stability index (\%)} = \left(1 - \frac{EC_1}{EC_2}\right) \times 100 \qquad (3)$$

The free proline concentration of leaf was determined using the method outlined by Bates et al. (1973). Initially, a 500 mg fresh leaf was sliced and ground into a paste which was then mixed with 10 ml sulfosalicylic acid (3%) solution and subjected to centrifugation at 6,000 rpm for 15 minutes. Subsequently, 2 ml sample was transferred into a test tube containing 2 ml of acid ninhydrin and 2 ml of glacial acetic acid that was exposed to hot water bath for 30 mins. Then, 2 ml mixture solution was added with 4 ml toluene and again centrifuged at 5,000 rpm for 5 minutes. After 30 minutes, precipitation was completed and the upper layer was separated using a separating funnel and the absorbance of the sample was measured at 520 nm using a spectrophotometer. Finally, the concentration of free proline was determined from same absorbance values of different standard solutions of proline.

3.6.3 Head yield and head quality attributes

Total head yield was counted by measuring individual head weight and finally obtained fresh seed weight. Head diameter was measured using digital slide calipers. Seed number per head was obtained by seed counter.

3.7 Statistical analysis

All of the collected data were subjected to a two-way analysis of variance (ANOVA) using Statistix v. 10 (Analytical Software 2105, Miller Landing Rd., Tallahassee, FL 32312, USA). Means for significant treatments effects were identified by F test and separated by Tukey's honest significant difference test ($p < 0.05$). Data were presented based on the highest order of factorial combination that was significant in the ANOVA.

CHAPTER IV

RESULTS AND DISCUSSION

Results

4.1 Growth parameters

The interactive effect between salinity and silicon doses, as well as Si and AMF were significant for plant height (Table 2). Plants inoculated with AMF showed maximum plant height compare to non-inoculated one across silicon doses. AMF inoculated plant significantly increased plant height by 16% compare to non-inoculated at control level of Si. Plant height was increased progressively with increasing Si doses in both AMF inoculated and non-inoculated plants (Table 3). Shoot diameter were increased with increasing silicon doses and reduced with increasing salinity level. At 12 dS m^{-1}, shoot diameter was increased by 33% at silicon doses Si_{450} compare to control where AMF was not inoculated. AMF inoculation significantly increase shoot diameter by 17% and 11% at Si_{150} and Si_{300} respectively at 12 dS m^{-1} (Table 4). Shoot and root dry weight, root volume was maintained significantly by the application of silicon compared to control but reduced with increasing water salinity levels. About 45% and 25% of shoot and root dry weight were decreased respectively with increasing salinity level but 47% and 38% of root and shoot dry weight were increased with increasing Si doses compare to control at 12 dSm^{-1} (Table 5). Among the treatment combination maximum root volume were observed at Si_{450}. Root volume was increased by 37% at Si_{450} compare to control and reduced by 26% at 12 dS m^{-1} (Table 6). The studied growth parameter shoot diameter showed statistically similar responses from 0.54 to 12 dS m^{-1} but shoot and root dry weight were significantly reduced at 12 dS m^{-1}.

Table 2. Significance level in the three-way ANOVA of the effect of Si dose (Si), salinity levels (S) and AMF inoculation (AMF) on morpho-physiological traits, seed yield and other attributes of sunflower.

Traits	Si doses	Salinity levels (S)	AMF inoculation (AMF)	Si × Salinity	Salinity × AMF	Si × AMF	Si × Salinity × AMF
Growth parameters							
Plant height (cm)	ns	*	ns	**	ns	**	ns
Stem diameter (cm)	**	**	**	**	ns	*	*
Shoot dry weight (g $plant^{-1}$)	ns	ns	ns	*	ns	ns	ns
Root dry weight (g $plant^{-1}$)	**	**	**	**	ns	ns	ns
Root volume (cm^3)	**	**	**	**	**	ns	ns
Physiological traits							
Leaf greenness (SPAD value)	**	**	ns	ns	*	**	*
Leaf relative water content (%)	ns	**	*	**	**	**	*
Electrolyte leakage (%)	**	**	**	**	**	ns	**
Cell membrane stability index (%)	**	**	**	**	ns	**	**
Proline concentration (μg g^{-1} fresh weight)	**	ns	**	ns	**	*	*
Yield attributes and yield							
Head weight (g $plant^{-1}$)	*	**	**	ns	ns	ns	ns
Head diameter (cm)	**	ns	ns	**	ns	**	*
Seed number $head^{-1}$	**	*	**	ns	ns	ns	ns
Seed yield (g $head^{-1}$)	**	*	*	ns	ns	**	*

**,* and ns indicate $p<0.01$, $p<0.05$ and not significant, respectively

Table 3. Effect of silicon dose and AMF inoculation on plant height of sunflower.

Si doses (kg ha^{-1})	Plant height (cm)	
	–AMF	+AMF
Si_0	110.5±5.8cB	128.6±3.06cA
Si_{150}	116.6±5.8cA	122.5±3.4cA
Si_{300}	130.8±0.8bB	142.8±0.8bA
Si_{450}	139.0±4.2aB	156.6±3.7aA

Means followed by same small case letters are statistically similar within a column and means followed by same capital letter are statistically similar within a row based on Tukey's honest significant difference test at $p < 0.05$; data are means of three replications ± standard errors.

Table 4. Effect of silicon dose, salinity levels and AMF inoculation on shoot diameter of sunflower.

Si dose (kg ha^{-1})	AMF inoculation	Shoot diameter (cm)			
		0.54 dS m^{-1}	4 dS m^{-1}	8 dS m^{-1}	12 dS m^{-1}
Si_0	–AMF	10.5±2.4bA	9.8.0±0.5bA	9.8± 1.3bA	8.3±0.6aA
	+AMF	11.1±0.3bA	9.8±0.9bA	9.8±0.4bA	8.5±0.1aA
Si_{150}	–AMF	11.0± 0.8bA	10.1±0.7bA	9.0±0.6bA	8.6±0.4aA
	+AMF	11.9±0.3abA	11.3±0.2bA	11.3±0.3abA	10.1±0.1aA
Si_{300}	–AMF	12.1±0.4abA	11.8±0.2abA	10.8±0.8abA	10.1±0.6aA
	+AMF	12.8±0.2abA	12.5±0.3abA	11.6±0.1abA	11.3±0.3aA
Si_{450}	–AMF	13.2±0.5abA	12.1±0.2abA	12.1±0.3abA	11.2±0.6aA
	+AMF	15.5±0.5aA	15.1±0.3aA	12.8±0.2abA	11.8±0.8aA

Means followed by same small case letters are statistically similar within a column and means followed by same capital letter are statistically similar within a row based on Tukey's honest significant difference test at $p < 0.05$; data are means of three replications ± standard errors.

Table 5. Shoot dry weight and root dry weight of sunflower was influenced by of silicon doses and salinity.

Si dose (kg ha^{-1})	Shoot dry weight (g $plant^{-1}$)				Root dry weight (g $plant^{-1}$)			
	0.54 dS m^{-1}	4 dS m^{-1}	8 dS m^{-1}	12 dS m^{-1}	0.54 dS m^{-1}	4 dS m^{-1}	8 dS m^{-1}	12 dS m^{-1}
Si_0	30.3±5.4bA	28.9±4.7aAB	28.4± 6.3aAB	18.7± 2.1bB	5.3±0.09bA	4.4±0.4bAB	4.4±0.6bAB	3.9±0.1bB
Si_{150}	36.1±1.3abA	30.8±4.9aB	26.1±1.4aBC	21.7± 5.4abC	5.7±0.2abA	5.1±0.2bAB	4.8±0.3bAB	4.5±0.06abB
Si_{300}	37.5± 4.2abAB	31.4±4.2aB	27.4±4.9aAB	23.2±6.9abA	6.2±0.5abA	5.8±0.3abA	5.5±0.2abA	5.4±0.1abA
Si_{450}	40.5± 3.9aB	31.1.0± 4.6aB	29.4±1.5aAB	27.9± 4.6aA	6.6±0.2aA	6.4±0.5aAB	6.1±0.2aAB	5.4±0.3aB

Means followed by same small case letters are statistically similar within a column and means followed by same capital letter are statistically similar within a row based on Tukey's honest significant difference test at $p < 0.05$; data are means of three replications ± standard errors.

Table 6. Effect of silicon doses and salinity levels on root volume of sunflower.

Si dose (kg ha^{-1})	Root volume (cm^3)			
	0.54 dS m^{-1}	4 dS m^{-1}	8 dS m^{-1}	12 dS m^{-1}
Si_0	19.6.0±1.1bA	18.8±0.6bAB	18.3±0.6abAB	14.5±1.3bB
Si_{150}	20.6±1.3bA	20.8±1.1bAB	18.0±1.5bAB	16.8±1.1abB
Si_{300}	22.5±1.4bA	21.3±1.1abAB	20.5±1.7abAB	18.6±1.7abB
Si_{450}	27.1±1.7aA	24.8±1.06aAB	22.1±2.0aB	19.3±1.7aB

Means followed by same small case letters are statistically similar within a column and means followed by same capital letter are statistically similar within a row based on Tukey's honest significant difference test at $p < 0.05$; data are means of three replications ± standard errors.

4.2 Physiological traits

The study investigated the three-way interaction among silicon doses, salinity levels and AMF inoculation was significant for physiological traits such as on various physiological traits including leaf greenness, LRWC, electrolyte leakage, membrane stability index and free proline concentration. The interactive effect of silicon doses, salinity and AMF inoculation were significant for the physiological traits (Table 2). Plants treated with silicon and AMF generally exhibited better performance than control plants regardless of water salinity. For example, at 12 dS m^{-1} water salinity level, plants treated with Si_{450} with +AMF had higher SPAD values (17%), LRWC (45%), membrane stability index (60%), and lower electrolyte leakage (15%) compare to the control plants (Table 7 and 8). Most of the physiological traits decline progressively with increasing salinity level and also increase with AMF inoculation. However, the leaf greenness of plant did not show a significant difference between 0 and 8 dS m^{-1} salinity level. At 12 dS m^{-1} AMF inoculation increased leaf greenness by 5% compare to non-inoculated one regardless of silicon doses and increase 10% increasing with silicon doses compare to control. Combined effect of Si and AMF increase leaf greenness by 8% compared to control. Leaf relative water content was decreased progressively with increasing salinity levels regardless of Si doses (Table 2) in which maximum water content was recorded at Si_{450} with AMF inoculated plants at 12 dSm^{-1}, salinity levels. AMF inoculated plants consisted of higher leaf relative water content over non inoculated plants regardless under various Si doses. Electrolyte leakage was significantly influenced by various Si doses in which AMF inoculated plants possess lower electrolyte leakage than non-inoculated plants under Si_{300} and Si_{450} at 12 dSm^{-1}. Similarly, membrane stability index was significantly affected by different salinity levels where Si450 application promoted 70% and 53 % electrolyte leakage in AMF inoculated plants and non-AMF inoculated plants over control (Si_o) at 12 dS m^{-1} salinity levels. Free proline concentration was significantly influenced by the three-way interaction among Si doses, salinity levels and AMF inoculation (Table 2). Proline concentration increased with higher salinity levels by 31% compared to control. At water salinity 12 dS m^{-1}, the maximum proline concentration was observed with Si_{450} which was 19% and 28% higher than the control (0.54 dS m^{-1}) for AMF-inoculated plants and non-inoculated plants, respectively (Table 9). Similarly, at Si_{450}, 14% more proline synthesis was found at mycorrhized plants over non-mycorrhized plant under extreme salt stressed plants (12 dS m^{-1}). In comparison, most of the AMF-treated plants had higher free proline concentrations than non-treated plants. Similarly, free proline concentration was also increase with increasing silicon doses.

Table 7. Effect of silicon dose, salinity levels and AMF inoculation on leaf greenness (SPAD value) and leaf relative water content of sunflower.

Si dose (kg ha^{-1})	AMF inoculation	Leaf greenness (SPAD value)				Leaf relative water content (%)			
		0.54 dS m^{-1}	4 dS m^{-1}	8 dS m^{-1}	12 dS m^{-1}	0.54 dS m^{-1}	4 dS m^{-1}	8 dS m^{-1}	12 dS m^{-1}
Si_0	–AMF	37.2±1.7bA	37.2±0.8bA	36.4±0.4aA	34.1± 0.8aA	74.1±1.9bA	65.5±4.1cAB	65.1±3.5bAB	54.3±1.9cB
	+AMF	37.9± 1.8bA	37.8±1.9abA	36.3±2.3aA	35.8±0.3aA	78.0±0.2bA	78.5±0.8dAB	70.8±0.4bAB	58.4±8.06bcB
Si_{150}	–AMF	38.5± 2.6bA	37.5±2.1abA	37.4±1.3aA	37.1±0.6aA	73.5±4.7bAB	75.4±9.5bA	75.3±9.2abAB	61.4±8.4bcB
	+AMF	40.1±0.6bA	40.5±0.9abA	38.0±0.8aA	37.4±1.9aA	79.1± 2.4bA	78.6±6.9abAB	75.1±0.6abAB	65.2±0.04bcB
Si_{300}	–AMF	40.5±0.4bA	39.9±0.9abA	37.2±0.9aA	37.1±0.4aA	81.2±1.6bA	76.7± 0.7abAB	74.6±1.3abAB	65.6±3.7bB
	+AMF	42.5±0.4abB	40.3±0.9abB	38.3±1.1aB	37.5±1.6aA	87.3±1.3abA	78.7± 2.9abAB	76.3±0.01abB	70.4± 4.4abB
Si_{450}	–AMF	40.6±0.6bA	38.9± 0.6abA	38.9±0.4aA	37.5± 1.4aA	86.9±0.4abA	79.4±0.5abAB	78.5± 2.7abAB	72.3±1.9abB
	+AMF	47.9±1.2aA	42.6± 0.4aAB	40.6± 0.6aB	39.9±2.3aB	92.3±2.8aA	85.3±0.6aAB	80.2± 1.05aB	79.1±1.0aB

Means followed by same small case letters are statistically similar within a column and means followed by same capital letter are statistically similar within a row based on Tukey's honest significant difference test at $p < 0.05$; data are means of three replications ± standard errors.

Table 8. Effect of silicon dose, salinity levels and AMF inoculation on electrolyte leakage and cell membrane stability index of sunflower.

Si dose (kg ha^{-1})	AMF inoculation	Electrolyte leakage (%)				Cell membrane stability index (%)			
		0.54 dS m^{-1}	4 dS m^{-1}	8 dS m^{-1}	12 dS m^{-1}	0.54 dS m^{-1}	4 dS m^{-1}	8 dS m^{-1}	12 dS m^{-1}
Si_0	–AMF	59.4±0.2aB	60.5±0.2bcAB	59.5±0.1cB	62.6±0.3cA	37.4±2.05cA	34.6±5.6bAB	28.4±1.2bB	12.5±0.6cC
	+AMF	55.8±0.2bC	58.3±0.4cBC	60.4±0.09cB	70.4±0.6bcA	39.1±0.2bcA	37.5±3.5bAB	30.5± 0.8bB	15.7±0.6bcC
Si_{150}	–AMF	59.2±0.2abC	62.0± 0.3bcC	67.7±0.06bB	72.9±0.2abA	39.8±2.05bcA	38.9±2.04abAB	32.3±0.9bB	15.1±1.09bcC
	+AMF	51.9±0.2cC	58.3±0.2cBC	61.8±0.08cB	73.3± 0.7abA	42.0±3.6bcA	38.3±3.4bbAB	28.1±3.4bB	16.7±0.3bcC
Si_{300}	–AMF	50.4±0.5cdC	62.4±0.4bB	70.9±0.1aAB	75.4± 0.5aA	42.5±0.8abA	41.5±0.09abAB	28.2±2.9bB	19.6±1.9bC
	+AMF	46.9± 0.2dC	55.9±0.1cdB	68.5±0.1abAB	70.5±0.8bcA	44.8± 0.6abA	42.1±3.3bbAB	32.4±2.5bB	28.4±3.3abAB
Si_{450}	–AMF	44.6±0.2dB	68.0± 0.3aAB	70.9±0.3aAB	71.9±0.7bA	42.4±2.7bA	39.9±0.2abAB	36.0±0.1abB	19.1±1.1bcC
	+AMF	44.1±0.3dC	50.1±0.2dB	59.7± 0.1cAB	61.1±0.4cA	48.9±3.8aA	42.8±2.3aB	40.2±0.02aBC	31.9±3.7aC

Means followed by same small case letters are statistically similar within a column and means followed by same capital letter are statistically similar within a row based on Tukey's honest significant difference test at $p < 0.05$; data are means of three replications ± standard errors.

Table 9. Effect of silicon dose, salinity levels and AMF inoculation on proline concentration of sunflower.

Si dose (kg ha^{-1})	AMF inoculation	Proline concentration (μg g^{-1} fresh weight)			
		0.54 dS m^{-1}	4 dS m^{-1}	8 dS m^{-1}	12 dS m^{-1}
Si_0	–AMF	450.0±11.5bA	565.0±20.2aA	570.0±5.7bA	595.0±2.8bA
	+AMF	525.0±14.4abB	570.0±25.8aAB	585.0±2.8bAB	725.0±2.8abA
Si_{150}	–AMF	615.0±60.6abA	625.0±20.2aA	648.3±43.3abA	690.0±14.3abA
	+AMF	675.0±129.9aA	695.0±43.3aA	815.0±42.6aA	825.0±5.7abA
Si_{300}	–AMF	565.0±11.5abB	575.0±5.7aAB	650.0±69.6abAB	750.0±20.2abA
	+AMF	575.0±31.7abB	590.0±8.6aAB	600.0±40.4bAB	761.6±34.6abA
Si_{450}	–AMF	580.0±17.3abA	610.0±17.3aA	640.0±40.4abA	760.0±28.8abA
	+AMF	650.0±20.2abB	685.0±37.5aAB	750.0±23.09abAB	865.0±20.2aA

Means followed by same small case letters are statistically similar within a column and means followed by same capital letter are statistically similar within a row based on Tukey's honest significant difference test at $p < 0.05$; data are means of three replications ± standard errors.

4.3 Yield attributes and seed yield

The interactive effect among silicon doses, salinity levels and AMF inoculation was significant for yield attributes and seed yield except for head weight and seed number (Table 2). Head diameter was influenced by the AMF inoculation and it was increased by 20% at 12 dS m^{-1} with Si_{450} dose compare to control. Individual effect of silicon doses, salinity and AMF inoculation was significant for head weight and seed number of plants (Table 10). Silicon dose Si_{450} increased the head weight and seed number $plant^{-1}$ by 12% and 17%, respectively compare to controlled plant. AMF inoculated plant increased the head weight and seed number $plant^{-1}$ by 23% and 41% respectively compare to non-inoculated plant. Head weight and seed number $plant^{-1}$ was declined with increasing salinity level where maximum head was observed at controlled level (0.54 dSm^{-1}) and minimum at 12 dSm^{-1}. Seed number was declined by 27% at 12 dS m^{-1} compared to control. Plants inoculated with AMF produced higher seed yield than control plants across silicon doses. At 12 dS m^{-1} salinity level, plants treated with Si_{450} had promoted seed yield by 56% and 50% in AMF inoculated and non-inoculated plant than Si_0 (Table 11).

Table 10. Individual effect of silicon dose, salinity levels and AMF inoculation on head weight and seed number head^{-1} of sunflower.

Factor	Head weight (g plant^{-1})	Seed number head^{-1}
Si dose (kg ha^{-1})		
Si_0	25.5±1.4b	453.6±14.7b
Si_{150}	26.2±0.7ab	460.3±7.5b
Si_{300}	27.8±2.1ab	506.5±8.2ab
Si_{450}	28.6±0.8a	531.3±11.7a
Salinity levels		
0.54 dS m^{-1}	29.9±1.9a	607.08±14.2a
4 dS m^{-1}	27.8±0.8ab	561.1±5.4b
8 dS m^{-1}	25.3±2.1b	491.5±3.5c
12 dS m^{-1}	21.3±1.1c	472±9.5c
AMF inoculation		
–AMF	23.8±1.3b	363.2±4.5b
+AMF	30.3±1.8a	512.6±6.2a

Means followed by same small case letters are statistically similar within a column and means followed by same capital letter are statistically similar within a row based on Tukey's honest significant difference test at $p < 0.05$; data are means of three replications ± standard errors.

Table 11. Effect of silicon dose, salinity levels and AMF inoculation on head diameter and seed yield of sunflower.

Si dose (kg ha^{-1})	AMF inoculation	Head diameter (cm)				Seed yield (g $head^{-1}$)			
		0.54 dS m^{-1}	4 dS m^{-1}	8 dS m^{-1}	12 dS m^{-1}	0.54 dS m^{-1}	4 dS m^{-1}	8 dS m^{-1}	12 dS m^{-1}
Si_0	–AMF	11±1.4cA	11.5±0.7bA	10.6±0.4bA	10.0±0.5bA	16.7±1.9bA	16.3±3.3bAB	16.5± 4.2aAB	11.6±3.2bB
	+AMF	12.5±0.3abA	11.8±0.3bA	10.9±0.3bB	10.8±0.6bB	18.1±0.4bA	16.4±0.8bAB	17.9±0.8aAB	12.5±1.1bB
Si_{150}	–AMF	11.5±0.3bcA	11.0±0.5bAB	11.4±0.4abAB	10.1±0.4bB	17.2± 2.8bA	16.5±1.3bA	15.0±3.4aA	12.8±1.9bA
	+AMF	12.3±0.3bcA	12.1±0.6bA	12.3±0.7abA	11.0±0.5bA	17.6±0.2bA	16.2± 0.9bA	15.5±0.8aA	14.1±0.9abA
Si_{300}	–AMF	12.6±0.3bA	12.1±0.2bA	10.6±0.9bA	11.6±0.3abA	17.4±0.9bA	17.8±2.3abA	16.4±2.3aA	15.3± 2.9abA
	+AMF	13.5±0.3abA	12.0± 0.6bA	10.9± 0.7bB	11.5±0.7abB	20.3±0.8bA	19.2± 0.3abA	20.1± 1.8aA	17.5±0.6aA
Si_{450}	–AMF	12.6±0.3bA	12.0±0.5bAB	12.3± 0.6abAB	10.5±0.3bB	19.4±1.4bA	20.2± 3.1abA	20.1± 1.3aA	18.1±0.7abA
	+AMF	14.3±0.3aA	13.8±0.2aAB	13.5±0.7aAB	12.7±0.7aB	25.9±0.6aA	22.1±0.7aAB	20.9±0.8aB	18.8±0.7aB

Means followed by same small case letters are statistically similar within a column and means followed by same capital letter are statistically similar within a row based on Tukey's honest significant difference test at $p < 0.05$; data are means of three replications ± standard errors.

Discussion

Soil salinity is the major limiting environmental variables that affects various physiological and biochemical process for deterring optimum crop growth and yield. Soluble salts interfere almost all of the physiological and metabolic process in plants, such as carbon fixation, nitrogen metabolism, nutrient uptake, stomatal conductance, transpiration and partitioning of assimilates (Lucini et al., 2015; Yin et al., 2010). Moreover, chlorophyll degradation as well as the structural and functional breakdown of cell membrane are attributed by salinity which was visualized in the present study in which leaf greenness was significantly declined with increasing salinity (Table 7). Salt strains stimulate the production of reactive oxygen species (ROS), which damage the functional stability of proteins, nucleic acids, and cell membranes (Lubitz et al., 2019). ROS are metabolic byproducts generated through partial oxygen reduction that cause oxidative damage in cellular particle under stress condition and as signaling molecules under normal environment (Huang et al. 2017). It is well known that application of different doses of beneficial element such as Si promotes morphological and physiological traits to combat salt stress in oil crop especially sunflower. In the current study, it was observed that root growth, physiological traits and yield of sunflower were drastically reduced under varying level of salt stress but the application of different doses of Si minimized the harmful effect of salt stress on sunflower.

Results in the current study implying that leaf greenness and LRWC were drastically reduced by increasing salinity levels irrespective of Si dose but AMF inoculation significantly augmented the parameters in compared with control (Table 7). Si caused high accumulation of proline, soluble sugar and glycine betaine in plants that resulted in higher osmotic adjustment, ultimately improved LRWC to maintain leaf pigments such as chlorophyll, carotenoids biosynthesis that ensure the integrity of photosynthetic apparatus. Salt stress also induce moisture stress in soil that causes chlorophyll degradation might be due to promoting the enzyme activity named chlorophyllase. Si fertilized plants exhibited higher leaf greenness might be due to the significant inhibition of this enzyme activity. It has been observed that silicon protects chlorophyll molecule by organizing pigment system stability and thylakoid membranes towards higher net photosynthetic rate (Feng et al. 2010). Moreover, exogenous application of Si significantly enhanced stomatal conductance (g_s) in sunflower by increasing stomatal number and size which facilitated gaseous exchange for efficient photosynthetic activity under salt stress towards higher root and shoot (Hohmann-Marriott and Blankenship 2012). Root and shoot biomass are indicators of plant tolerance against salt stress (Badem and

Söylemez, 2022). In this experiment, it was found that plant height, shoot and root biomass of silicon treated plants were significantly enhanced over control (Table 3) which might be attributed by rapid cell division and elongation in apical meristem that promote root length and leaf length ultimately surface area increased. This higher leaf surface area might fix more carbon through photosynthesis towards higher shoot biomass (Das et al., 2021).

Osmotic regulation is a pivotal metabolic adaptation for most of the crop plants to minimize the detrimental effects of salt stress by reducing cellular osmotic potential through synthesizing diversified organic and inorganic osmolytes in cytoplasm (Begum et al., 2020). The present study showed a higher concentration of leaf proline that was recorded in Si_{450} treated plants with AMF inoculation over control even at 12 dS m^{-1} salinity level (Table 8). These results depicting that proline content gradually enhanced by increasing salt stress levels and also considerable influenced by Si fertilization (Table 8). Proline is a compatible organic osmolyte that acts as a free radical scavenger for protecting biomolecules against oxidative stress (Kaur and Asthir, 2015). Biosynthesis of various osmolytes, such as proline, soluble sugar and glycine betaine are driver for osmotic adjustment that nullify the lethal effect of oxidative damage through quenching ROS, maintaining membrane integrity, and stabilizing enzymes (Kumar et al., 2017; Abdelaziz et al., 2017). Proline accumulation has been connected to stress tolerance, and stress-tolerant plants possess higher levels of proline content than stress-sensitive ones (Ahmed et al., 2023). Proline is a stress marker and compatible osmolyte that exponentially pile up in plants under stressful condition to maintain internal water balance in plant tissue. Higher seed yield in sunflower with Si fertilization could be attributed to more chlorophyll biosynthesis, improved stomata regulation, greater enzyme activity, and enhanced biomass formation resulting in more carbohydrate accumulation and translocation for seed formation towards seed yield (Islam & Muttaleb 2016; Hasanuzzaman et al., 2018).

CHAPTER V

SUMMARY AND CONCLUSION

The results of the present investigation revealed that the morpho-physiological characteristics and yield of sunflower exposed to salt stress manifested downregulation but synergistic interaction between Si dose and AMF minimized the harmful effects of salt stress on sunflower. Plant height of sunflower inoculated with AMF was increased by 16% at Si_{300} compare to non-inoculated plant. Shoot diameter also increased by 45% in the interaction of AMF and Si_{450} in comparison with controlled plant. Similarly shoot and root dry weight was increased by a surprising amount such as 49% and 38% respectively at Si_{450} compared to control in the salinity level 12 dSm^{-1}. Application of Si to plants resulted in a notable improvement in leaf relative water content, greenness, and membrane stability index by 45%, 17% and 60% respectively, while also reducing electrolyte leakage to 15% at a water salinity of 12 $dS\ m^{-1}$. These enhancements were significantly higher compared to non-treated plants. The metabolic fingerprint linked with salinity stress involved accumulation of stress-relieving compounds, mainly proline which was increased by 45% through exogeneous application of Si and AMF. Moreover, plants inoculated with AMF produced higher seed yield than control plants across silicon doses and it has been recorded those plants treated with Si_{450} had promoted seed yield by 56% and 50% in AMF inoculated and non-inoculated plant than Si_0 at 12 $dS\ m^{-1}$ salinity level. So, integrated application of Si coupled with AMF along with standard fertilizer dose protected salt stressed plants by improving and upregulation of physiological traits, seed yield and ion homeostasis in sunflower.

RECOMENDATIONS

- Application of MSA at 450 kg ha^{-1} (90 kg ha^{-1} soluble Si) along with the recommended field application dose of fertilizer is advocated for maximizing seed yield of sunflower under salt stress.
- Introduction of AMF inoculation as a biofertilizer in sunflower should be highly encouraged to farmers practice to restore soil health, fertility and productivity under salt stress.

REFERENCES

Adhikary, D., Das, D., Ali, M.Y., Ullah, H., Datta, A. (2022). Growth, grain yield, and water productivity of traditional rice landraces from coastal Bangladesh, as affected by salt stress. J Crop Improv., doi: 10.1080/15427528.2022.2048765

Ahmed, S., Umar, I., & Sardar, R. (2023). Glutathione Alleviates Chromium Stress and Promotes Growth of Pisum sativum L. by Improving Nutrient Orchestration, Proline, and Physiochemical Attributes. Journal of Soil Science and Plant Nutrition, 23(3), 3780-3796.

Abdelaziz, M. N., Xuan, T. D., Mekawy, A. M. M., Wang, H., & Khanh, T. D. (2017). Relationship of salinity tolerance to Na+ exclusion, proline accumulation, and antioxidant enzyme activity in rice seedlings. Agriculture, 8(11), 166.

Agubosi, O. C. P., WIKA, B. K., & Alagbe, J. O. (2022). Effect of dietary inclusion of Sunflower (Helianthus annus) oil on the growth performance of broiler finisher chickens. Texas Journal of Multidisciplinary Studies, 8, 1-8.

Ahmed, M., Ullah, H., Attia, A. Tisarum, R., Chaum, S, Datta, A., (2011). Interactive effects of Ascophyllum nodosum seaweed extract and silicon on growth, fruit yield and quality, and water productivity of tomato under water stress. Silicon 15: 2263–2278.

Alam, A., Hariyanto, B., Ullah, H., Salin K.R. & Datta, A. (2021). Effects of silicon on growth, yield and fruit quality of cantaloupe under drought stress. Silicon, 13, 3153–3162.

Al-Amri, S. M. (2019). Mitigation of salinity stress of pepper (*Capsicum annuum* L.) by arbuscular mycorrhizal fungus, *Glomus constrictum*. Applied Ecology and Environmental Research, 17(4).

Bates, L.S., Waldren, R.P., Teare, I.D. (1973). Rapid determination of free proline for water-stress studies. Plant and Soil, 39, 205–207.

Begum, N., Qin, C., Ahanger, M. A., Raza, S., Khan, M. I., Ashraf, M. & Zhang, L. (2020). Role of arbuscular mycorrhizal fungi in plant growth regulation: implications in abiotic stress tolerance. Frontiers in Plant Science, 10, 1068.

Bishnoi, A., Jangir, P., Shekhawat, P. K., Ram, H., & Soni, P. (2023). Silicon supplementation as a promising approach to induce thermotolerance in plants: current understanding and future perspectives. Journal of Soil Science and Plant Nutrition, 23(1), 34-55.

Camejo, D., Rodriguez, P., Morales, M.A., Amico, J.M.D., Torrecillas, A. Alarcon, J.J. (2005). High temperature effects on photosynthetic activities of two tomato cultivars with different heat susceptibility. Plant Physiology, 162, 281-289.

Chen, S., Zhao, H., Zou, C., Li, Y., Chen, Y., Wang, Z., Jiang, Y., Liu, A., Zhao, P., Wang, M. et al. (2017). Combined inoculation with multiple arbuscular mycorrhizal fungi improves growth, nutrient uptake and photosynthesis in cucumber seedlings. Front. Microbiol. 8, 2516.

Das, D., Basar, N.U., Ullah, H., Attia, A., Salin, K.R. Datta, A. (2021b). Growth, yield and water productivity of rice as influenced by seed priming under alternate wetting and drying irrigation. Archives of Agronomy and Soil Science, 68: 1515-1529.

Das, D., Basar, N.U., Ullah, H., Salin, K.R. Datta, A. (2021a). Interactive effect of silicon and mycorrhizal inoculation on growth, yield and water productivity of rice under water-deficit stress. Journal Plant Nutri, 44, 2756–2769.

Das, D., Ullah, H., Tisarum, R., Cha-um, S., Datta, A. (2021c). Morpho-physiological responses of tropical rice to potassium and silicon fertilization under water-deficit stress. Journal of Soil Sci, and Plant Nutr, 23, 220–237.

De Melo Peixoto, M., Flores, R. A., do Couto, C. A., Pacheco, H. D. N., de Mello Prado, R., Souza-Junior, J. P., ... & Graciano-Ribeiro, D. (2022). Silicon application increases biomass yield in sunflower by improving the photosynthesizing leaf area. Silicon, 14(1), 275-280.

De Souza, T. A. F., & Santos, D. (2018). Effects of using different host plants and long-term fertilization systems on population sizes of infective arbuscular mycorrhizal fungi. Symbiosis, 76, 139-149.

Diagne, N., Ngom, M., Djighaly, P. I., Fall, D., Hocher, V., and Svistoonoff, S. (2020). Roles of arbuscular mycorrhizal fungi on plant growth and performance: Importance in biotic and abiotic stressed regulation. Diversity, 12(10), 370.

Ebbisa, A. (2022). Arbuscular mycorrhizal fungi (AMF) in optimizing nutrient bioavailability and reducing agrochemicals for maintaining sustainable agroecosystems. In Arbuscular mycorrhizal fungi in agriculture-new insights. Intech Open.

Egamberdieva, D., Wirth, S., Bellingrath-Kimura, S. D., Mishra, J., and Arora, N. K. (2019). Salt-tolerant plant growth promoting rhizobacteria for enhancing crop productivity of saline soils. Frontiers in Microbiology, 10, 2791.

Etesami, H., Li, Z., Maathuis, F. J., & Cooke, J. (2022). The combined use of silicon and arbuscular mycorrhizas to mitigate salinity and drought stress in rice. Environmental and Experimental Botany, 201, 104955.

Evelin, H., Giri, B., Kapoor, R. (2013). Ultrastructural evidence for AMF mediated salt stress mitigation in Trigonella foenum-graecum. Mycorrhiza 23, 71–86.

Feng, J., Shi, Q., Wang, X., Wei, M., Yang, F., & Xu, H. (2010). Silicon supplementation ameliorated the inhibition of photosynthesis and nitrate metabolism by cadmium (Cd) toxicity in *Cucumis sativus* L. Scientia Horticulturae, 123(4), 521-530.

Guntzer, F., Keller, C., Meunier, J.-D., (2012). Benefits of plant silicon for crops: areview. Agron. Sustain. Dev. 32, 201–213.

Haghighi, T. M., Saharkhiz, M. J., & Zarei, M. (2022). Mycorrhiza and silicon Influence Glycyrrhiza glabra L. under water-deficit; antioxidant activity, medicinal metabolites and mineralogical analysis.

Hasanuzzaman, M., Bhuyan, M. H. M. B., Nahar, K. Hossain, M. S. Mahmud, J. Al, Hossen, Masud, M. S., Moumita, A. A. C. & Fujita, M. (2018). Potassium: A vital regulator of plant responses and tolerance to abiotic stresses. Agronomy, 8(3), 31.

Hayat, S., Hasan, S.A., Fariduddin, Q. Ahmad, A. (2008). Growth of tomato (Lycopersicon esculentum) in response to salicylic acid under water stress. Plant Interaction, 3, 297–304.

Hegazi, A.M., El-Shraiy, A.M., Ghoname, A.A (2015). Alleviation of salt stress adverse effect and enhancing phenolic anti-oxidant content of eggplant by seaweed extract. Gesunde Pflanzen 67(1), 21–31

Hohmann-Marriott, M. F., & Blankenship, R. E. (2012). The photosynthetic world. Photosynthesis: Plastid Biology, Energy Conversion and Carbon Assimilation, 3-32.

Huang, H., Ullah, F., Zhou, D. X., Yi, M., & Zhao, Y. (2017). Mechanisms of ROS regulation of plant development and stress responses. Frontiers in Plant Science, 10, 800.

Islam. A. & Muttaleb, A. (2016.) Effect of potassium fertilization on yield and potassium nutrition of Boro rice in a wetland ecosystem of Bangladesh. Archives of Agronomy and Soil Science, 62, 1530-1540.

Jouyban, Z. (2016). The effects of salt stress on plant growth. Technical Journal of Engineering and Applied Sciences, 2(1), 7-10.

Kaur, G., & Asthir, B. J. B. P. (2015). Proline: a key player in plant abiotic stress tolerance. Biologia Plantarum, 59, 609-619.

Krishi Projukti Hatboi (Handbook of Agrotechnology).2nd Edition, Bangladesh Agricultural Research Institute, Gazipur, Bangladesh.

Kumar, A., Verma H, Singh, V.K., Singh P.P., Singh, S.K., Ansari. W.A, Yadav, A., Singh. P.K & Pandey, K.D. (2017). Role of Pseudomonas sp. in Sustainable Agriculture and Disease Management. In: Agriculturally Important Microbes for Sustainable Agricultural. Springer, Singapore, pp. 195-215.

Läuchli, A., and Grattan, S. R. (2017). Plant growth and development under salinity stress. Advances in molecular breeding toward drought and salt tolerant crops, 1-32.

Lubitz, W., Chrysina, M., & Cox, N. (2019). Water oxidation in photosystem II. Photosynthesis Research, 142(1), 105-125.

Lucini, L., Pellizzoni, M., Pellegrino, R., Molinari, G. P., & Colla, G. (2015). Phytochemical constituents and in vitro radical scavenging activity of different Aloe species. Food Chemistry, 170, 501-507.

Mohammadi, M, Modarres-Sanavy S.A.M, Pirdashti H, Zand, B., Tahmasebi-Sarvestan, Z. (2019). Arbuscular mycorrhizae alleviate water deficit stress and improve antioxidant response, more than nitrogen fixing bacteria or chemical fertilizer in the evening primrose. Rhizosphere, 9, 76–89.

Nguyen, H. D. (2018). Impact of arbuscular mycorrhizal fungi on plant tolerance to some abiotic stresses and phytopathogens (Doctoral dissertation, Szent István Egyetem (2000-2020)).

Pal, S. C., Hossain, M. B., Mallick, D., Bushra, F., Abdullah, S. R., Dash, P. K., & Das, D. (2024). Combined use of seaweed extract and arbuscular mycorrhizal fungi for alleviating salt stress in bell pepper (*Capsicum annuum* L.). Scientia Horticulturae, 325, 112597.

Parça, F., & Ünay, A. (2021). A Preliminary Study on the Response of Confectionery Sunflower (*Helianthus annuus* L.) to Arbuscular Mycorrhizal Fungi (AMF). ANADOLU Ege Tarımsal Araştırma Enstitüsü Dergisi, 31(1), 1-8.

Rabbi, S.M.F., Tighe, M.K., Warren, C.R., Zhou, Y., Denton, M.D., Barbour, M.M., Young, I.M., (2021). High water availability in drought tolerant crops is driven by root engineering of the soil micro-habitat. Geoderma 383, 114738.

Raven, J.A., 2023. Cycling silicon-the role of accumulation in plants. New Phytologist, 158(3): 419-430.

Rizwan, M., (2016). Silicon-mediated heavy-metal tolerance in durum wheat: Evidences of combined effects at the plant and soil levels. These pour obtenir le grade de Docteur d'Aix-Marseille universite, Faculté des Sciences et Techniques, Discipline: Géosciences de l'Environnement, France. (www.theses.fr/2012AIXM4335/abes) (Date of Access: 20.03.2017).

Roy, S. D., D. Das, and M. E. Kabir. (2017). Seed germination and seedling growth of sunflower (*Helianthus annuus* L) under salt stressed conditions. Khulna University Studies 14, 39–47

Savić, J., Marjanović-Jeromela, A., (2013). Effect of silicon on sunflower growth and nutrient accumulation under low boron supply. Helia, 36: 61-68.

Savvas, D., & Ntatsi, G. (2015). Biostimulant activity of silicon in horticulture. Scientia Horticulturae, 196, 66-81.

Scavo, A., Abbate, C., Mauromicale, G. (2019). Plant allelochemicals: agronomic, nutritional and ecological relevance in the soil system. Plant and Soil, 442, 23–48.

Shah, T., Khan, H., Ali, A., Khan, Z., Alsahli, A. A., Dewil, R., & Ahmad, P. (2024). Silicon and arbuscular mycorrhizal fungi alleviate chromium toxicity in *Brassica rapa* by regulating Cr uptake, antioxidant defense expression, the glyoxalase system, and secondary metabolites. Plant Physiology and Biochemistry, 206, 108286.