



## Enhancing salinity tolerance in ornamental sunflower (*Helianthus annuus* L.) through mycorrhizal symbiosis

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### ABSTRACT

Salt accumulation in the agricultural soils are inevitable when irrigation is performed by saline water. This phenomenon is a global challenge especially in the drylands. Vesicular-arbuscularmycorrhizal fungi (VAM) as biofertilizers that form symbiotic associations with plant roots are natural and suitable amendments for somehow overcoming this problem. The current study aimed to investigate the potential of mycorrhizal fungi (VAM) to enhance the growth of ornamental sunflowers (*Helianthus annuus* L.) cultivated under salinity stress conditions in a pot culture system. The overarching objective was to identify sustainable strategies that mitigate environmental challenges, such as soil salinity, and decrease reliance on chemical fertilizers. A 3×4 factorial experiment was conducted using a completely randomized design with three replications. The experimental factors included three salinity levels (a control without NaCl, 50 and 100 mM NaCl) and four VAM species (uninoculated plant as the control, and plants inoculated with *Rhizophagus irregularis*, *Funneliformis mosseae*, and *Glomus howei* species). Plant growth parameters, including flower diameter, fresh and dry weights of flowers, shoot and root biomasses, proline level, chlorophyll content, relative water content, malondialdehyde content, ion leakage, and root colonization percentage were measured after plant harvest. The results showed that salinity stress, VAM inoculation, and their interaction significantly affected most plant growth indices. Higher salinity levels led to a decrease root and flower weights, flower diameter, and chlorophyll content, while proline contents and ion leakage were positively affected by high soil salt content. At high salinity levels (100 mM), inoculation with *F. mosseae* had the greatest effect on improving plant growth. The fresh weight of flowers (16.4 g), shoots (27.6 g pot<sup>-1</sup>), roots (6.57 g pot<sup>-1</sup>), and total chlorophyll (36.0 mg g<sup>-1</sup> of fresh weight) were higher in the treatment inoculated with *F. mosseae* and without salinity. The highest proline content (5.6 μmol g<sup>-1</sup> fresh weight) and ion leakage (76.4%) values were observed in the non-VAM and 100 mM NaCl treatments. The symbiotic relationship between arbuscular mycorrhizal fungi and plants constitutes a mutually advantageous biological interaction, substantially enhanced plant growth and productivity. The findings suggest that VAM inoculation can improve plant growth and ornamental value under salinity stress. Compared to other species, *Funneliformis mosseae* showed greater capacity to enhance the quantity and quality of ornamental sunflower production.

**Keywords:** Mycorrhizal functions; Oxidative stress; Plant tolerance; Sunflower; Proline; Symbiotic relationship.

### 1. Introduction

Salt stress is an important environmental challenge that adversely influences productivity and quality, and thereby plant growth and production, especially in the arid and semi-arid regions (Machado and Serralheiro, 2017). Salt stress has affected approximately 33% of irrigated agricultural fields and 20% of the total farmed lands worldwide (Negacz *et al.*, 2022). High salinity stress impairs the growth and development of plant through specific ion toxicity, nutrient imbalance and reduced osmotic potential and water uptake by plants (Zhao *et al.*, 2021). Salt stress is known to reduce photosynthesis rate and trigger unfavorable oxidative damage to versatile biomolecules such as cell's lipids, proteins and DNA (Nakamichi, 2016). The major effect of Salt stress in the plant is the enhanced generation and accumulation of reactive oxygen species, which are extremely toxic to the plant and induce oxidative stress/damage in plants

(Puttamanayaka *et al.*, 2024). Accumulation of malondialdehyde (MDA), a product of lipid peroxidation, in plant tissues is a potential indicator of oxidative damage under metal stress. It has been shown that salinity over a critical level increases the MDA content in plants (Shuyskaya *et al.*, 2023). Nevertheless, plants under salinity-induced stress can produce enzymatic or non-enzymatic antioxidants to detoxify and eliminate the reactive oxygen species and to avoid oxidative stress (Hasanuzzaman *et al.*, 2021). The biosynthesis of proline has frequently been advocated to be used by plants to scavenge and detoxify the reactive oxygen species (ROS) formed during salt stress (Renzetti *et al.*, 2025). Proline is considered as an important detoxifying non-enzymatic scavenger for plants to survive in stress environments (Raza *et al.*, 2023).

VAM are promising soil microorganisms that establish strong symbiosis with most terrestrial plants (Valenzuela-

Aragon *et al.*, 2025). They can enhance plant growth, nutrient uptake, and stress tolerance (Larrainzar and Wienkoop, 2017; Khaliq *et al.*, 2022; Fall *et al.*, 2022). Previous researches have shown that VAM inoculation can improve the growth, nutrient acquisition and ornamental characters of different plant species (Chen *et al.*, 2023), including *Limonium sinuatum* (Sheikh-Assadi *et al.*, 2023). VAM can also help plants resist salinity stress by improving nutrient uptake and inducing physiological and biochemical changes in plant metabolites (Bencherif *et al.*, 2015; Kakabouki *et al.*, 2023; Boorboori and Lackóová, 2025). Plant inoculation with VAM has been widely used to improve plant tolerance to salt stress through either an increase in nutrient uptake or an increase in the levels of plant's enzymatic and non-enzymatic antioxidants or promoting plant growth (Hashem *et al.*, 2018; Dastogeer *et al.*, 2020). The use of VAM in combination with several plant species has shown the beneficial effects of these fungi on plant performance under saline conditions. Among others, VAM inoculation increased phenolic compounds in the roots of Rose Balsam (*Impatiens balsamina*) grown under salinity stress (Banuelos *et al.*, 2014). Inoculation of Bearded iris (*Iris germanica*) with VAM reduced the negative effects of salinity stress and increased plant yield (Ziaei *et al.*, 2019). Similarly, VAM inoculation improved salinity tolerance of *Teucrium chamaedrys*, with a greater impact in the presence of *Funneliformis mosseae* (Dianti *et al.*, 2019). In *Lolium perenne*, VAM inoculation enhanced photosynthetic capacity, growth, and salinity stress tolerance (Wei *et al.*, 2023). It also positively affected malondialdehyde, proline, soluble sugar, and soluble protein content (Li *et al.*, 2023a). However, the interaction between salinity stress and VAM inoculation seems complicated, and depends largely on plant species, VAM species, and the type and level of salinity (Porcel *et al.*, 2012). Different VAM species may have variable capacity and capability to mitigate the effects of salinity stress by altering leaf water content (Zou and Wu, 2011). In addition to their role in nutrient acquisition and stress alleviation, VAM are essential for improving flower production (White and Hammond, 2008). The availability of soil nutrients positively affects flower traits such as size, nectar volume, and pollen count (Vaudo *et al.*, 2022). Furthermore, direct evidence for the role of VAM to minimize salinity stress by mitigating oxidative stress in ornamental sunflowers remains unavailable.

Selecting appropriate ornamental plants is crucial to the increasing popularity of urban green spaces. The ornamental sector must be adequately prepared for high-standard production, and enhancing growth conditions in greenhouses will facilitate the production of quality and sustainable cut flowers (Paiva *et al.*, 2024). The global demand for cut flowers is driving significant growth in this sector (Ahmed *et al.*, 2018). Ornamental sunflowers (*H. annuus* L.) are a valuable ornamental plant native to western North America. They are widely used in

landscaping, as cut flowers, and as potted plants (Ferrante and Ferrini, 2023). The quality of cut flowers, including ornamental sunflowers, is a crucial factor in their success. Traditional methods of increasing yield and quality often involve the use of fertilizers and other chemicals (Krasilnikov *et al.*, 2022). However, excessive use of these chemicals can be harmful to the environment and soil health (Wahid *et al.*, 2020). The use of sustainable agricultural practices, including biofertilizers like VAM, is essential for minimizing environmental damage and reducing reliance on chemical fertilizers. In recent decades, organic agriculture has gained significant popularity in many developed countries (Sheikh-Assadi *et al.*, 2023; Nejati-Sini *et al.*, 2024).

Research limitations can include: 1. the experiment is conducted under controlled greenhouse conditions, which do not fully replicate the complex and variable environmental dynamics of field settings, 2. this study examines only a limited consortium of VAM at fixed inoculation densities. Different VAM isolates vary considerably in their salt tolerance, colonization efficiency, and functional compatibility with host plants.

Therefore, this study aimed to investigate the effects of VAM inoculation on the vegetative and reproductive growth of ornamental sunflowers under salt stress conditions. It is hypothesized that inoculating ornamental sunflowers with VAM would mitigate the salinity stress by an increase in proline level, which could be reflected by improvements of photosynthetic pigments, plant biomass and flower quality.

## 2. Experimental Section

### 2.1. Experimental layout, treatments and plant inoculation

This controlled experiment was conducted under greenhouse conditions in the Research Greenhouse of Shahrekord University (longitude: 50.827558, latitude: 32.352346, altitude: 2092 m above sea level) with an average temperature of 25±2 and relative humidity of 60±5 to explore the impact of VAM inoculation on the growth and salinity tolerance of ornamental sunflowers (*H. annuus* L.) grown under saline conditions. The soil used for the experiment had loamy sand texture with an available phosphorus content of 10.8 mg kg<sup>-1</sup>, an electrical conductivity (EC) of 1.05 dS m<sup>-1</sup>, pH of 7.24 and an OM of 1.85 %. A completely randomized (3×4) factorial design with three replicates (n=3) was implemented in a pot (15 cm diameter, 20 cm height and 1.4 Kg soil ( $p_b=1.31 \text{ g.cm}^{-3}$ )) culture setup. The experiment consisted of three irrigation water salinity levels (control, 50 mM NaCl, and 100 mM NaCl) and inoculation of three VAM species (a control without VAM inoculation, plants inoculated with *Rhizophagus irregularis*, *Funneliformis mosseae*, and *Glomus howei*). The fungal inoculum comprised a diverse array of propagules, including spores and fungal hyphae (about 120 spores/g inoculum), which

were applied at a rate of 10 grams per kilogram of potting substrate.

The pot medium consisted of a sterilized (autoclaved at 121°C, 1.5 atm for 20 minutes) mixture of 15% decomposed litter, 30% sand and 55% soil. The mixture was first passed through a 4 mm sieve for homogeneity. F1-type ornamental sunflower seeds were procured from the Takii Seed brand in Japan. The seeds were sown at a depth of 2–3 cm within the soil. Following sowing, the pots were irrigated with an equal volume of municipal water, with subsequent irrigations applied once the soil surface dried. Additionally, the surface of the pot soil was leveled to ensure uniform water distribution and prevent water accumulation that could lead to flooding conditions.

Salinity stress was introduced via irrigation water approximately 30 days after planting, at the 4-leaf stage. To avoid osmotic shock and prevent plasmolysis, salt stress was imposed incrementally by gradually increasing the NaCl concentration. Specifically, plants were first treated with 50 mM NaCl, followed by 100 mM NaCl. Control plants were irrigated with non-salinized water throughout the experiment. The irrigation regimen was designed such that approximately 10% of the applied salt solution drained from the bottom of each pot; additionally, pots were flushed with non-salinized water once per week. The ornamental sunflower (*H. annuus* L.) requires approximately 10 weeks from planting to anthesis.

The mean annual potential evapotranspiration (Annual PET) at the study location was calculated using the Penman - Monteith equation - based on long-term meteorological records. The Annual PET for the experimental site was determined to be 1255 mm year<sup>-1</sup>. This PET characterization is essential for contextualizing the water demand regime under which the salinity treatments were imposed, as evaporative demand directly influences the concentration of salts in the root zone and the severity of osmotic stress experienced by the plants.

## 2.2. Plant measurements and analysis

In this study, , plant flower diameter, leaf area, fresh and dry weights of flowers, shoots, and roots were measured at the flowering stage (Pandit et al., 2024). Chlorophyll content was determined using the method of Lichtenthaler and Wellburn (1983) by randomly sampling mature leaves and extracting them with acetone. Ion leakage was measured according to the method described by Lutts *et al.* (1995). Relative water content (RWC) was measured using the procedure described in Smart and Bingham (1974), MDA level was measured using the method of Heath and Packer (1968), and leaf proline content was determined using the method of Bates *et al.* (1973). To assess the percentage of root colonization, the fine roots were stained using a 1% aniline blue solution following the method of Phillips and Hayman (1970). Then, the stained organs were observed under a microscope at 40x magnification using the method of Giovanti and Moss

(1980) to determine the percentage of root colonization by the gridline intersection method. The symbiosis was classified into five classes based on the percentage of root colonization: 0-5%, 6- 25%, 26-50%, 51-75%, 76-100%.

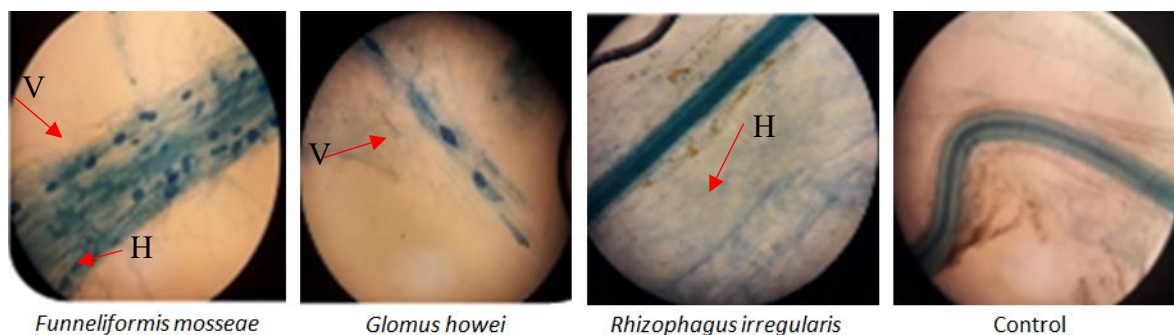
## 2.3. Statistical analysis

Statistical analysis of the data was conducted using analysis of variance (ANOVA) in SPSS version 26. Mean comparisons were performed using Duncan's multiple range test at a significance level of 5% ( $p \leq 0.05$ ). A biplot principal component analysis (PCA) was generated to visualize the correlations between traits and their contributions to the principal components. Individuals (based on discrete colors) and variables were grouped according to their contributions to the main components. R-studio software (RStudio Suite V February 1, 5033) was used for this analysis.

## 3. Results and Discussion

### 3.1. Plant root colonization

The root system of ornamental sunflowers was successfully colonized by VAM in Figure 1 and the root colonization differed among the VAM species in Table 1. Root VAM colonization by *Rhizophagus irregularis*, *Funneliformis mosseae*, and *Glomus howei* was 56.3%, 25.8% and 47.7%, respectively. This suggests that these fungal species are salinity tolerant and able to maintain an efficient symbiosis with root systems of ornamental sunflowers under high salinity stress in the medium. This is consistent with research conducted by Porras-Soriano *et al.* (2009) and Witzel *et al.*, (2025). The symbiosis between inoculated VAM and host plants can facilitate the adaptation of plants to various biotic and abiotic stress conditions, including salinity stress (Tang *et al.*, 2024). The plant root colonization by VAM tended to decrease (10.3%) with increasing salinity level from 31.0% at low salinity level to 28.1% at high salinity level (Table 1). Similarly, previous studies indicated that, arbuscular mycorrhizal fungal (VAM) symbionts can mediate plant stress responses by enhancing salinity tolerance (Dastogeer *et al.*, 2020; Porras-Soriano *et al.*, 2009), and mycorrhizal colonization rate and mycorrhizal dependence of *Glomus mosseae* inoculation were significantly higher than those of *Glomus etunicatum* inoculation (Xu *et al.*, 2024). A higher rate of mycorrhizal colonization reflects a stronger symbiotic relationship between plants and arbuscular mycorrhizal fungi (VAM). Studies indicate that salt stress adversely affects mycorrhizal colonization by inhibiting spore germination and mycelial growth (Zhang *et al.*, 2024). In this experiment, mycorrhizal dependence exhibited a trend of initially increasing and then decreasing with rising salt concentrations, paralleling findings observed in maize (Ahmed *et al.*, 2023). This pattern may be attributed to the plant's ability to tolerate low salt levels, while VAM gradually assume a more prominent role as salt concentration increases up to 50 mmol/L.



**Figure 1.** Establishment of symbiosis between VAM species and roots of *H. annuus* at the four-leaf stage (V: vesicles, H: Hyphae).

**Table 1.** Comparative analysis of the combined effects of mycorrhizal fungi and salinity treatment on the morphological and biochemical characteristics of *H. annuus*.

| Treatments/                                  | <i>Funneliformis mosseae</i> | <i>Rhizophagus irregularis</i> | <i>Glomus howei</i> | Control            | (No salt)          | 50 (mmol NaCl/l)    | 100 (mmol NaCl/l)  |
|----------------------------------------------|------------------------------|--------------------------------|---------------------|--------------------|--------------------|---------------------|--------------------|
| morphological and biochemical parameters     |                              |                                |                     |                    |                    |                     |                    |
| Flower fresh weight (g plant <sup>-1</sup> ) | 14.70 <sup>a</sup>           | 7.63 <sup>b</sup>              | 8.94 <sup>b</sup>   | 4.48 <sup>c</sup>  | 10.91 <sup>a</sup> | 8.68 <sup>b</sup>   | 7.22 <sup>c</sup>  |
| Flower dry weight (g plant <sup>-1</sup> )   | 3.63 <sup>a</sup>            | 2.14 <sup>b</sup>              | 2.32 <sup>b</sup>   | 0.97 <sup>c</sup>  | 2.63 <sup>a</sup>  | 2.30 <sup>ab</sup>  | 1.86 <sup>b</sup>  |
| Shoot fresh weight (g plant <sup>-1</sup> )  | 21.81 <sup>a</sup>           | 11.89 <sup>c</sup>             | 14.52 <sup>b</sup>  | 7.45 <sup>d</sup>  | 16.55 <sup>a</sup> | 13.18 <sup>b</sup>  | 11.28 <sup>c</sup> |
| Shoot dry weight (g plant <sup>-1</sup> )    | 8.04 <sup>a</sup>            | 4.22 <sup>c</sup>              | 5.63 <sup>b</sup>   | 3.38 <sup>c</sup>  | 6.46 <sup>a</sup>  | 5.34 <sup>b</sup>   | 4.16 <sup>c</sup>  |
| Root fresh weight (g plant <sup>-1</sup> )   | 6.57 <sup>a</sup>            | 3.35 <sup>b</sup>              | 2.82 <sup>bc</sup>  | 1.61 <sup>c</sup>  | 4.73 <sup>a</sup>  | 3.63 <sup>b</sup>   | 3.16 <sup>bc</sup> |
| Root dry weight (g plant <sup>-1</sup> )     | 2.55 <sup>a</sup>            | 1.46 <sup>b</sup>              | 1.86 <sup>b</sup>   | 0.67 <sup>c</sup>  | 2.23 <sup>a</sup>  | 1.61 <sup>b</sup>   | 1.07 <sup>bc</sup> |
| Flower diameter (cm)                         | 10.55 <sup>a</sup>           | 8.23 <sup>b</sup>              | 10.11 <sup>a</sup>  | 8.07 <sup>c</sup>  | 10.40 <sup>a</sup> | 9.21 <sup>b</sup>   | 8.47 <sup>c</sup>  |
| Stem diameter (mm)                           | 5.64 <sup>a</sup>            | 3.85 <sup>c</sup>              | 3.96 <sup>bc</sup>  | 4.14 <sup>b</sup>  | 5.11 <sup>a</sup>  | 4.59 <sup>a</sup>   | 3.86 <sup>b</sup>  |
| Leaf area (cm <sup>2</sup> )                 | 10.52 <sup>a</sup>           | 6.69 <sup>c</sup>              | 8.83 <sup>b</sup>   | 5.64 <sup>d</sup>  | 8.95 <sup>a</sup>  | 7.88 <sup>b</sup>   | 6.90 <sup>c</sup>  |
| flowering stem length (cm)                   | 5.61 <sup>a</sup>            | 46.92 <sup>b</sup>             | 45.72 <sup>bc</sup> | 37.71 <sup>c</sup> | 47.41 <sup>a</sup> | 46.87 <sup>ab</sup> | 41.51 <sup>c</sup> |
| Relative leaf water content (%)              | 34.29 <sup>a</sup>           | 14.48 <sup>c</sup>             | 17.84 <sup>b</sup>  | 13.30 <sup>c</sup> | 27.30 <sup>a</sup> | 19.17 <sup>b</sup>  | 13.48 <sup>c</sup> |
| Ion leakage (%)                              | 40.31 <sup>d</sup>           | 64.89 <sup>b</sup>             | 58.89 <sup>c</sup>  | 76.42 <sup>a</sup> | 46.31 <sup>c</sup> | 60.70 <sup>b</sup>  | 72.61 <sup>a</sup> |
| Proline (μmol/g fw)                          | 2.57 <sup>d</sup>            | 4.65 <sup>b</sup>              | 3.92 <sup>c</sup>   | 5.68 <sup>a</sup>  | 2.60 <sup>bc</sup> | 5.07 <sup>a</sup>   | 3.94 <sup>b</sup>  |
| Chlorophyll a (mg/g fw)                      | 0.14 <sup>a</sup>            | 0.11 <sup>b</sup>              | 0.12 <sup>b</sup>   | 0.08 <sup>c</sup>  | 0.13 <sup>a</sup>  | 0.11 <sup>a</sup>   | 0.09 <sup>b</sup>  |
| Chlorophyll b (mg/g fw)                      | 0.13 <sup>a</sup>            | 0.09 <sup>b</sup>              | 0.09 <sup>b</sup>   | 0.06 <sup>c</sup>  | 0.13 <sup>a</sup>  | 0.08 <sup>b</sup>   | 0.06 <sup>c</sup>  |
| Total chlorophyll (mg/g fw)                  | 0.24 <sup>a</sup>            | 0.18 <sup>b</sup>              | 0.24 <sup>a</sup>   | 0.18 <sup>b</sup>  | 0.27 <sup>a</sup>  | 0.19 <sup>b</sup>   | 0.16 <sup>c</sup>  |
| Carotenoid (μg/g fw)                         | 2.63 <sup>b</sup>            | 2.09 <sup>c</sup>              | 3.09 <sup>a</sup>   | 3.06 <sup>a</sup>  | 2.80 <sup>a</sup>  | 2.40 <sup>b</sup>   | 1.91 <sup>c</sup>  |
| Root colonization percentage (%)             | 56.3 <sup>a</sup>            | 25.8 <sup>c</sup>              | 47.72 <sup>b</sup>  | 0.00 <sup>d</sup>  | 31.0 <sup>a</sup>  | 31.31 <sup>a</sup>  | 28.1 <sup>b</sup>  |
| Malondialdehyde (μmol/g fw)                  | 0.068 <sup>d</sup>           | 0.084 <sup>b</sup>             | 0.079 <sup>c</sup>  | 0.10 <sup>a</sup>  | 0.05 <sup>c</sup>  | 0.09 <sup>b</sup>   | 0.11 <sup>a</sup>  |

\* Means with the same letters in each column and each group indicate no significant difference based on Duncan's test at 5% level.

### 3.2. VAM impact on plant growth and biomass and flower traits

The results of the ANOVA revealed that VAM inoculation, salinity, and their interaction significantly

affected the plant flower fresh weight ( $p \leq 0.01$ ) (Table 2). Additionally, the interaction of salinity and VAM treatment, as well as VAM significantly influenced the dry weight ( $p \leq 0.01$ ). The highest flower fresh weight was observed in the *Funneliformis mosseae* treatment without

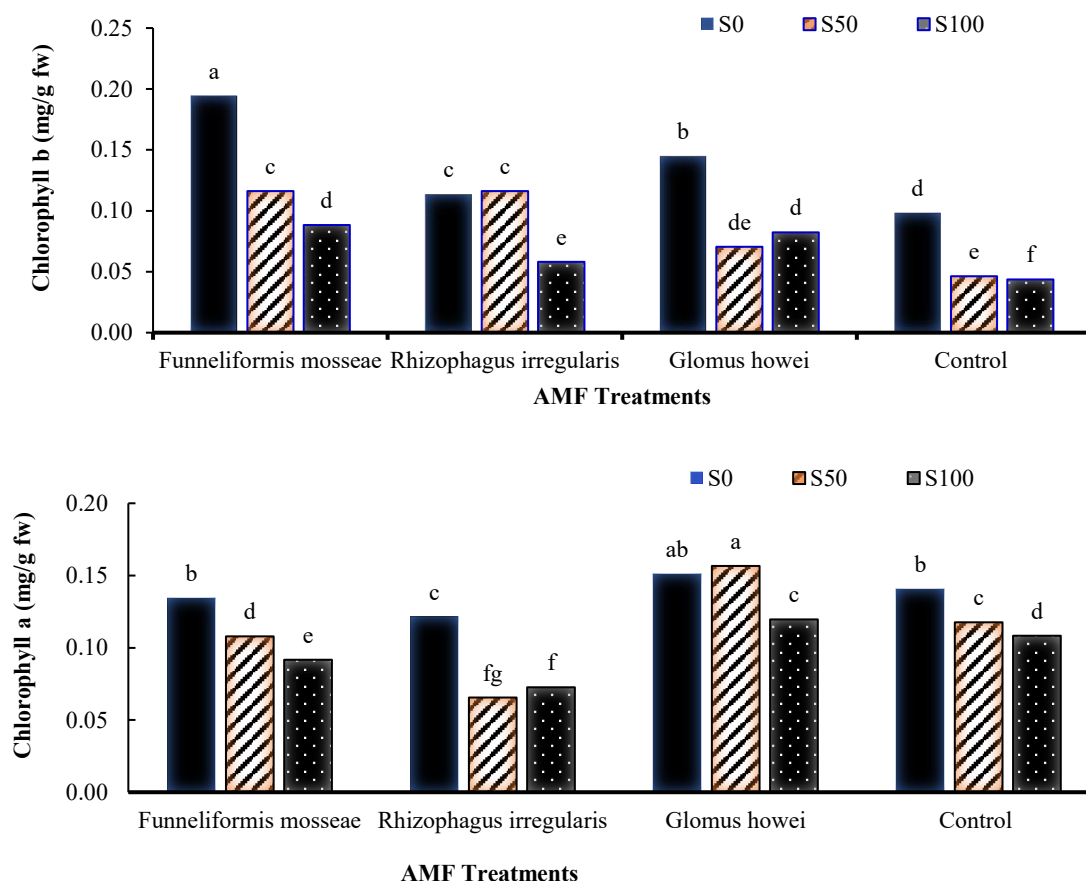
**Table 2.** The results of analysis of variance (Mean squares) for morphological and biochemical parameters of *H. annuus* plants exposed to salinity and inoculated with VAM.

| Source of variation/<br>Df/              | VAM treatment<br>3 | Salinity treatment<br>2 | Salinity×VAM<br>6 | Error<br>24 | C.V (%)<br>- |
|------------------------------------------|--------------------|-------------------------|-------------------|-------------|--------------|
| morphological and biochemical parameters |                    |                         |                   |             |              |
| Root colonization percentage             | 704.1**            | 196.3**                 | 30.18**           | 12.4        | 10.85        |
| Flower fresh weight                      | 164.4**            | 41.4**                  | 0.641**           | 0.513       | 5.73         |
| Flower dry weight                        | 10.65**            | 1.82 **                 | 0.633**           | 0.063       | 2.78         |
| Shoot fresh weight                       | 339.9**            | 85.36**                 | 10.18**           | 1.02        | 7.46         |
| Shoot dry weight                         | 37.46**            | 15.93**                 | 0.481**           | 0.152       | 2.83         |
| Root fresh weight                        | 37.94**            | 7.79 **                 | 0.403**           | 0.081       | 2.10         |
| Root dry weight                          | 5.54**             | 4.03**                  | 0.842**           | 0.079       | 4.81         |
| Flower diameter                          | 14.56**            | 7.37**                  | 0.122 ns          | 0.123       | 1.33         |
| Stem diameter                            | 5.16**             | 4.96**                  | 0.123ns           | 0.075       | 1.62         |
| Leaf area                                | 42.62**            | 12.59**                 | 0.105**           | 0.023       | 2.29         |
| Length of flowering stem                 | 263.5**            | 124.2**                 | 28.87**           | 5.42        | 8.35         |
| Relative leaf water content              | 851.3**            | 578.2**                 | 17.7**            | 1.56        | 7.53         |
| Ion leakage                              | 2025.0**           | 2089.7**                | 103.2**           | 22.73       | 28.52        |
| Proline                                  | 15.37**            | 6.92**                  | 3.92**            | 0.143       | 8.86         |
| Chlorophyll a                            | 0.005**            | 0.005**                 | 0.001**           | 8.82        | 1.25         |
| Chlorophyll b                            | 0.007**            | 0.016**                 | 0.001**           | 0.001       | 11.11        |
| Total chlorophyll                        | 0.011 ns           | 0.037 ns                | 0.001**           | 0.009       | 4.28         |
| Carotenoid                               | 2.71**             | 2.23**                  | 0.682**           | 0.017       | 0.701        |
| Malondialdehyde                          | 0.003**            | 0.011**                 | 0.004**           | 0.004       | 12.53        |

\*\*, \* and ns are significant at 1% and 5 % levels and non-significant, respectively.

salinity (14.70 g/plant), while the lowest flower weight was recorded in the treatment without mycorrhizal inoculation and 100 mM salinity (4.48 g/plant) (Table 1). Similarly, the highest flower dry weight was found in the *Funneliformis mosseae* treatment with control salinity (3.63 g/plant), and the lowest dry weight was in the treatment without inoculation and 100 mM salinity (0.974 g/plant) (Table 1). Moreover, the ANOVA indicated that the interaction of VAM and salinity significantly influenced root weight ( $p \leq 0.01$ ). The main effects of mycorrhizal treatment, salinity, and their interaction on root dry weight were also significant ( $p \leq 0.01$ ) in Table 2. The highest root fresh weight was obtained in the *Funneliformis mosseae* treatment with control salinity (6.57 g/plant), and the lowest root fresh weight was recorded in the treatment without inoculation and 100 mM salinity. The highest root dry weight was also observed in the *Funneliformis mosseae* treatment with control salinity (2.55 g/plant), significantly outperforming other

mycorrhizal fungi species (Table 1). The lowest root dry weight was found in the treatment without inoculation and 100 mM salinity. Mycorrhizal fungus significantly increased root fresh weight compared to the control treatment. Salinity imposes both osmotic and ionic stress on plants. Initially, osmotic stress resulting from salinity causes water deficit in the root zone, directly impairing the plant's water status. Nevertheless, plants typically recover over subsequent hours, resuming a gradual and steady growth rate. Over time, a second phase ensues, characterized by the toxic accumulation of  $\text{Na}^+$  and  $\text{Cl}^-$  ions within the cytoplasm. Additionally, under salinity stress, plants must allocate extra energy to mitigate the deleterious effects of excess  $\text{Na}^+$  ions and often experience nutrient deficiencies. Collectively, these physiological challenges adversely impact overall plant growth (Isayenkov and Maathuis, 2019). VAM association in plants influences plant shoot biomass and root biomass more than plant height (Dastogeer et al., 2020). This



**Figure 2.** Average comparison of salinity stress and mycorrhizal fungi (VAM) interaction on chlorophyll a and chlorophyll b in *H. annuus* [S50: 50 mM NaCl and S100: 100 mM NaCl].

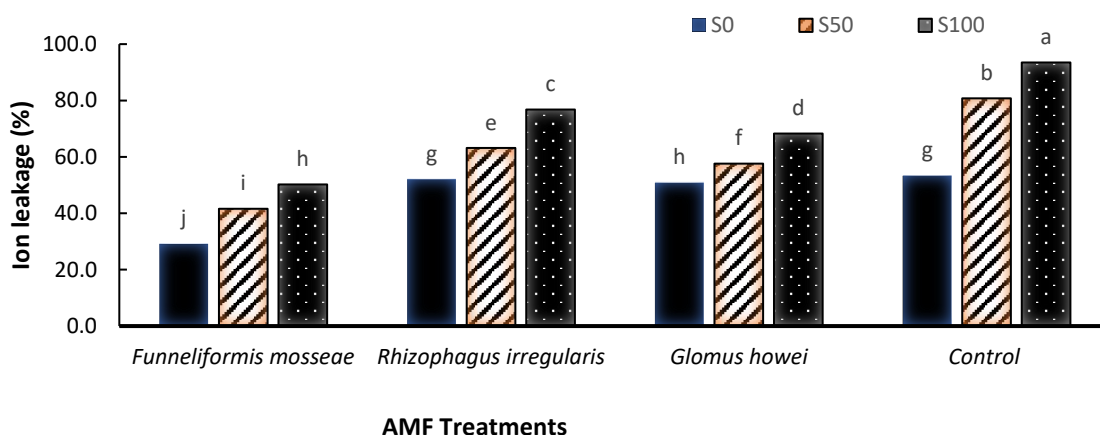
suggests that VAM can enhance nutrient transfer between the root and stem, leading to increased plant growth and dry weight of aerial organs (Schubert and Hayman, 1986).

The ANOVA results revealed that both mycorrhizal fungus and salinity treatments significantly affected flower diameter ( $p \leq 0.01$ ), but their interaction was not significant in Table 2. The highest flower diameter was observed in the *Funneliformis mosseae* treatment with control salinity (10.55 cm), while the lowest flower diameter was recorded in the no-inoculation and *Rhizophagus irregularis* treatments with 100 mM salinity (8.07 cm) (Table 1). Previous studies have reported that salinity can reduce flower diameter, stem length, root and leaf dry weight, leaf area, and the number and length of secondary stems in various plants, including *Zinia* (Carter and Grieve, 2010), *Geranium* (Li *et al.*, 2023b), sage (Zeidali *et al.*, 2018), and tomato (Parida and Das, 2005). In this research, the use of mycorrhizal fungi improved plant performance by increasing root surface area, leading to enhanced salt absorption. This resulted in increased growth of roots and aerial parts in most treatments and reduced salinity stress in the inoculated treatments.

### 3.3. Physiological response to VAM inoculation

The interaction of mycorrhizal fungus and salinity significantly affected chlorophyll a, chlorophyll b, and total chlorophyll in ornamental sunflowers ( $p \leq 0.01$ ) in Table 2. Additionally, mycorrhizal fungus treatment alone significantly influenced chlorophyll a and b ( $p \leq 0.01$ ). The highest chlorophyll a content was observed in the *Funneliformis mosseae* treatment with control salinity (0.14 mg/g fresh weight), while the lowest content was found in the treatment without inoculation and 100 mM salinity (0.086 mg/g fresh weight) (Figure 2). Similarly, the highest chlorophyll b content was in the *Funneliformis mosseae* treatment with control salinity (0.13 mg/g fresh weight), and the lowest was in the treatment without inoculation and 100 mM salinity (0.06 mg/g fresh weight) (Figure 2). The highest total chlorophyll content was also observed in the *Funneliformis mosseae* treatment with control salinity (0.24 mg/g fresh weight), and the lowest was in the treatment without inoculation and 100 mM salinity (0.18 mg/g fresh weight) (Table 1). In previous studies, pepper plants subjected to salinity stress and inoculated with *Glomus intraradices* showed increased





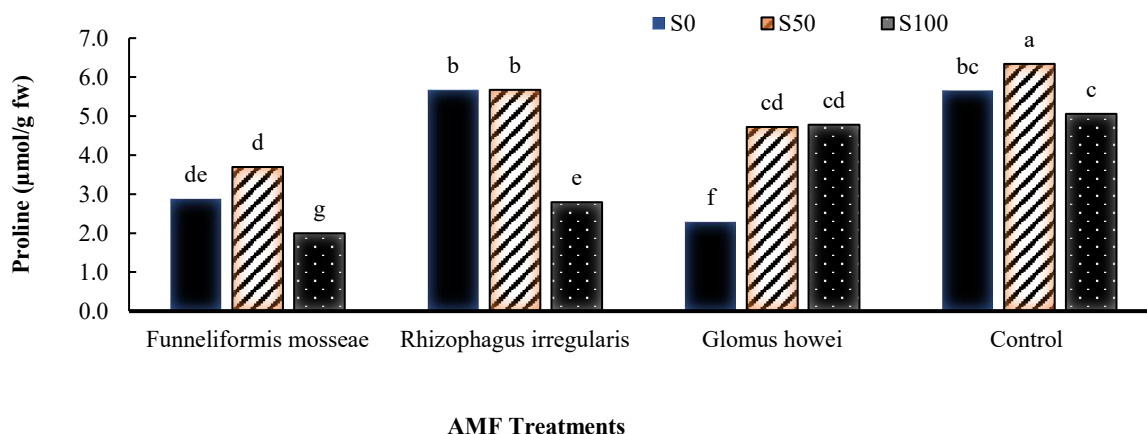
**Figure 3.** Average comparison of salinity stress and mycorrhizal fungi (VAM) interaction on Ion leakage and leaf area in *H. annuus*. [S50: 50 mM NaCl and S100: 100 mM NaCl].

RWC, chlorophyll, and carotenoid content (Parida and Das, 2005). The decrease in chlorophyll content under salinity stress can be attributed to the accumulation of sodium and chlorine ions, which inhibits pigment production (Ozlem *et al.*, 2012). In spinach, chlorophyll a, b, and total chlorophyll have been found to decrease with increasing salinity (Sabra *et al.*, 2012). Salinity affects plant growth by altering various physiological processes, including photosynthesis, reducing chlorophyll content, decreasing pore conductance, reducing Rubisco activity, and increasing the chlorophyll a to b ratio (Kalaji *et al.*, 2011). Under stress conditions like salinity, plants produce reactive oxygen species that damage mitochondria and chloroplasts, leading to cellular structure destruction (Ratnakar and Rai, 2013). In this study, higher salinity concentrations led to a decrease in chlorophyll content due to the accumulation of sodium and chlorine ions. This reduction in chlorophyll has also been reported in other plants, such as corn, safflower, and beans (Pandit *et al.*, 2024). However, the inoculated treatments exhibited a less severe reduction in chlorophyll compared to the non-inoculated treatments.

The ANOVA results revealed that both mycorrhizal fungus and salinity treatments significantly affected ion leakage ( $p \leq 0.01$ ), as did their interaction ( $p \leq 0.01$ ) in Table 2. The highest leakage rate was observed in the treatment without mycorrhizal inoculation and 100 mM salinity (76.42%), while the lowest leakage rate was found in the *Glomus howei* and *Funneliformis mosseae* treatments with control salinity (40.30%) in Figure 3. Salinity stress can damage the cell membrane, leading to the leakage of intracellular and vacuolar fluids into the environment, increasing the electrical conductivity of the solution (Alia-Mohanty and Matysik, 2001). Many mycorrhizal species are tolerant to severe osmotic stresses, with some fungi capable of withstanding salinity levels up to 65 MPa (Galal and Lindell, 2010). In this study, increasing salinity

levels led to a significant increase in ion leakage, particularly at 100 mM salinity compared to the control. Increased ion leakage has also been reported in flax (Khan *et al.*, 2007), mustard (Siddiqui *et al.*, 2008), and three cultivars of coneflower (Sabra *et al.*, 2012). Mycorrhizal fungus inoculation decreased ion leakage in the treatments under salinity stress compared to the control treatment. This suggests that VAM can enhance cell membrane resistance in ornamental sunflower plants against salinity stress.

The ANOVA results indicated that the interaction of VAM and salinity significantly affected the proline content in leaves ( $p \leq 0.01$ ) in Table 2. The highest proline content (5.6  $\mu\text{mol/g}$  fresh weight) was observed in the plants without inoculation under high salinity level (100 mM NaCl), while the lowest content (2.57  $\mu\text{mol/g}$  fresh weight) was found with inoculating the *Funneliformis mosseae* species under no salinity (Figure 4). The synthesis and accumulation of osmolytes like amino acid proline is a mechanism used by plants to resist salinity (Zhang *et al.*, 2024; Yoshida *et al.*, 1997). Proline is a storage compound, a soluble substance with a low molecular weight, and a neutral pH. Its osmotic performance is attributed to its chemical properties (Parida and Das, 2005). Proline accumulation as an osmotic regulator occurs in plants under dry conditions, low temperatures, salinity, and other abiotic stresses that reduce cell water potential (Joshani, 2020). Osmotic regulation is associated with an increase in proline, which also acts as a free radical scavenger (Alia-Mohanty and Matysik, 2001). In this study, salinity caused oxidative damage to the plant, leading to the activation of proline as a membrane amino acid to enhance plant resistance. *Rhizophagus irregularis* showed a greater decrease in proline content compared with other species, indicating its higher sensitivity to salinity stress. The symbiosis between the plant and *F. mosseae* was associated with reduced proline



**Figure 4.** Average comparison of salinity stress and mycorrhizal fungi (VAM) interaction on proline in *H. annuus*. [S50: 50 mM NaCl and S100: 100 mM NaCl].

accumulation compared to the interactions with the other two fungal strains, suggesting that *F. mosseae* conferred greater protection against salt stress, thereby resulting in lower proline synthesis by the plant. Overall, mycorrhizal fungi reduced stress in ornamental sunflowers, and proline levels were lower in plants coexisting with the fungus compared to those without. Proline levels increased significantly under moderate salinity conditions in VAM-inoculated plants. Proline can play important roles as an osmoregulatory compound as well as an ROS scavenger (Yoshida *et al.*, 1997). However, it is still unresolved whether its presence is an adaptive response that provides greater stress tolerance or if its increase is a symptom of stress injury (Ashraf and Foolad, 2007). Relatively high levels of proline in the presence of VAM could, therefore, indicate less damage to a moderately stressed plant in the presence of VAM. Xu *et al.* (2024) observed that, under increasing salt concentrations, inoculation with mycorrhizal fungi led to enhancements in plant height, fresh biomass, chlorophyll content, and the levels of proline, soluble sugars, soluble proteins, as well as activities of antioxidant enzymes such as peroxidase, superoxide dismutase, and catalase. Conversely, this treatment was associated with reductions in malondialdehyde content and superoxide anion production rate, indicating a mitigation of oxidative stress.

Climate change projections indicate a global increase in the frequency, duration, and intensity of droughts, coupled with rising sea levels and altered precipitation patterns. These factors collectively exacerbate soil salinization - particularly in arid, semi-arid, and coastal agricultural regions - through reduced leaching, capillary rise of saline groundwater, and seawater intrusion into freshwater aquifers. The salinity levels tested in this study (which ranged from moderate to severe) may therefore become more pervasive and more extreme under future climate scenarios (Bennett and Classen, 2020). While *Funnelformis mosseae* demonstrated resilience at the

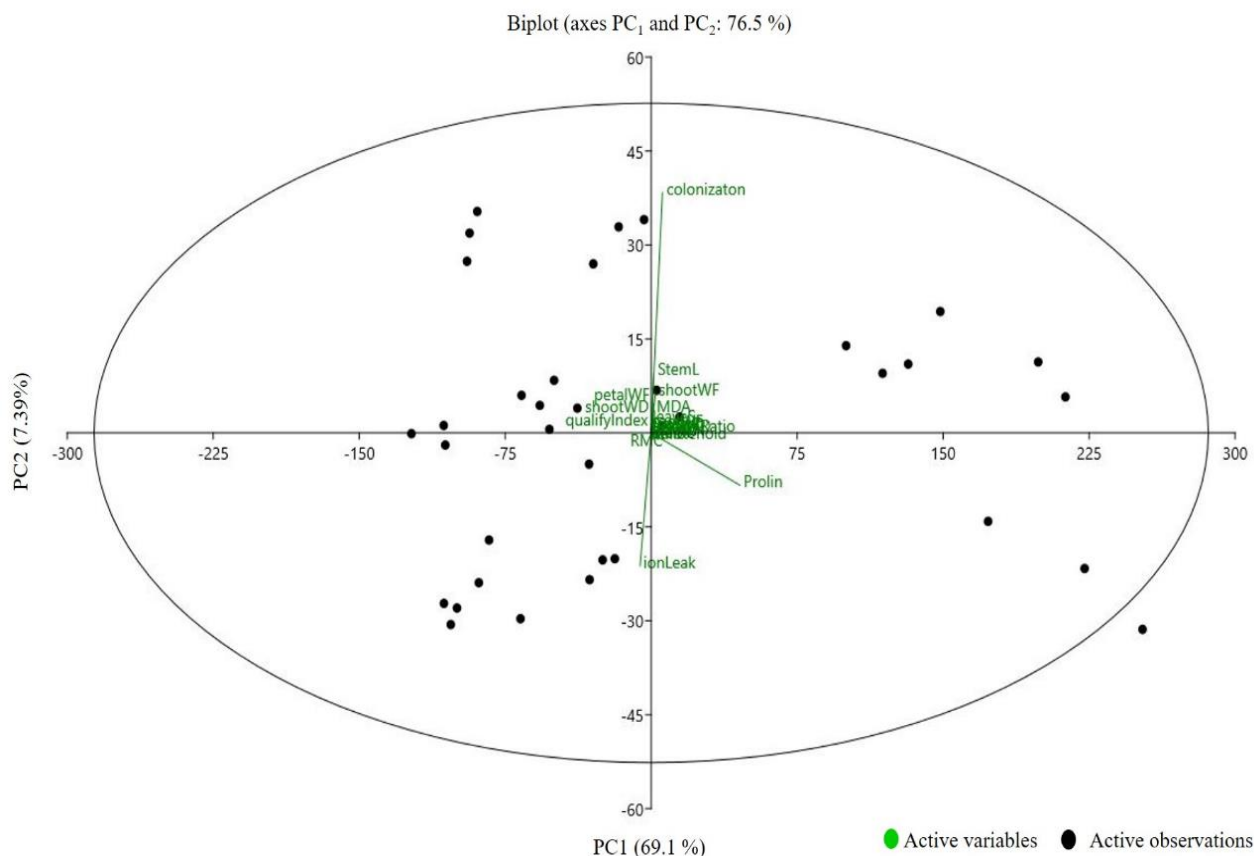
salinity levels examined, the efficacy of VAM-mediated protection may diminish if salinity concentrations surpass critical physiological thresholds beyond those tested here. The present study controlled temperature as a fixed variable, yet climate change is driving upward shifts in average temperatures and an increase in heatwave frequency. Elevated temperatures can independently stress both the host plant and the mycorrhizal symbiont. For AMF, high soil temperatures may reduce spore germination, hyphal extension, and root colonization efficiency, potentially undermining the very establishment of symbiosis that the study identifies as critical for salt tolerance.

Rising atmospheric CO<sub>2</sub> concentrations, a primary driver of climate change, have complex and often synergistic effects on mycorrhizal symbioses. Elevated CO<sub>2</sub> generally increases photosynthetic rates and carbon allocation belowground, potentially enhancing the carbon supply available to mycorrhizal fungi. This could, in theory, strengthen the symbiotic partnership and magnify the salinity-alleviating benefits observed in this study (Knapp *et al.* 2015).

### 3.4. Principal component analysis

To analyze the factors influencing the responses of ornamental sunflowers to salt stress and VAM inoculation, principal component analysis (PCA) was also performed. The analysis included all measured parameters (Table 2). Eigenvalues were examined to determine the most important principal components (PCs). The first two PCs accounted for more than 76.5% of the total variance (Figure 5). The first PC highlighted the significant influence of biochemical traits such as proline, ion leakage and chlorophyll content. The second PC emphasized the effects of VAM colonization percentage and stem length. Additionally, positive and negative correlations between traits were observed. The biplot revealed that mycorrhizal





**Figure 5.** Principal Component Analysis (PCA) biplot depicting Principal Components (PC) scores of treatments (dots) and loadings of vector variables. The terms “stemL” (Stem length), “stemD” (Stem diameter), “leavesN” (Number of leaves), “flowerD” (Flower diameter), “petalL” (Petal length), “leaveS” (Leaf area), “petalWF” (Flower fresh weight), “petalWD” (Flower dry weight), “shootWF” (Shoot fresh weight), “shootWD” (Shoot dry weight), “rootWF” (Root fresh weight), “rootWD” (Root dry weight), “Chl.a” (Chlorophyll a), “Chl.b” (Chlorophyll b).

inoculation treatment was more effective in increasing the growth and overall performance of ornamental sunflower plants under salinity stress.

#### 4. Conclusion

This study demonstrates that salinity stress negatively affected the quantitative and qualitative yield of ornamental sunflowers. Mycorrhizal fungi (VAM) significantly improved vegetative and biochemical and physiological traits in these plants under salinity stress. This study showed that the roots of ornamental sunflower were successfully colonized by VAM and the root colonization sites were significantly different among different VAM species, with *R. irregularis*, *F. mosseae* and *G. howei* being highly valued in terms of high salinity stress tolerance and symbiotic fitness. These fungal species facilitated fungal adaptation and enhanced stress tolerance by maintaining effective symbiosis even in high salinity environments. Although the colonization percentage slightly decreased with increasing salinity,

these inoculated fungi stimulated plant growth and development, and among them, *F. mosseae* was more adaptable, showing significant improvement in plant morphological and physiological traits even at high salinity levels. Also, inoculation with *F. mosseae* effectively mitigated the adverse effects of salinity stress by maintaining higher levels of chlorophyll a and chlorophyll b, thereby supporting photosynthetic efficiency under saline conditions. Moreover, VAM inoculation, particularly with *F. mosseae*, was associated with reduced ion leakage and enhanced osmolyte accumulation, as evidenced by lower ion leakage rates and modulated proline content, respectively. Notably, plants inoculated with VAM exhibited less membrane damage and better osmotic regulation under salinity stress, indicating improved stress tolerance. The increased proline synthesis observed in non-inoculated plants under high salinity further underscores the role of osmolytes in salinity resistance, while VAM inoculation appeared to optimize this response. The VAM inoculation increased the yield of ornamental sunflowers by enhancing root

biomass, improving water and salt absorption, and promoting plant growth. Additionally, VAM increased the resistance of ornamental sunflowers to salinity stress, offering a valuable tool for mitigating the negative impacts of salinity on plant productivity. VAM-mediated plant salinity tolerance has broad ecological and agricultural implications. Overall, these findings suggest that VAM inoculation, especially with *F. mosseae*, can be an effective strategy to enhance salinity tolerance in ornamental sunflowers, promoting healthier growth and maintaining physiological stability under saline conditions. Future research should explore the underlying molecular mechanisms and evaluate the practical applications of VAM in salt-affected horticultural practices.

### Author Contributions

S. Reezi, the corresponding author, participated in supervising the second author in data analysis with SPSS, interpreted the results, and preparing the manuscript. S.S. Mashae, also corresponding author, participated in laboratory work, data analysis, and interpreted the results. A. Sadeghi participated in conducting greenhouse and laboratory experiments, data collection, and manuscript preparation.

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### Conflict of Interest

The author declares that there is no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been completely observed by the authors.

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