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RESEARCH PAPER

# Combined Use of Arbuscular Mycorrhizal Fungi Inoculants and Potassium Silicate for Improving Drought Tolerance in Cucumber

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# الاستخدام المشترك لفطريات المايكورايزا وسيليكات البوتاسيوم لتحسين تحمل الجفاف في نبات الخيار

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## ABSTRACT.

Drought stress is one of the most serious abiotic stresses that cause significant reductions in plant growth and yield in many areas around the world. Arbuscular mycorrhizal fungi (AMF) improve plant drought tolerance by extending the root zone through extraradical hyphae exploration of soil micropores. This study evaluated the combined application of AMF and potassium silicate (PS) to improve the drought tolerance of cucumber plants. Cucumber plants were grown under two watering regimes: drought-stressed 60% water holding capacity (WHC) and unstressed 80%WHC, and three mycorrhizal inoculation treatments (control, native AMF: quick root colonizer species, and commercial AMF) with or without potassium silicate application (100 mL/pot, con. 40 mL/L). Drought stress significantly reduced plant height, biomass, and photosystem II efficiency, while it increased the content of leaf glycine betaine and catalase activity. Generally, AMF inoculation significantly improved plant growth, SPAD, photosystem II efficiency, concentration of proline, glycine betaine, and catalase activity, and the native species exhibited comparable effects to commercial species for most variables studied. Contrary to our expectations, PS application alone did not improve plant growth, except for increased photosystem II efficiency, but root fresh and dry weights were reduced. Under drought stress, combination of PS and AMF significantly improved plant height, shoot fresh weight, SPAD value, glycine betaine, and catalase activity compared to PS alone. Our results highlight that the combined application of PS and AMF inoculants can protect cucumber seedlings from drought stress; however, further research is needed to optimize the PS application method and dosage and to investigate their long-term effects on plant growth and yield under field conditions.

**KEYWORDS:** biomass, photosystem, osmolytes, antioxidants, water field capacity

## الخلاصة:

يعد الإجهاد الناتج عن الجفاف أحد أخطر العوامل اللاأحيائية التي تسبب انخفاضًا كبيرًا في نمو النبات وإنتاجيته في العديد من المناطق حول العالم. تعمل الفطريات الجذرية المايكورايزا (AMF) على تحسين تحمل النبات للجفاف عن طريق توسيع منطقة الجذور من خلال استكشاف خيوط الفطر للماء ووصولها لمسامات التربة الدقيقة التي يصعب للجذور الوصول لها. قامت هذه الدراسة بتقييم التطبيق المشترك لـ AMF وسيليكات البوتاسيوم (PS) لتحسين تحمل نباتات الخيار للجفاف. تمت زراعة نباتات الخيار في ظل نظامين للري: الجفاف المجهد بنسبة ٦٠٪ من القدرة على الاحتفاظ بالمياه (WHC) وغير المجهد بنسبة ٨٠٪، وثلاثة معاملات للتلقيح بفطريات المايكورايزا ( الشاهد، ، والمايكورايزا المحلية: سلالات مستعمرة سريعة للجذور، و المايكورايزا المنتجة تجاريا) مع أو بدون سيليكات البوتاسيوم ( ١٠٠مل/ وعاء، وبتركيز ٤٠ مل / لتر). أدى إجهاد الجفاف إلى انخفاض كبير في ارتفاع النبات والكتلة الحيوية وكفاءة النظام الضوئي II ، في حين أنه زاد من محتوى البيتين جلايسين ونشاط الكاتلاز في الاوراق. وبشكل عام، أدى تلقيح AMF إلى تحسين نمو النبات بشكل كبير، ومحتوى الكلوروفيل SPAD، وكفاءة النظام الضوئي II، وتركيز البرولين، والجليسين البيتين، ونشاط الكاتلاز ، وأظهرت الأنواع المحلية من المايكورايزا تأثيرات مماثلة للأنواع التجارية لمعظم المتغيرات التي تمت دراستها. وعلى عكس توقعاتنا، فإن استخدام PS وحده لم يحسن نمو النبات، باستثناء زيادة كفاءة النظام الضوئي II، ولكن قلل من الأوزان الطازجة والجافة للجذور. تحت إجهاد الجفاف، أدى الجمع بين AMF و PS إلى تحسين كبير في ارتفاع النبات والوزن الطازج وقيمة SPAD والبيتين الجليسين ونشاط الكاتيلاز مقارنة بـ PS وحده. تسلط نتائجنا الضوء على أن التطبيق المشترك للمايكورايزا وسيليكات البوتاسيم يمكن أن يحمي شتلات الخيار من إجهاد الجفاف؛ ومع ذلك، هناك حاجة إلى مزيد من البحث لتحسين طريقة تطبيق سيليكات البوتاسيوم للتركيز المناسب وللتحقق من آثارها طويلة المدى على نمو النبات وإنتاجيته تحت الظروف الحقلية

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## Introduction

Drought stress is one of the most serious abiotic stresses that cause significant reductions in plant growth and yield in many areas around the world (Farooq et al., 2009). This is expected to be a future problem in many regions due to climate change, which causes a reduction in precipitation and an increase in evaporation (Marchin et al., 2020), which is increasingly intensifying and occurs more frequently in arid and semi-arid areas (Zou et al., 2021). In Oman, drought is a serious issue that affects agriculture, food production, water resources, energy supply, biodiversity, human health, the economy, and society (El Kenawy et al., 2020). Agriculture in Oman is highly dependent on groundwater: 92% of irrigation is sourced from wells, and the natural recharge of groundwater aquifers by rainfall is below the extraction rate (Al-Alawi, 2014). Oman is classified as one of the driest regions in the world, with an average rainfall of 100 mm and an evaporation rate of over 2000 mm (MAF, 2008; Al-Alawi, 2014). Furthermore, the soil texture is sandy with high infiltration rates, high alkaline, and with very low organic matter content (FAO, 2008). The Oman government has many strategies to optimize water use such as to conserve the water resources, reuse the treated wastewater, combat desertification, use modern irrigation system and dam construction (Sustainable Agriculture and Rural Development Strategy: Towards 2040 (SARDS2040)) (Al-Alawi, 2014, FAO, 2018).

Drought in agriculture is as defined a period when water availability in the plant rhizosphere is lower than the level required for plant growth and biomass production due to low precipitation, decreased ground water level and low retention of water by soil pore structure (Osmolovskaya et al., 2018; Kapoor et al., 2020). Water deficiency in soil affects the physiological processes of plants, such as photosynthesis, including the function of the photosynthesis (PSII) reaction center, respiration, and mineral nutrient uptake (Farooq et al., 2009; Cheng et al., 2021). Moreover, these lead to a negative effect on stomatal function, gas exchange, reduced cell division, growth of stems and roots, leaf number and area, a reduction in fresh and dry biomass production, reduced water-use efficiency, and increased levels of reactive oxygen species (ROS) and oxidative stress (Anjum et al., 2011; Kapoor et al., 2020). Reactive oxygen species are inevitable toxic by-products of aerobic metabolism, and they have different forms, such as singlet oxygen, hydrogen peroxide, superoxide anion free radicals, and hydroxyl radicals, and they mainly exist in the chloroplast, mitochondria, peroxisomes, cell membrane, cell wall, and other parts of cells (Das et al., 2014; Choudhury et al., 2017; Zou et al., 2021). Excess ROS can cause lipid peroxidation, damage to proteins and nucleic acids, degradation of enzymes and even cause cell death (Shah et al., 2001; Sharma et al., 2012; Choudhury et al., 2017). In addition, drought stress limits potassium absorption, which is an important element in the physiological and molecular mechanisms of plant drought tolerance, such as stomatal regulation and the control of plant water loss via transpiration (Wang et al., 2013).

Plants have strategies to mitigate the effects of drought, such as osmotic adjustment, regulation of stomatal conductance and photosynthesis, production of antioxidant and scavenger compounds to scavenge ROS, and regulation of water uptake and flow in their tissues (Quiroga et al., 2017). These responses are dependent on plant genotype-specific, stress intensity and duration (Farooq et al., 2009; Kapoor et al., 2020). Osmotic adjustment is a process of accumulation of solutes (osmolyte accumulation) such as proline, glycine betaine, sucrose, soluble carbohydrates, and other solutes (Anjum et al., 2011). In addition, phytohormones such as abscisic acid, jasmonic acid, salicylic acid, ethylene, auxin, gibberellin, cytokinin and brassinosteroid can be expressed to mitigate drought stress (Farooq et al., 2009; Ullah et al., 2018). Numerous genes and transcription factors that are responsive to drought have been discovered at the molecular level, including the dehydration-responsive element-binding gene, aquaporin, numerous proteins during late embryogenesis, and dehydrins (Farooq et al., 2009).

Arbuscular mycorrhizal fungi enhance soil water availability to plants (Zhang et al., 2019). They improve plant drought tolerance by helping plants take up water, and nutrients through the use of extraradical hyphae, 2-5 µm in diameter, from the soil microspores (Wu et al., 2017; Cheng et al., 2021) and by increasing the surface area of absorption (Sharifian et al., 2022). AMF can enhance host plants' photosynthesis under drought stress by increasing net photosynthetic rate, transpiration rate, stomatal conductance, and decreasing intercellular $CO_2$ concentration (Cheng et al., 2021). AMF help plants tolerate drought stress by adjusting their water potential, increasing their antioxidant defense system, producing polyamines and fatty acids, acquiring essential minerals, and inducing the expression of genes involved in drought tolerance (Cheng et al., 2021). According to Chen et al. (2017), incorporating multiple AMF strains can induce higher photosynthetic ability and nutrient uptake in cucumber seedlings, leading to improved plant biomass production, suggesting that the use of multiple AMF species is more beneficial than that of a single species. Ma et al. (2019) found that AMF can alleviate the negative effect of cold stress on cucumber photosynthesis by increasing the chlorophyll content, net photosynthetic rate, and parameters related to photochemical quenching, while decreasing non-photochemical quenching and sugar contents in leaves. Zhang et al. (2019) reported inoculated *Zenia insignis* seedlings by arbuscular mycorrhizal fungi improved plant growth (height 13.6-32.1%), phosphorus content and antioxidant enzymes such as superoxide dismutase and catalase activity under drought stress. However, the performance and effectiveness of mycorrhizae can vary depending on a number of factors, including the type of AMF species, plant species, growing conditions, developmental stage of the plant, soil properties, and the presence of other microorganisms (Teranishi and Kobae, 2020). In addition, drought stress can also reduce the diversity of mycorrhizal fungi, and native AMF species are more adaptable to drought conditions (Cheng et al., 2021).

Potassium silicate is a water-soluble compound comprising potassium (K) and silicon (Si). It is a white solid or colorless solution readily available to plants. According to the United States Environmental Protection Agency (EPA, 2007), PS is generally considered safe for both humans and the environment. Despite Si is not a necessary element for higher plants, but many plants are capable of accumulating significant levels of Si in their tissues via the transpiration stream (Samuels et al., 1991), which has beneficial effects on plant growth, biomass, and yield and helps plants cope with biotic and abiotic stresses (Sharifian et al., 2022; Anitha et al., 2023). In addition, Si application has been shown to improve cucumber resistance to biotic stresses, such as powdery mildew (Liang et al., 2005). Potassium silicate can reduce drought stress in plants by enhancing photosynthesis efficiency, water use efficiency, reducing transpiration, and strengthening plant cell walls (Gomaa et al., 2021; Hafez et al., 2021). Overall, silicon boosts root endodermal silicification and improves cell water balance, while potassium ions aid in osmotic adjustment (Farooq et al., 2009). In organic farming practices, incorporating silicon sources into fruit crops can potentially enhance yields while reducing the need for chemical fertilizers, pesticides, and fungicides (Patil et al., 2017).

Cucumber (*Cucumis sattivus* L.) is sensitive to drought stress, which alters its physiology and biochemistry, restricting its growth and decreasing yield (Farag et al., 2019; Shehata et al., 2022). To date, no studies have been conducted that combine AMF and PS application in cucumber plants under stress. The current study aimed to investigate the synergistic effects of

AMF, particularly quick root colonizing native AMF, in combination with PS, to enhance their underlying mechanisms for alleviating drought stress in plants. This study sought to provide insights into whether the performance of native AMF, with their specific quick root-colonizing properties, can surpass or equal the benefits of a commercial AMF inoculum under drought conditions for cucumber plants.

## Materials and Methods

### Experimental Design

Seeds of a cucumber (*Cucumis sativus* L.*)*, genotype 'Diva' (Johnny's Selected Seed, USA) were grown in the glasshouse of the Agricultural Experimental Station (AES), from Sultan Qaboos University, Oman (23°35'53.2"N 58°09'51.7"E). The growth conditions included a temperature of 28 °C and natural sunlight. Seeds were planted on December 18, 2022, in 50 cell seedling trays (≈ 60 mL/cell) with sterilized potting soil. The seeds were divided into three treatments based on mycorrhizal inoculum: control (no inoculant), native AMF (quick root colonizer species obtained from a previous experiment), and commercial AMF inoculum (SYMBIVIT, Symbiom Ltd., Czech Republic) M1, M2, and M3, respectively. Native AMF included three species identified in a previous study (Al Hinai et al. unpublished) that were maintained in the glasshouse, colonizing sorghum and alfalfa. The AMF in this mix were: *Rhizoglomus arabicum, R. custos* and *Septoglomus viscosum.* The commercial AMF inoculum included five species according to the label, but were not independently confirmed, including *Claroideoglomus etunicatum, C. claroideum, Rhizoglomus irregularis, Funneliformis geosporus, and F. mosseae*. Additionally, the commercial inoculant contained natural clay carriers and bio-additives supporting the development of mycorrhizal symbiosis (chitin, keratin, natural humates, and seaweed extract ground minerals). The native and commercial inoculum was added to each potting soil (5% v: v) before sowing the seed. First, sterilized potting soil was added to fill half of the well of try, then 3 mL of AMF inoculant was added to the seed, and the well was filled with sterilized potting soil. One seed was sown in each tray cell, and the plants were grown in trays for 14 days to allow AMF to colonize the cucumber roots. On the 3rd of January 2023, the seedlings were transplanted into pots (1 L), filled with sterilized substrate containing sterilized greenhouse soil, potting soil, and perlite (1:1:1 v:v:v). Each pot was filled with the same mass of substrate (900 g/pot). The pots were arranged in a randomized complete block design on glasshouse benches, with four replicates totaling 48 pots (one plant per pot). Plants were allowed to grow for another two weeks to establish their root systems before the application of further treatments.

The potassium silicate treatment was applied once at a rate of 100 mL/pot, with concentration: 40 mL/liter. The watering regimes were based on the water holding capacity (WHC) of soil and were maintained under full unstressed conditions (80% WHC) or under drought stress (60% WHC). The WHC was determined by saturating each pot filled with 900 g of substrate and measuring the weight after 4 hours. The difference in weight was then multiplied by 80% or 60% to determine the amount of water to ap% ply for maintaining full or stressed conditions, respectively. The pots were weighed every two days, and water was applied to adjust the weight of each pot for both treatments. Sixty days after sowing, various parameter measurements were taken, and the plants were harvested.

### Plant Biomass Parameters

At the end of the experiment (60 days from planting), plant height of 48 plants was measured with a ruler from the base to the tip, and the roots were washed gently to remove soil. The fresh weights (FW) of both shoots and roots were recorded using an electric balance. Fresh shoots and roots were oven-dried at 80°C until a constant weight was reached, allowing for determination of dry weight (DW). A portion of the fresh leaves was frozen at -30 °C for subsequent biochemical analyses. Concurrently, selected fresh roots from each pot were placed in a solution of acetic acid and ethanol (1:3; v: v) for microscopy and calculation of the mycorrhizal root colonization percentage.

### Mycorrhizal Root Colonization Percentage

A subsample of the roots of each plant was stained as described by Phillips and Hyman (1970). Fine cucumber roots were cut into 1 cm lengths from each pot. Roots were washed, placed in test tubes, and treated with 10% KOH in a water bath at 90 °C for 1h. Then the roots were washed twice and covered with 2% (v/v) HCl for 30 min. The HCl was discarded and then the roots were subjected to a treatment involving immersion in a 0.05% (w/v) trypan blue solution in lactoglycerol, followed by incubation in a water bath set at 90°C for a duration of 20-30 min. Subsequently, the treated roots were transferred to Petri dishes containing a 50% glycerol solution for de-staining and viewing under an Olympus SZ51 microscope (Olympus Corporation, Japan). The colonization percentage was calculated by counting at least 20 root pieces per treatment using the following formula:

$$Root\ colonization\ (\%) = \frac{No.of\ colonized\ roots\ pieces}{No.of\ observed\ roots\ pieces} \times 100$$

### Chlorophyll Contents and Chlorophyll Fluorescence

Before harvesting, measurements were collected from the most recent fully expanded leaves of each plant. Leaf color was estimated using a SPAD-502 Plus device (Konica Minolta Inc., Japan) as a proxy for leaf chlorophyll. Leaf chlorophyll fluorescence was recorded using a multifunction plant efficiency analyzer (M-PEA+; Hansatech Instruments Ltd., Norfolk, UK). The ratio of variable (Fv) to maximum fluorescence (Fm) was recorded as photosystem II efficiency (PSII), whereas the ratio of minimum (F0) to maximum (Fm) fluorescence was recorded as the chlorophyll fluorescence index.

### Proline

Free proline was determined from the leaves following the method described by Bates et al. (1973). Fresh leaf samples (0.25 g) of each plant were ground in a pestle and mortar with 10 mL aqueous sulfosalicylic acid (3% w/v) until homogenized and then centrifuged at 3000 rpm for 10 minutes. Then, 2 mL of the supernatant was mixed with 2 mL of acid ninhydrin and 2 mL glacial acetic acid and incubated in a water bath at 100 °C for 1h. The reaction was terminated in an ice bath and 4 mL of toluene was added. The upper layer was separated and read using a spectrophotometer at a wavelength of 520 nm.

### Glycine Betaine

Glycine betaine was determined from 1 g of fresh leaves and homogenized in 10 mL of distilled water using a mortar and pestle. After filtration, sulfuric acid (1 mL; 2 *N*) was added to 1 mL of the sample extract. The mixture was then added to a tube with 0.2 mL of potassium tri-iodide solution and then was cooled in an ice bath for 1.5 h. Subsequently, 20 mL of cooled of 1, 2 dichloroethane along 2 mL of distilled water were added to the mixture. Two layers were formed in the mixture while the tube was still in an ice bath and mixed by passing a continuous stream of air for 1-2 mins. The absorbance of the lower layer was recorded at 365 nm (Grieve and Gratan, 1983).

### Catalase Activity

To determine catalase (CAT) activity, homogenized leaf tissue (using mortar and pestle) was added to phosphate buffer, 35% hydrogen peroxide ($H_2O_2$) was added, and CAT activity was determined by the disappearance of $H_2O_2$ (Chance and Maehly, 1955). Absorbance was recorded using a UV-VIS spectrophotometer to estimate CAT activity at 240 nm.

### Lipid Peroxidation

Lipid peroxidation was measured as the malondialdehyde (MDA) content and was determined as described by Heath and Packer (1968). First, 0.1 g the leaf tissue was homogenized in trichloroacetic acid (TCA) using a mortar and pestle. The homogenate was centrifuged for 15 min

(4500 rpm, 4 °C), and 0.5 mL of the supernatant was mixed with 1.5 mL 0.5% TBA (2-thiobarbituric acid) diluted in 20% TCA. The mixture was then incubated in a water bath at 95 °C for 25 min and submerged in ice to end the reaction. The MDA contents were estimated by measuring the difference in absorbance between 532 and 600 nm.

### Statistical Analysis

Analysis of variance technique was used for data analysis using the statistical program Minitab (Minitab® 17.3.1). For mean separation, Tukey's test was used.

## Results

### Plant Height and Biomass

The adverse effects of drought on plant height and biomass were significant ($p \leq 0.05$; Tables 1 and 4). In general, mycorrhizal plants had significantly greater plant height, shoot fresh weight and shoot dry weight compared to non-inoculated plants. PS treatment did not show any effect on plant height or shoot biomass (Tables 1 and 4). However, the root biomass (fresh and dry weight) was lower in the PS treatment. In addition, there were significant 2- and 3-way interac-

**Table 1.** Influence of arbuscular mycorrhizal fungi inoculation and potassium silicate application on plant height and biomass of cucumber plants under drought stress.

| **Watering regimes (W)** | **Plant height (cm)** | **Shoot fresh weight (g)** | **Shoot dry weight (g)** | **Root fresh weight (g)** | **Root dry weight (g)** | **Root colonization (%)** |
|---|---|---|---|---|---|---|
| Full | 95.3 a | 38 a | 5.5 a | 13 a | 0.72 a | 34 a |
| Stress | 81 b | 33 b | 4.7 b | 11 b | 0.62 b | 29 a |
| P value | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.091 |
| **Arbuscular mycorrhizal fungi (AMF)** | | | | | | |
| None (M1) | 85 b | 33 b | 4.8 b | 11.7 a | 0.7 a | 0 b |
| Native (M2) | 86 b | 35ab | 5 ab | 11.3 a | 0.7 a | 44 a |
| Com. (M3) | 99 a | 38 a | 5.5a | 12.2 a | 0.64 a | 50 a |
| P value | 0.001 | 0.018 | 0.029 | 0.181 | 0.070 | 0.001 |
| **Potassium silicate (PS)** | | | | | | |
| PS- | 89 a | 35 a | 5.3 a | 12.6 a | 0.7 a | 30 a |
| PS+ | 91a | 36 a | 4.9 a | 10.8 b | 0.6 b | 33 a |
| P value | 0.558 | 0.544 | 0.058 | 0.001 | 0.001 | 0.208 |
| **Interaction ($P \leq 0.05$)** | | | | | | |
| W × AMF | 0.667 | 0.600 | 0.652 | 0.186 | 0.025 | 0.476 |
| W × PS | 0.389 | 0.142 | 0.495 | 0.103 | 0.004 | 0.208 |
| AMF × PS | 0.071 | 0.411 | 0.865 | 0.050 | 0.019 | 0.641 |
| W × AMF× PS | 0.002 | 0.006 | 0.492 | 0.384 | 0.392 | 0.115 |

*Means sharing same letter do not differ significantly ($p \leq 0.05$).*

**Table 2.** Influence of arbuscular mycorrhizal fungi inoculation and potassium silicate application on SPAD value, PSII efficiency and chlorophyll fluorescence of cucumber plants under drought stress.

| **Watering regimes (W)** | **SPAD value** | **PSII efficiency** | **Chlorophyll fluorescence (F0/Fm)** |
|---|---|---|---|
| Full | 34 a | 0.504 a | 0.507 b |
| Stress | 33 a | 0.480 b | 0.530 a |
| P value | 0.506 | 0.011 | 0.026 |
| **Arbuscular mycorrhizal fungi (AMF)** | | | |
| None (M1) | 27 b | 0.514 a | 0.483 b |
| Native (M2) | 34.5 a | 0.489 ab | 0.539 a |
| Com. (M3) | 39 a | 0.473 b | 0.532 a |
| P value | 0.001 | 0.003 | 0.001 |
| **Potassium silicate (PS)** | | | |
| PS- | 33.7 a | 0.475 b | 0.530 a |
| PS+ | 33.3 a | 0.510 a | 0.507 b |
| P value | 0.863 | 0.001 | 0.034 |
| **Interaction ($P \leq 0.05$)** | | | |
| W × AMF | 0.505 | 0.062 | 0.006 |
| W × PS | 0.805 | 0.001 | 0.002 |
| AMF × PS | 0.001 | 0.004 | 0.001 |
| W × AMF× C | 0.012 | 0.388 | 0.860 |

*Means sharing same letter do not differ significantly (p≤0.05).*

**Table 3.** Influence of arbuscular mycorrhizal fungi inoculation and potassium silicate application on leaf free proline contents, glycine betaine, catalase activity, malondialdehyde contents and root colonization of cucumber plants under drought stress.

| Watering regimes (W) | Proline (mmol $g^{-1}$ FW) | Glycine betaine (mg/l) | CAT (units $mg^{-1}$ protein) | MDA (nmol $g^{-1}$ FW) |
|---|---|---|---|---|
| Full | 2.06 a | 17.5 b | 12.5 b | 30.11 a |
| Stress | 1.73 a | 18.8 a | 12.7 a | 30.94 a |
| P value | 0.101 | 0.050 | 0.001 | 0.436 |
| **Arbuscular mycorrhizal fungi (AMF)** | | | | |
| None (M1) | 1.56 b | 8.7 b | 12.5 b | 29.39 a |
| Native (M2) | 1.76 ab | 23.6 a | 12.7 a | 31.02 a |
| Com. (M3) | 2.35 a | 22.3 a | 12.6 a b | 31.17 a |
| P value | 0.007 | 0.001 | 0.003 | 0.320 |
| **Potassium silicate (PS)** | | | | |
| PS- | 1.8 a | 18.5 a | 12.6 a | 29.50 a |
| PS+ | 2 a | 17.9 a | 12.6 a | 31.55 a |

| P value | 0.266 | 0.403 | 0.113 | 0.059 |
|---|---|---|---|---|
| **Interaction (P ≤ 0.05)** | | | | |
| W × AMF | 0.068 | 0.001 | 0.098 | 0.001 |
| W × PS | 0.057 | 0.510 | 0.642 | 0.495 |
| AMF × PS | 0.058 | 0.001 | 0.001 | 0.003 |
| W × AMF× PS | 0.031 | 0.001 | 0.006 | 0.004 |

*Means sharing same letter do not differ significantly (p≤0.05).*

**Table 4.** Analysis of variance for influence of arbuscular mycorrhizal fungi inoculation and potassium silicate application on plant height and biomass of cucumber plants under drought stress.

| SOV | Mean sum of squares | | | | | | |
|---|---|---|---|---|---|---|---|
| | **df** | **Plant height** | **Shoot fresh weight** | **Shoot dry weight** | **Root fresh weight** | **Root dry weight** | **Root colonization** |
| W | 1 | 4002*** | 349*** | 8 *** | 64*** | 0.13 *** | 276NS |
| AMF | 2 | 922*** | 90* | 2.2* | 3NS | 0.014 NS | 11938*** |
| PS | 1 | 22NS | 7.5NS | 2NS | 41*** | 0.08*** | 151NS |
| W × AMF | 2 | 25NS | 10NS | 0.24 NS | 3.2 NS | 0.02* | 69NS |
| W × PS | 1 | 47NS | 45NS | 0.26 NS | 5 NS | 0.05** | 151NS |
| AMF × PS | 2 | 177NS | 18NS | 0.08NS | 6* | 0.02* | 41NS |
| W × AMF× PS | 2 | 464** | 116** | 0.4 NS | 2NS | 0.005NS | 210 NS |
| Error | 36 | 62 | 20 | 0.56 | 1.8 | 0.005 | 92 |

*SOV= Source of variation; df= Degree of freedom; W= watering regimes; AMF= Arbuscular mycorrhizal fungi; PS= potassium silicate. NS=Non-Significant at probability levels of 0.05; ***=Significant at probability level of 0.001.*

tions between watering regime, AMF, and PS on plant height, shoot FW, root FW, and DW ($p \leq 0.05$; Tables 1 and 4).

The interaction effect of the watering regime, AMF, and PS on plant height was significant ($p \leq 0.05$). Plants inoculated with commercial AMF and grown under the full watering regime without PS had the highest plant height values (Figure 1). In addition, under stress conditions, plants inoculated with commercial AMF and treated with PS had significantly higher values for all measured parameters compared to native AMF and non-inoculated plants (Figure 1).

The interaction effect of watering regime, AMF, and PS on shoot fresh weight was significant ($p \leq 0.05$) (Figure 2). In general, AMF-inoculated plants, regardless of the type of AMF, had significantly higher shoot fresh weight values than non-inoculated plants with the exception of commercial AMF with PS under stress condition.

The interaction effect of AMF and PS on root fresh weight was significant (Figure 3a) showed that non-inoculated plants treated with PS had the lowest root weight. This effect was

**Figure 1.** Influence of arbuscular mycorrhizal fungi (AMF) inoculation and potassium silicate application on plant height of cucumber plants under drought stress. Bars sharing the same letter do not differ significantly (p≤0.05). M1=No AMF, M2=Native AMF, M3=Commercial AMF. PS+= potassium silicate and PS-= No potassium silicate.

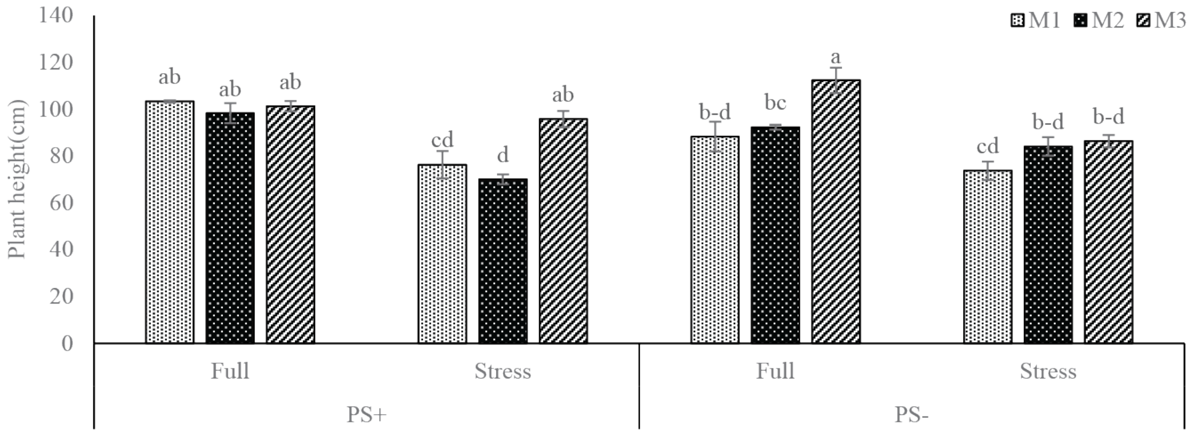
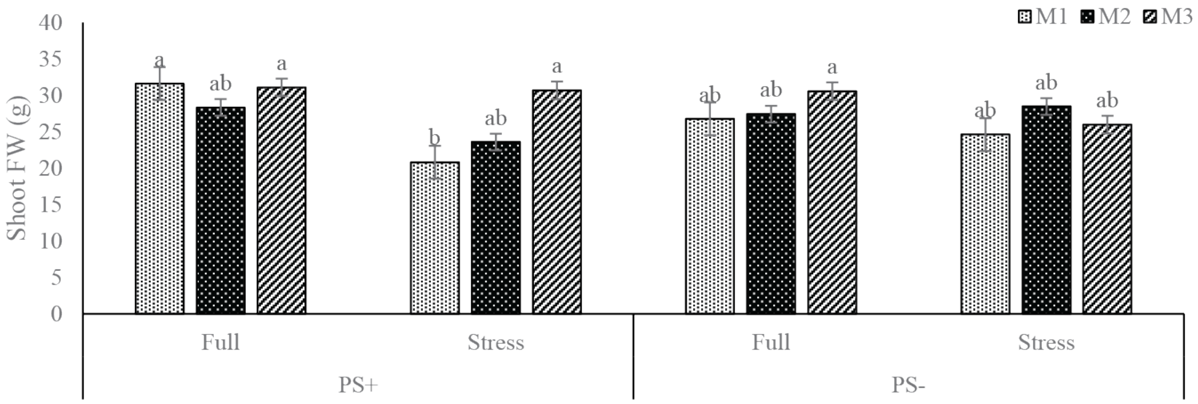


**Figure 2.** Influence of arbuscular mycorrhizal fungi (AMF) inoculation and potassium silicate application on shoot fresh weight (FW) of cucumber plants under drought stress. Bars sharing the same letter do not differ significantly (p≤0.05). M1=No AMF, M2=Native AMF, M3=Commercial AMF. PS+= potassium silicate and PS-= No potassium silicate.



not significantly different among the inoculation treatments. In addition, the interaction effect of watering regime with AMF or PS, on root dry weight was significant (Figure 3b and 3c) and showed that non-inoculated plants in the full watering regime had the highest values.

## Percentage of Mycorrhizal Root Colonization

All fungal structures were observed including arbuscules, vesicles and hyphae within the cortex cells of cucumber roots on the cucumber plants inoculated with mycorrhiza, whereas these structures were not observed in non-inoculated plants. The analysis of variance indicated that the effects of the watering regime, AMF, and PS were not significant (Tables 1 and 4). In addition, the interaction effect of watering regime, AMF, and PS on root colonization was not significant (Tables 1 and 4).

## Chlorophyll Content and Chlorophyll Fluorescence

The effects of the watering regime, AMF, and PS were significant for the chlorophyll fluores-

**Figure 3.**Influence of arbuscular mycorrhizal fungi (AMF) inoculation and potassium silicate application on root fresh and dry weight (FW) of cucumber plants under drought stress (a: AMF and PS interaction on Root FW, b: watering regime and AMF interaction on root DW, c: watering regime and PS interaction on root DW and d: AMF and PS interaction on root DW). Bars sharing the same letter do not differ significantly (p≤0.05). M1=No AMF, M2=Native AMF, M3=Commercial AMF. PS+= potassium silicate and PS-= No potassium silicate.

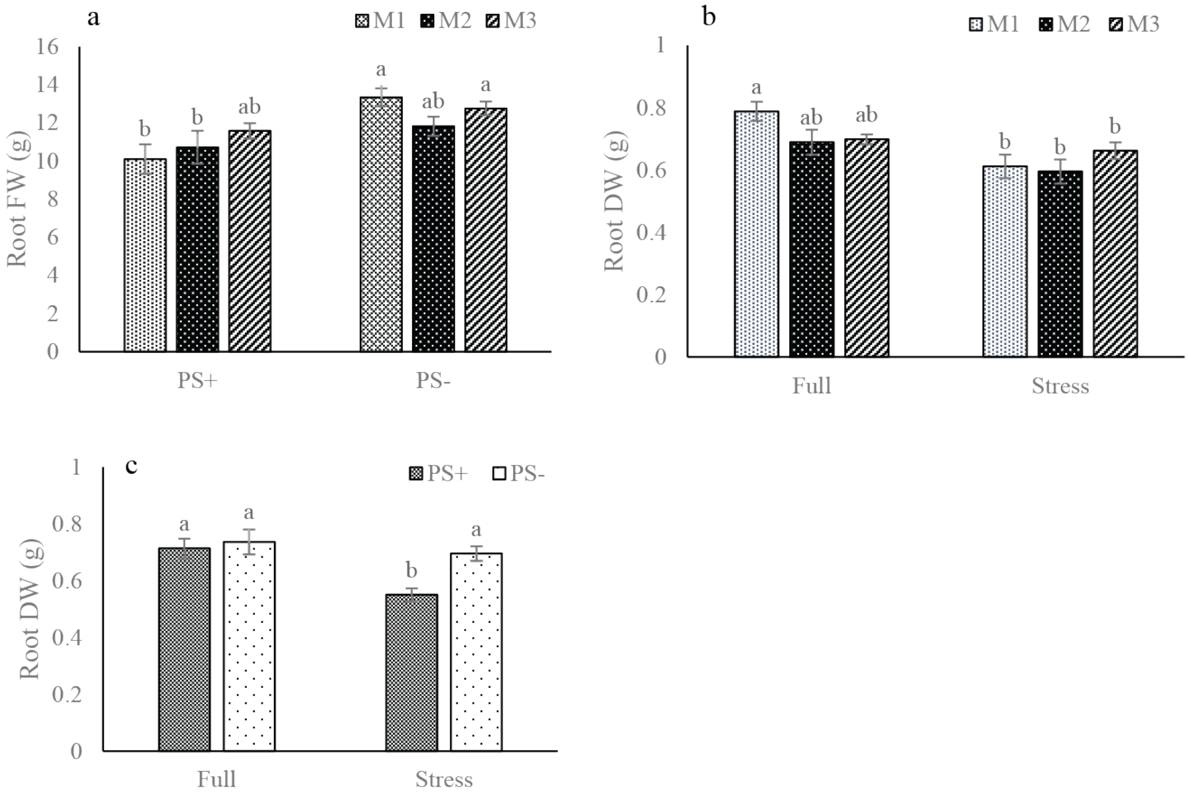


**Table 5.** Analysis of variance for influence of arbuscular mycorrhizal fungi inoculation and potassium silicate application on SPAD value, PSII efficiency and chlorophyll fluorescence of cucumber plants under drought stress.

| SOV | Mean sum of squares | | | |
|---|---|---|---|---|
| | **df** | **SPAD value** | **PSII efficiency** | **Chlorophyll fluorescence (F0/Fm)** |
| W | 1 | 17.3 NS | 0.007* | 0.006 * |
| AMF | 2 | 585*** | 0.007** | 0.015*** |
| PS | 1 | 1 NS | 0.015*** | 0.006* |
| W × AMF | 2 | 27 NS | 0.003 NS | 0.007** |
| W × PS | 1 | 2.4 NS | 0.0197*** | 0.0125** |
| AMF × PS | 2 | 1055 *** | 0.006** | 0.0145*** |
| W × AMF× PS | 2 | 193 ** | 0.001 NS | 0.0002 NS |
| Error | 36 | 38 | 0.001 | 0.0011 |

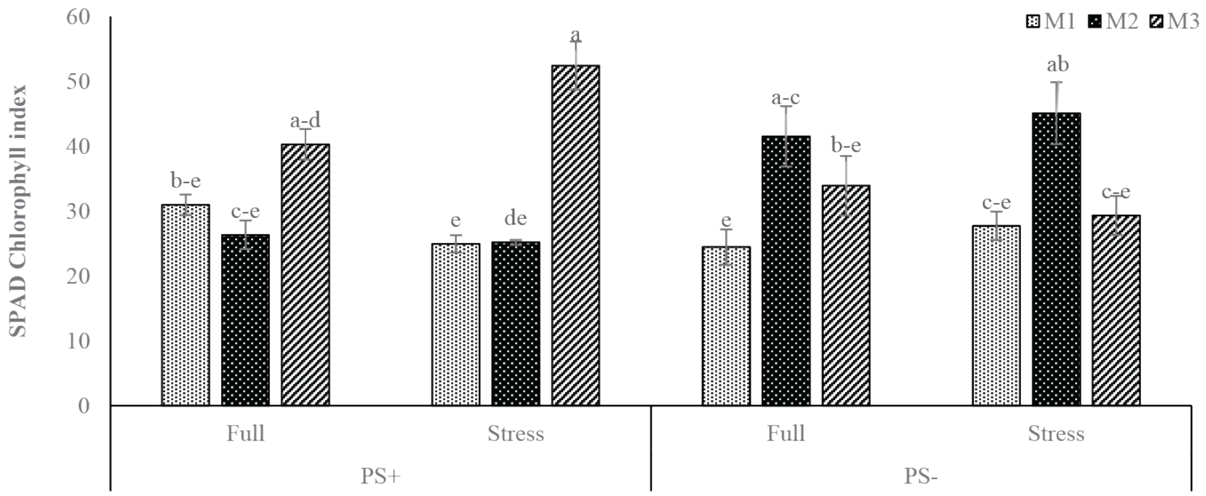
*SOV= Source of variation; df= Degree of freedom; W= watering regimes; AMF= Arbuscular mycorrhizal fungi; PS= potassium silicate. NS=Non-Significant at probability levels of 0.05; ***=Significant at probability level of 0.001.*

**Table 6.** Analysis of variance for influence of arbuscular mycorrhizal fungi inoculation and potassium silicate application on leaf free proline contents, glycine betaine, catalase activity, malondialdehyde contents and root colonization of cucumber plants under drought stress.

| SOV | Mean sum of squares | | | | |
|---|---|---|---|---|---|
| | **df** | **Leaf free proline** | **Glycine betaine** | **CAT** | **MDA** |
| W | 1 | 1.34 NS | 21 * | 0.2465 *** | 8.26 NS |
| AMF | 2 | 2.7071** | 1087 *** | 0.1250 ** | 15.65 NS |
| PS | 1 | 0.6053NS | 3.62 NS | 0.0469NS | 50.73 NS |
| W × AMF | 2 | 1.3693NS | 90 *** | 0.0442NS | 162.73 *** |
| W × PS | 1 | 1.8291 NS | 2.2 NS | 0.0039 NS | 6.32 NS |
| AMF × PS | 2 | 1.4638 NS | 657.84*** | 0.1466*** | 92.18 ** |
| W × AMF× PS | 2 | 1.8077* | 227.29 *** | 0.1056** | 84.22 ** |
| Error | 36 | 0.4736 | 5 | 0.0178 | 13.29 |

*SOV= Source of variation; df= Degree of freedom; W= watering regimes; AMF= Arbuscular mycorrhizal fungi; PS= potassium silicate. NS=Non-Significant at probability levels of 0.05; ***=Significant at probability level of 0.001.*

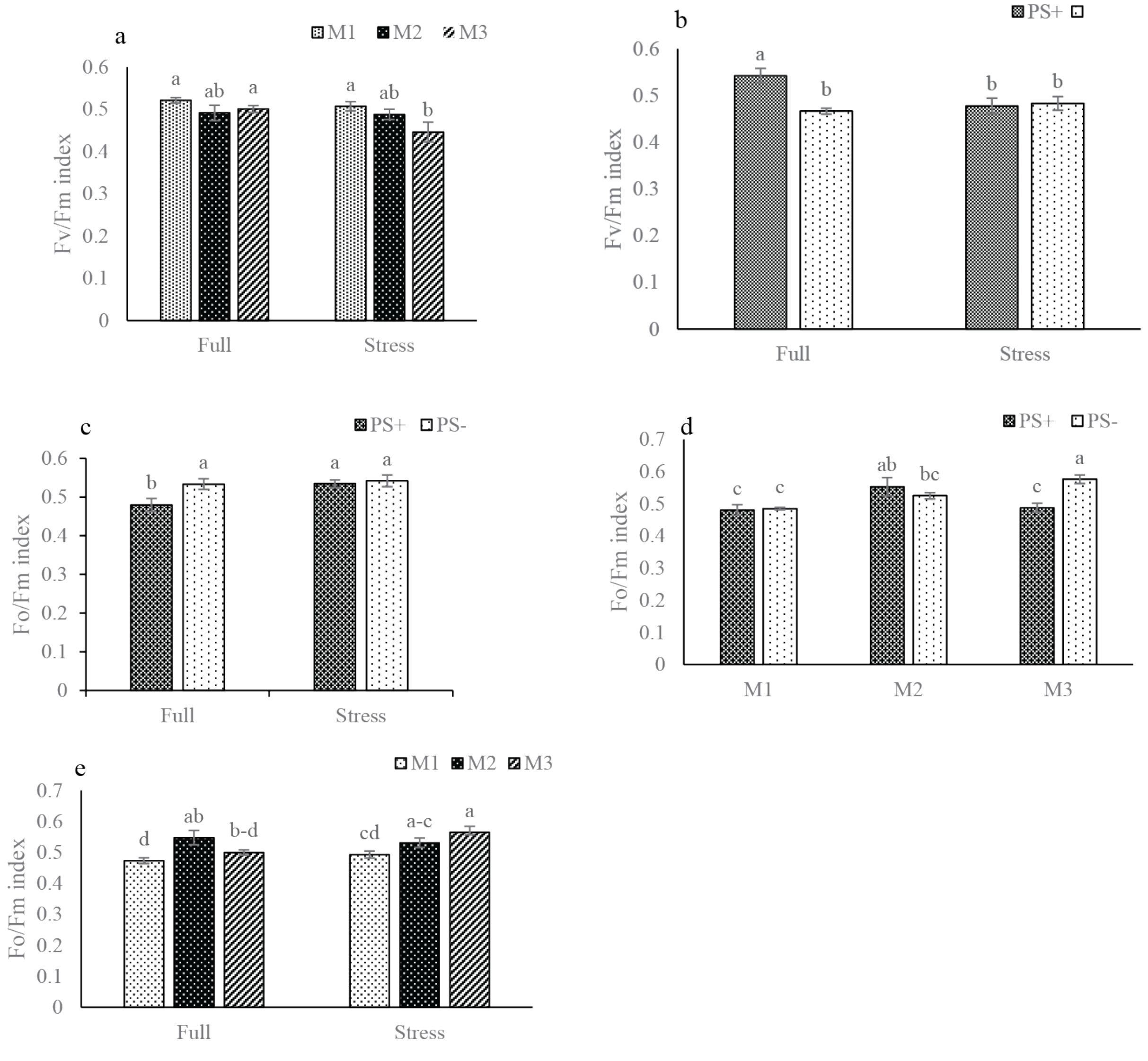
**Figure 4.** Influence of arbuscular mycorrhizal fungi (AMF) inoculation and potassium silicate application on SPAD chlorophyll value of cucumber plants under drought stress. Bars sharing the same letter do not differ significantly (p≤0.05). M1=No AMF, M2=Native AMF, M3=Commercial AMF. PS+= potassium silicate and PS-= No potassium silicate.



cence parameters Fv/Fm (PSII efficiency) and F0/Fm (Tables 2 and 5). In addition, AMF was significant for chlorophyll content (SPAD value) (Table 5). Watering regime had a differential effect on the chlorophyll fluorescence parameters, with PSII efficiency having the highest value under full watering regime and F0/Fm having the highest value under stress. PS treatment also showed a differential effect on chlorophyll fluorescence readings, with treated plants having the highest values of PSII efficiency and the lowest value of F0/Fm. AMF inoculation significantly increased SPAD and F0/Fm, but not for PSII efficiency (Table 2).

The interaction effect of the watering regime, AMF, and PS on SPAD was significant (p

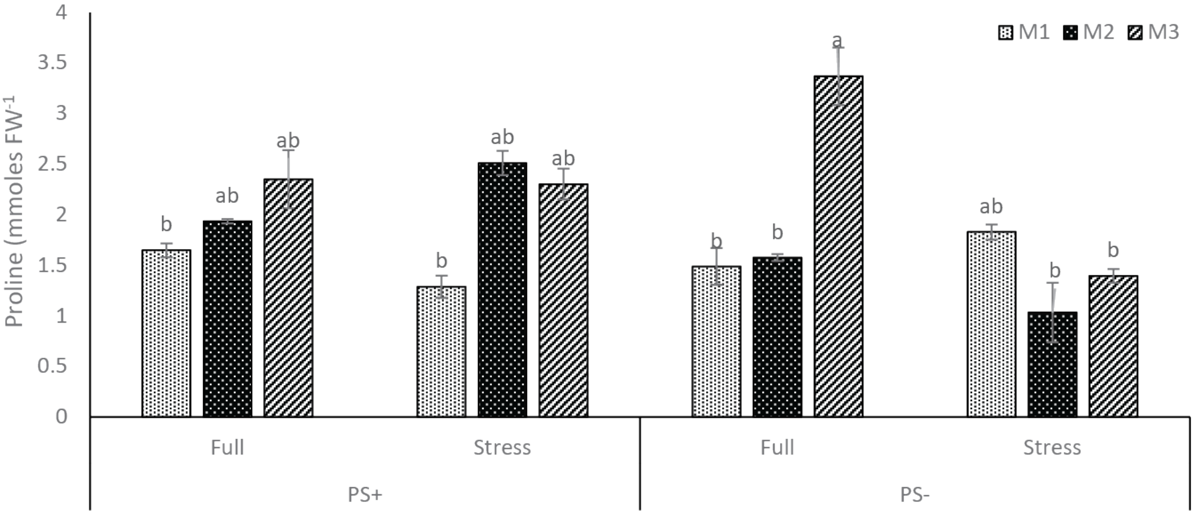
**Figure 5.** Influence of arbuscular mycorrhizal fungi (AMF) inoculation and potassium silicate application on photosynthesis fluorescence (Fv/Fm and F0/Fm) of cucumber plants under drought stress (a: AMF and watering regime interaction on Fv/Fm index, b: watering regime interaction on Fv/Fm index, c: watering regime and PS interaction on F0/Fm index, d: AMF and PS interaction on F0/Fm index and e: AMF and watering regime on F0/Fm index). Bars sharing the same letter do not differ significantly ($p \leq 0.05$). M1=No AMF, M2=Native AMF, M3=Commercial AMF. PS+= potassium silicate and PS-= No potassium silicate.



$\leq 0.05$) (Table 2, Table 5 and Figure 4). Plants inoculated with commercial AMF grown under both watering regimes and treated with PS had higher SPAD values than those of native AMF and non-inoculated plants (Figure 4). In contrast, plants inoculated with native AMF grown under both watering regimes and untreated with PS had significantly higher SPAD values than the commercial AMF and non-inoculated plants.

The interaction effect of watering regime and AMF on Fv/Fm (PSII efficiency) was significant (Figure 5a) and showed that plants inoculated with commercial AMF under stress conditions had the lowest values. The interaction effect of watering regime and PS on PSII efficiency was significant (Figure 5b), and plants treated with PS under the full watering regime had the highest values.

**Figure 6.** Influence of arbuscular mycorrhizal fungi (AMF) inoculation and potassium silicate application on leaf proline of cucumber plants under drought stress. Bars sharing the same letter do not differ significantly ($p \leq 0.05$). M1=No AMF, M2=Native AMF, M3=Commercial AMF. PS+= potassium silicate and PS-= No potassium silicate.



For F0/Fm, the interaction effect of watering regime and PS was significant (Figure 5c) and showed that plants treated with PS under the full watering regime had the lowest value. In addition, the interaction effect of AMF and PS on F0/Fm was significant (Figure 5d), plants inoculated with commercial AMF and untreated plants treated with PS had the highest values. In addition, the interaction effect of watering regime and AMF on F0/Fm was significant (Figure 5e). Inoculated plants with commercial AMF under stress condition had the highest value of F0/Fm, while non-inoculated plant with full watering regime had the lowest vale of F0/Fm (Figure 5e).

## Proline

Leaf proline content was significantly higher in plants inoculated with AMF than in non-inoculated plants, especially for commercial inoculants ($p \leq 0.05$; Tables 3 and 6). The effects of the watering regime and PS treatment on leaf proline content were not significant. The interaction effect of the watering regime, AMF, and PS on leaf proline content was significant ($p \leq 0.05$; Table 3 and Figure 6). Plants inoculated with commercial AMF grown under the full watering regime and not treated with PS had the highest leaf proline content (Figure 6). Other treatments comparisons for proline were not significantly different.

## Glycine Betaine

Drought stress significantly increased the leaf glycine betaine content compared to that in the full watering regime ($p \leq 0.05$; Tables 3 and 6). Plants inoculated with native and commercial AMF had significantly increased levels of glycine betaine compared to non-inoculated plants ($p \leq 0.05$; Table 3 and Table 6). The effect of the PS treatment was not significant.

The interaction effect of the watering regime, AMF, and PS on leaf glycine betaine was significant ($p \leq 0.05$; Table 3 and Figure 7). Plants inoculated with commercial AMF grown under the full watering regime and treated with PS had the highest values of leaf glycine betaine. Moreover, both inoculants with PS under stees conditions showed higher leaf glycine compared

**Figure 7.** Influence of arbuscular mycorrhizal fungi (AMF) inoculation and potassium silicate on leaf glycine betaine of cucumber plants under drought stress. Bars sharing the same letter do not differ significantly (p≤0.05). M1=No AMF, M2=Native AMF, M3=Commercial AMF. PS+= potassium silicate and PS-= No potassium silicate.

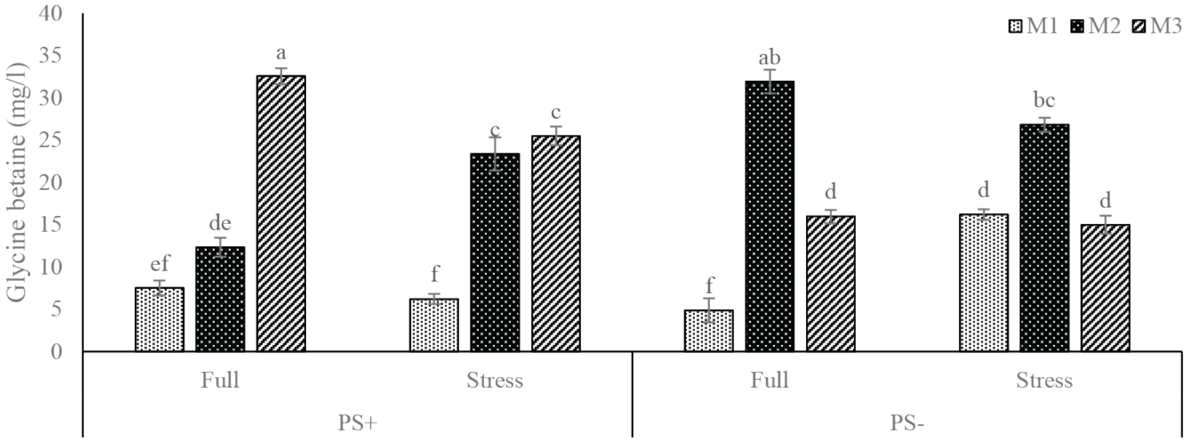
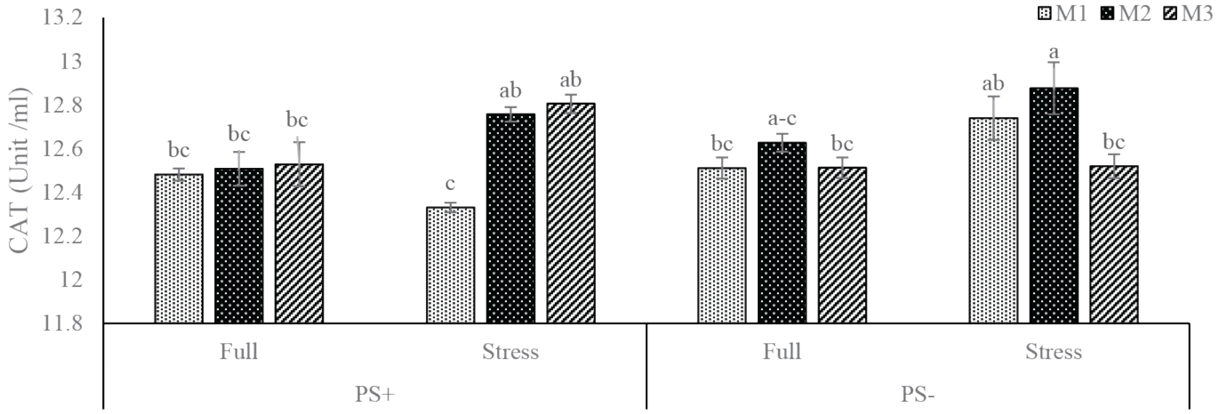


**Figure 8.** Influence of arbuscular mycorrhizal fungi (AMF) inoculation and potassium silicate application on leaf CAT of cucumber plants under drought stress. Bars sharing the same letter do not differ significantly (p≤0.05). M1=No AMF, M2=Native AMF, M3=Commercial AMF. PS+= potassium silicate and PS-= No potassium silicate.



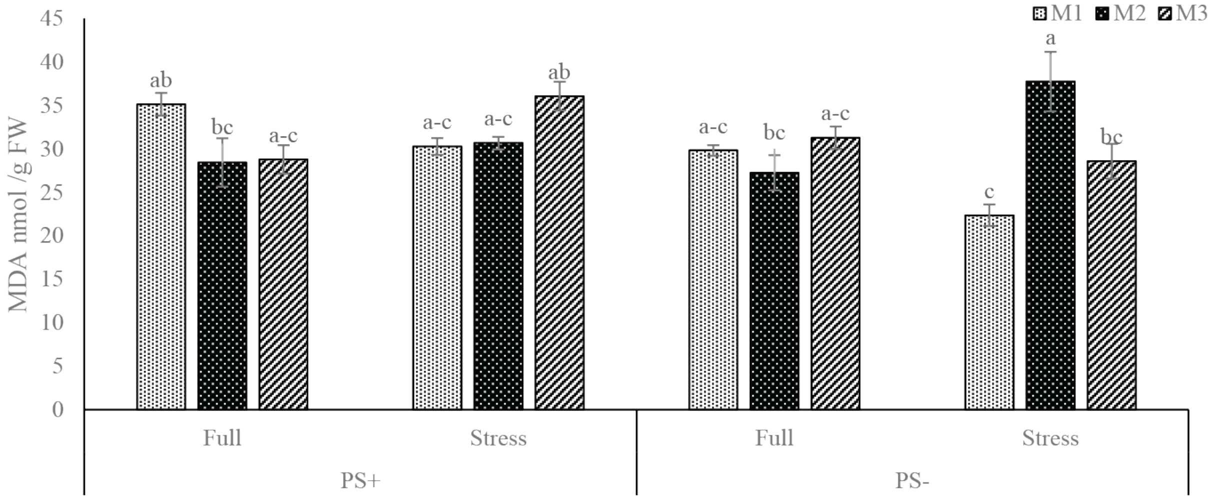
with non-inoculated plants. In addition, under stress conditions and without PS treatment, plants with native AMF showed the highest glycine betaine value compared to AMF commercial and non-inoculated plants (Figure 7).

## Catalase Activity

The effects of drought stress and AMF on CAT activity were significant (p ≤ 0.05; Tables 3 and 6). The interaction effect of watering regime, AMF, and PS was also significant (Tables 3 and 6). Drought stress significantly increased CAT values compared to full watering plants (p ≤ 0.05; Table 3). Plants inoculated with native AMF had the highest value, followed by commercial AMF, and the non-inoculated plants had the lowest value (Table 3). The effect of the PS treatment was not significant.

The interaction effect of watering regime, AMF, and PS on CAT showed that plants inoculated with native AMF grown under stress conditions and not treated with PS had the hi-

**Figure 9.** Influence of arbuscular mycorrhizal fungi (AMF) inoculation and potassium silicate application on leaf MDA of cucumber plants under drought stress. Bars sharing the same letter do not differ significantly (p≤0.05). M1=No AMF, M2=Native AMF, M3=Commercial AMF. PS+= potassium silicate and PS-= No potassium silicate.



ghest CAT values (Figure 8). In addition, mycorrhizal plants under stress conditions with PS showed significantly higher CAT values than the non-inoculated plants (Figure 8).

### Lipid Peroxidation (MDA)

There was no significant main effect of the watering regime, AMF, or PS on malondialdehyde content (MDA) ($p \leq 0.05$; Tables 3 and 6). However, the interaction effect of these factors was significant ($p \leq 0.05$; Tables 3 and 6). Plants inoculated with native AMF grown under drought stress and untreated with PS showed the highest MDA values (Figure 9).

## Discussion

As expected, this study revealed that drought had a strong significant effect on the responses of several plant parameters to water deficit (Table 1). Soil water deficit negatively affects plant physiology, reducing photosynthesis, growth, and nutrient uptake, while increasing oxidative stress, leading to decreased biomass production and water-use efficiency (Anjum et al., 2011; Kapoor et al., 2020).

The AMF, regardless of the type of inoculant, increased plant height, shoot fresh weight, and shoot dry weight (Table 1, Figures 1 and 2). Previous studies have demonstrated that when subjected to drought stress, plants inoculated with AMF exhibit a substantial increase in growth compared to plants without mycorrhizal association (Chandrasekaran, 2022). This is because AMF enhance water and nutrient uptake from soil and soil micropores by establishing a network of mycelium that extends from the plant's roots into the surrounding soil. This extensive network of fungal threads creates a closer contact between soil particles and the plant's roots, enabling water and nutrients to move more easily (Wu et al., 2017; Cheng et al., 2021; Sharifian et al., 2022). The interaction between AMF and PS was also significant. Plants inoculated with commercial AMF under drought stress with PS had the highest plant height and shoot fresh weight compared to native and non-inoculated plants (Figures 1 and 2) and the commercial AMF inoculum also increased plant height in the full water and non-PS treatments (Figure 1). This in for some points agree with Sharma et al. (2022) found that the combined application of PS and *Funneliformis mosseae* significantly increased the growth and biomass of onions grown under drought stress.

Drought stress impairs PSII function by reducing stomatal closure and $CO_2$ influx, increasing photoinhibition, and damaging the oxygen-evolving complex and the electron transport chain (Farooq et al., 2017; Zhao, 2019). The Fv/Fm ratio is a sensitive indicator of plant photosynthetic performance, and a decline in this ratio reflects a reduction in PSII efficiency, or photoinhibition induced by stress conditions (Guidi et al., 2019; Xia et al., 2023). This leads plants to close PSII reaction centers to prevent damage and, in some cases, leads to an increase in the F0/Fm ratio due to high light energy. In the present study, drought decreased the efficiency of PSII (Fv/Fm) (Table 2) but increased the value of chlorophyll fluorescence (F0/Fm).

Mycorrhizal inoculation was found to have a significant positive effect on SPAD value and chlorophyll fluorescence (F0/Fm), regardless of inoculant type, compared to non-inoculated plants (Table 2). This is likely due to the beneficial effects of AMF on plant nutrient uptake and water relations, which can help mitigate the negative effects of drought stress (Jadrane et al., 2021). In addition, the present study found no significant differences between native and commercial AMF in SPAD, PSII, and chlorophyll fluorescence. This suggests that both types of AMF can be used to improve plant growth and physiology. The findings of the current study may challenge those of Pellegrino et al. (2022), who reported that a mixture of foreign arbuscular mycorrhizal fungi (AMF) isolates provided greater benefits to alfalfa than a local mixture of AMF isolates. The commercial inoculum contained in addition to AMF also some other plant growth promoting substances which may have led to the significant increased root growth under non-stressed conditions. Such root growth promoting effects are in particular known from sea-weed extracts and humates.

The PS can reduce drought stress in plants by enhancing photosynthesis, water use efficiency, and cell wall strength (Farrooq et al., 2009; Gomaa et al., 2021; Hafez et al., 2021). The continued deposition of silicon in the transpiration organs forms a double layer, which leads to lower stomatal opening and less water loss via leaf transpiration (Araújo et al., 2019). In the current study, potassium silicate increased PSII efficiency (Fv/Fm), but decreased chlorophyll fluorescence (F0/Fm) (Table 2). This variation in results may be due to PS dosage (Alkharpotly et al., 2019), or application method e.g., foliar sprays may be more effective than soil application (Abdou et al., 2022; El-Okkiah et al., 2022).

The present study showed that the combined use of commercial AMF and PS enhanced plant SPAD under drought stress compared to non-inoculated plants that were only treated with PS. Thus, PS may not be able to enhance SPAD readings in the absence of AMF under stress conditions. This finding contradicts Abd El-Gawad et al. (2017), who reported that applying foliar PS (200 ppm) to potato plants under drought stress increased the SPAD reading.

The results in this study showed that the leaf proline content was significantly higher in plants inoculated with commercial AMF than in non-inoculated plants (Table 3). AMF are known to induce proline accumulation and regulate nitric oxide (NO), which is an important signal for proline metabolism in plants (Liu et al., 2022). The AMF regulate the root uptake of available nutrients and enhance growth, even under stress conditions (Chun et al., 2018). In addition, AMF are also associated with increased biosynthesis of proline through the glutamate and ornithine pathways (Alotaibi et al., 2021). The current study is not consistent with previous studies, which have shown that higher proline levels are found in rice under drought stress (Hu et al., 1992), rice exposed to low temperatures (Liu et al., 2022), lotus (Alotaibi et al., 2021), and cucumber (Hashem et al., 2018; Liu et al.,2023).In addition, Begum et al. (2019)found that AMF inoculation increased the content of the compatible solute proline in maize plants, which helped to maintain the relative water content of plants under drought stress. The effects of the watering regime and PS treatment on leaf proline content were not significant. However, the interaction between AMF and PS was significant, with plants that received both commercial AMF and without PS treatment having the highest leaf proline content under the full water regime (Figure 6).

The results showed that leaf glycine betaine content was significantly affected by the watering regime and AMF treatments. Drought stress significantly increased the leaf glycine betaine content compared to that in the full watering regime (Table 3). Glycine betaine is a compatible solute synthesized by plants in response to environmental constraints e.g., salinity, drought, cold temperature, and heavy metals (Anjum et al., 2011). Osmoregulation and osmoprotection are functions of glycine betaine, which accumulates as osmolytes in plant cells (Giri, 2011). Glycine betaine enhances plant growth, the photosynthetic system, and antioxidant function, while also safeguarding crucial enzymes involved in the dicarboxylic acid cycle and photosystem, which are pivotal for the efficient operation of photosynthesis and respiration in plants (Ma et al., 2007; Tang et al., 2022). The accumulation of glycine betaine has been documented to protect photosynthesis by improving the efficiency of Rubisco enzyme activity (Begum et al., 2019). The exogenous applications of glycine betaine enhance plant tolerance to cold temperatures (Mickelbart and Boine, 2020), saline conditions (García-Caparrós et al., 2020), and drought (Tisarum et al., 2019).

In the present study, drought stress induced glycine betaine production in cucumber plants that were able to cope with drought stress (Table 3). In addition, mycorrhizal inoculation significantly increased leaf glycine betaine content, regardless of the type of AMF used, compared to non-inoculated plants (Table 3). Accumulation of osmolytes (e.g. glycine betaine) as found in our study (Tab 3) is considered a ubiquitous response that accelerates water uptake under drought conditions (Begum et al., 2019). AMF-mediated nitrogen, phosphorus, and potassium uptake and water absorption can help plants produce glycine betaine, which protects plants against reactive oxygen species as a result of stress (Rasouli et al., 2023). In the present study, the interaction between AMF and PS was also significant, with plants that received both commercial AMF and PS treatments without water stress showing the highest leaf glycine betaine content compared with native AMF and non-inoculated control plants (Figure 7). This suggests that PS may enhance the synergistic effect of AMF in inducing glycine betaine accumulation in plants (Figure 7). In drought-stressed plants, the combination of PS with both AMF inoculants resulted in significantly higher glycine betaine levels (Figure 7). In general, potassium silicate has been reported to help plants cope with the adverse effects of drought stress (Farooq et al., 2009; Gomaa et al., 2021; Hafez et al., 2021).

Drought stress significantly increased the CAT value compared to plants under full watering (Table 3). Catalase is an antioxidant enzyme responsible for breaking down hydrogen peroxide ($H_2O_2$), a harmful byproduct of ROS, into hydrogen and oxygen to maintain cellular processes, and is considered a biomarker for oxidative stress in plants (Mahomoodally, 2022). The results revealed that inoculation with either the native inoculant or the commercial one significantly improved the antioxidant defense system “CAT” of cucumber subjected to drought stress (Table 3). Mycorrhizal plants exhibit significantly elevated activities of the antioxidant enzymes catalase, peroxidase, and superoxide dismutase compared to non-mycorrhizal plants under drought stress (Ma et al., 2022). Moreover, in present study, mycorrhizal plants under stress conditions with PS showed significantly higher CAT values than the non-inoculated plants. Silicon highly effective in stimulating growth and increasing the activity of antioxidant enzymes (Hajiboland et al., 2017). By improving photosynthetic efficiency, water use efficiency, lowering transpiration, and fortifying plant cell walls, potassium silicate may mitigate drought stress in plants (Gomaa et al., 2021; Hafez et al., 2021). This interactive effect of AMF and PS suggests combining use of them can increase the catalase activity of cucumber plants to enhance drought tolerance.

Our results highlighted that the combined application of PS and AMF inoculants can protect cucumber seedlings from drought stress; however, further research is needed to optimize the PS application method and dosage and to investigate their long-term effects on plant growth and yield under field conditions.

## Conclusion

This study found that AMF inoculants can be used to improve the growth, photosynthesis, and stress tolerance mechanisms of cucumber plants under drought stress. AMF increased the SPAD value, proline and glycine betaine accumulation, and catalase activity. In many cases, the results revealed that native AMF species were just as effective as commercial AMF species, resulting in similar plant responses; therefore, both AMF inoculants used were effective in ameliorating the drought stress. Use of PS alone reduced root fresh and dry weight. However, the synergism between AMF and PS under drought stress significantly improved plant height, shoot fresh weight, SPAD value, glycine betaine, and catalase activity compared to PS alone. Our central hypothesis stands accepted, since our body of evidence shows that the protection of cucumber seedlings from drought stress was achieved by using a combination of PS and both AMF inoculants. Further research is needed to optimize the application method and dosage of PS and to investigate the long-term effects of this combined application on cucumber plant growth and yield in field conditions.

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