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Compost and arbuscular mycorrhizal fungi: A synergistic approach to improve drought tolerance in *Capsicum annuum* L.

Compost y hongos micorrízicos arbusculares: Un enfoque sinérgico para mejorar la tolerancia a la sequía en *Capsicum annuum* L.

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ABSTRACT

Drought stress significantly limits the growth and productivity of crops such as *Capsicum annuum* L. This study evaluated the synergistic effects of compost and arbuscular mycorrhizal fungi (AMF) on enhancing drought tolerance in capsicum plants. Two AMF species, *Funneliformis mosseae* and *Rhizophagus intraradices*, were applied in conjunction with compost to assess their combined impact on physiological and biochemical responses under drought stress. Key parameters such as relative water content (RWC), photosynthetic pigments, mycorrhizal colonization, nitrogen and phosphorus uptake, and antioxidant enzyme activities (SOD, CAT, APX, GR, and DHAR), were analyzed. The results showed that the combined application of AMF and compost significantly improved plant growth, nutrient acquisition, and antioxidant defense mechanisms compared with individual treatments. Activities of SOD, CAT, APX, GR, and DHAR increased substantially in treated plants, with the greatest upregulation observed under combined AMF + compost application during drought. The synergistic interaction of AMF and compost enhanced shoot and root biomass, chlorophyll content, and oxidative stress mitigation. These findings suggest that integrating AMF and compost can be a sustainable strategy to improve drought resilience and productivity in capsicum cultivation.

Palabras clave:
Enzimas antioxidantes
Absorción de nutrientes
Estrés oxidativo
Interacciones en la rizosfera
Estado hídrico

RESUMEN

El estrés por sequía limita significativamente el crecimiento y la productividad de cultivos como *Capsicum annuum* L. Este estudio evaluó los efectos sinérgicos del compost y de los hongos micorrízicos arbusculares (HMA) en la mejora de la tolerancia a la sequía en plantas de pimiento. Se aplicaron dos especies de HMA, *Funneliformis mosseae* y *Rhizophagus intraradices*, junto con compost, para evaluar su efecto combinado sobre las respuestas fisiológicas y bioquímicas bajo condiciones de estrés hídrico. Se analizaron parámetros clave como el contenido relativo de agua (CRA), los pigmentos fotosintéticos, la colonización micorrízica, la absorción de nitrógeno y fósforo, y las actividades de las enzimas antioxidantes (SOD, CAT, APX, GR y DHAR). Los resultados mostraron que la aplicación combinada de HMA y compost mejoró significativamente el crecimiento vegetal, la adquisición de nutrientes y los mecanismos de defensa antioxidante en comparación con los tratamientos individuales. Las actividades de SOD, CAT, APX, GR y DHAR aumentaron considerablemente en las plantas tratadas, observándose la mayor regulación positiva bajo la aplicación combinada de HMA + compost durante condiciones de sequía. La interacción sinérgica entre HMA y compost aumentó la biomasa de brotes y raíces, el contenido de clorofila y la mitigación del estrés oxidativo. Estos hallazgos sugieren que la integración de HMA y compost puede ser una estrategia sostenible para mejorar la resiliencia a la sequía y la productividad en el cultivo de pimiento.

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Drought stress poses a critical threat to global agriculture by reducing both yield and quality of crops. Capsicum (*Capsicum annuum* L.), an economically important vegetable, is particularly sensitive to water deficit, which adversely affects photosynthesis, water relations, and nutrient uptake, leading to substantial yield losses. Given the increasing frequency of drought events due to climate variability, improving drought tolerance in capsicum is critical sustainable crop production (Singh et al. 2025).

Among recent eco-friendly strategies, the application organic amendments such as compost and the use of beneficial microorganisms, especially arbuscular mycorrhizal fungi (AMF), have shown promise in mitigating drought induced stress (Benaffar et al. 2022; Singh and Singh 2024). AMF form symbiotic associations with the roots of most terrestrial plants, enhancing nutrient and water uptake while improving plant stress tolerance. These fungi increase the root's effective absorptive area and play a vital role in regulating hormonal balance and antioxidant defense systems (Begum et al. 2019; Singh et al. 2024a).

Compost serves as a rich source of organic matter and nutrients, improving soil structure, moisture retention, and microbial. When combined with AMF, it can create favorable conditions for fungal colonization and enhance plant performance under water-limited environments (Lahbouki et al. 2024; Ikan et al. 2025). The combined application of compost and AMF has been shown to exert synergistic effects in several crop species by integrating physical soil improvement with biological enhancement of nutrient and water uptake (El-Maghraby et al. 2024). Organic amendments can promote AMF establishment by increasing soil carbon availability and improving soil structure, thereby supporting hyphal growth and colonization efficiency (Park et al. 2024).

One of the primary physiological consequences of drought stress is the induction of oxidative stress. Water deficit conditions accelerate the generation of reactive oxygen species (ROS), including superoxide radicals, hydrogen peroxide, and hydroxyl radicals, which can damage cellular membranes, proteins, and nucleic acids (Fathi et al. 2025). Excessive ROS accumulation disrupts chloroplast function, degrades photosynthetic pigments, and reduces enzymatic activity. Plants counteract this oxidative imbalance through a coordinated antioxidant defense system involving enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), and dehydroascorbate reductase (DHAR), which collectively regulate redox homeostasis under stress conditions (Rao et al. 2025).

The synergistic potential of compost and AMF in enhancing drought tolerance in capsicum; however, remains insufficiently explored. Therefore, it was hypothesized that the combined application of compost and AMF would confer greater drought tolerance in *C. annuum* than individual applications by improving plant water status, nutrient uptake, photosynthetic pigment stability, and antioxidant enzyme activity. The specific aims were to assess changes in plant growth, nutrient acquisition, chlorophyll content, and antioxidant enzyme activity, and to determine whether combined treatment provides superior drought resilience compared with individual applications.

MATERIALS AND METHODS

Plant material

Seeds of capsicum (*Capsicum annuum* L.) were procured from a local supplier. Seeds were surface sterilized with 0.1% sodium hypochlorite solution for 10 minutes, followed by thorough rinsing with sterile distilled water. The disinfected seeds were germinated on moist blotter paper at 25 °C in the dark for 5 days. Uniform seedlings were transferred to 3 kg plastic pots containing a mixture of soil, sand, and compost in a 1:1:1 ratio. The 1:1:1 (soil:sand:compost) mixture was selected to provide a balanced growth medium that ensures adequate aeration and drainage. The experiment followed a randomized block design (RBD) with five replications per treatment; each pot contained a single plant.

Compost and arbuscular mycorrhizal fungal inoculum

Compost was prepared on-site from a homogenized mixture of plant residues and well-decomposed farmyard manure. The mature compost was characterized by pH=7.4, organic matter content of 28%, and a C/N ratio of 18:1. The AMF species *Funneliformis mosseae* (CMCC/AM - 1401) and *Rhizophagus intraradices* (CMCC/AM-1101) were obtained from the Centre

for Mycorrhiza Culture Collection (CMCC), TERI, India. The two AMF species were combined to mimic natural multispecies mycorrhizal communities and to harness complementary functional traits that enhance colonization efficiency, nutrient uptake, and drought tolerance compared with single-species inoculation. Inoculum was propagated using maize (*Zea mays*) as a host plant in trap cultures maintained under greenhouse conditions. At transplantation, 50 g of trap culture soil containing AMF spores and colonized root fragments was applied to each experimental pot. The AMF dose (50 g trap soil per pot) was selected based on the spore density of the inoculum (15 spores g^{-1}), which was quantified prior to application to ensure a biologically effective propagule load.

Experimental conditions and drought treatment
Plants were maintained under controlled greenhouse conditions (25±2 °C, 16-h photoperiod). Drought stress was imposed six weeks after planting by withholding irrigation until the soil moisture content reached approximately 50% of field capacity, monitored using a soil moisture meter. Control plants were maintained at 100% field capacity. Drought stress was maintained for six weeks.

Treatments
Eight treatments were established as given in Table 1. All treatments included five replicates. Plants were harvested six weeks after drought initiation and shoot, and root lengths were measured. Leaf area was determined following Ray and Singh (1989).

Table 1. Treatment combinations of AMF, compost, and drought stress under normal and deficit irrigation conditions.

Treatments	Description
T	Control (no AMF, no compost, irrigated normally)
T1	AMF only (irrigated normally)
T2	Compost only (irrigated normally)
T1 + T2	AMF + compost (irrigated normally)
T3	Drought (no AMF, no compost)
T1 + T3	Drought + AMF
T2 + T3	Drought + compost
T1 + T2 + T3	Drought + AMF + compost

Physicochemical characterization of the growth substrate
The physicochemical properties of the growth substrate were determined prior to pot filling. The substrate consisted of a 1:1:1 (w/w) mixture of field soil, river sand, and mature compost. Soil texture was determined by the hydrometer method and classified as sandy loam. The pH and electrical conductivity (EC) were measured in a 1:2.5 (soil:distilled water) suspension using a digital pH-meter and conductivity meter, respectively. The initial pH of the soil fraction was 7.2 and EC was 0.38 dS m^{-1}.

Organic carbon was determined by the Walkley and Black wet oxidation method (Walkley and Black 1934), recorded as 0.74% in the soil fraction. Available nitrogen was determined using the alkaline permanganate method (Subbiah and Asija 1956), yielding 228 mg kg^{-1} soil. Available phosphorus was measured by the Olsen extraction method (Olsen et al. 1954) and quantified colorimetrically, with a value of 16.4 mg kg^{-1} soil.

The sand component was washed thoroughly and air-dried before mixing. The composite soil–sand mixture (excluding compost and AMF inoculum) was sterilized by autoclaving at 121 °C and 15 psi for 1 h on two consecutive days to eliminate native microbial populations. Compost was added after sterilization to preserve its nutrient integrity, and AMF inoculum was introduced at transplantation as described in the previous subsection.

These baseline physicochemical characteristics ensured uniform growth conditions across treatments and provided a defined substrate context for interpreting plant responses to AMF inoculation and drought stress.

Relative leaf water content (RWC)
Relative leaf water content (RWC) was determined by standard gravimetric method with slight modifications using fresh leaf discs (Ghanbary et al. 2021). Fresh weight (FW) was recorded, after which samples were floated on distilled water for 24 hours to obtain turgid weight (TW),

then oven-dried at 70 °C for 48 hours to record dry weight (DW). RWC was calculated using Equation 1:

$$RWC(\%) = \frac{TW - DW}{FW - DW} \times 100 \quad (1)$$

Where TW is the turgid weight, DW is the dry weight, and FW is the fresh weight.

Photosynthetic pigments

Chlorophyll a, chlorophyll b, and carotenoid contents were quantified 80% extracts (Arnon 1949). Leaf samples (0.5 g) were homogenized in acetone, and absorbance was measured at 663, 645, and 470 nm, respectively. Pigment concentrations were calculated using standard Equations 2 to 5:

Chlorophyll a (mg g^{-1} FW):

$$Chl\ a = \frac{(12.7 \times A_{663} - 2.69 \times A_{645}) \times V}{1000 \times W} \quad (2)$$

Chlorophyll b (mg g^{-1} FW):

$$Chl\ b = \frac{(22.9 \times A_{645} - 4.68 \times A_{663}) \times V}{1000 \times W} \quad (3)$$

Total chlorophyll (mg g^{-1} FW):

$$Total\ Chl = \frac{(20.2 \times A_{645} + 8.02 \times A_{663}) \times V}{1000 \times W} \quad (4)$$

Carotenoids (mg g^{-1} FW):

$$Carotenoids = \frac{\left[1000 \times A_{470} - 1.82(Chal\ a \times 1000) - 85.02(Chal\ b \times 1000)\right] \times V}{198 \times 1000 \times W} \quad (5)$$

Where V is the final volume of extract (mL), and W is the fresh weight of tissue (g).

Mycorrhizal colonization

Root samples were thoroughly washed, cut into approximately 1 cm segments, and cleared in 10% (w/v) KOH at 90 °C for 30 min to remove cytoplasmic contents. After rinsing with distilled water, roots were acidified in 1% HCl for a few minutes and stained with 0.05% trypan blue prepared in lactoglycerol (lactic acid:glycerol:water, 1:1:1, v/v/v) following the method of Phillips and Hayman (1970). Excess stain was removed by destaining in lactoglycerol prior to microscopic examination.

Mycorrhizal colonization was quantified using the gridline intersect method described by Giovannetti and Mosse (1980). Stained root segments were mounted on slides, and at least 100 intersects per plant were observed under a compound microscope at 40× magnification. The presence of hyphae, arbuscules, or vesicles at each intersect was recorded, and percent root colonization was calculated following Equation 6:

$$Colonization(\%) = \frac{Number\ of\ infected\ intersects}{Total\ intersects\ observed} \times 100 \quad (6)$$

For spore enumeration, AMF spores were isolated from rhizosphere soil samples (50 g) using wet sieving followed by decanting and sucrose density gradient centrifugation (Nicolson and Gerdemann 1963). Spores retained on sieves (45–250 µm) were collected, transferred onto filter paper, and counted under a stereomicroscope.

Nutrient analysis

Plant tissues were oven-dried at 65–70 °C to constant weight, ground to a fine powder, and stored in airtight containers prior to analysis. For nutrient determination, 0.5 g of dried tissue was digested using concentrated H_2SO_4 followed by gradual addition of H_2O_2 until a clear digest was obtained. The digest was filtered and the volume adjusted with distilled water for subsequent analyses. Nitrogen was estimated by the Kjeldahl method (Piper 1966), and phosphorus was determined colorimetrically using the Vanadomolybdate method (Jackson 1973).

Antioxidant enzyme

Fresh leaf tissue (0.5 g) was homogenized in ice-cold buffer and centrifuged at 12,000 × g for 20 min at 4 °C.

The supernatant was used to determine the activities of Superoxide dismutase (SOD) activity by measuring the inhibition of nitroblue tetrazolium (NBT) reduction at 560 nm following the method of Beauchamp and Fridovich (1971). Catalase (CAT) activity was assayed by monitoring the decomposition of H_2O_2 at 240 nm according to Aebi (1984). Ascorbate peroxidase (APX) activity was estimated by recording the oxidation of ascorbate at 290 nm as described by Nakano and Asada (1981). Glutathione reductase (GR) activity was measured based on the reduction of oxidized glutathione (GSSG) at 412 nm following Smith et al. (1988). Dehydroascorbate reductase (DHAR) activity was assayed by monitoring the reduction of dehydroascorbate at 265 nm according to Nakano and Asada (1981).

Statistical analysis

Data were analyzed using ANOVA in SPSS. Mean separation was conducted using Duncan's Multiple Range Test (DMRT) at $P \leq 5\%$. All values are presented as mean ± SE (n=5). In addition to ANOVA, a Principal Component Analysis (PCA) was performed using standardized (z-score normalized) data to evaluate the overall relationships among growth traits, relative water content, photosynthetic pigments, nitrogen and phosphorus concentrations, mycorrhizal colonization parameters, and antioxidant enzyme activities. PCA was conducted in SPSS using the covariance matrix, and components with eigenvalues >1 were retained. Biplots were generated to visualize treatment clustering and variable loadings.

RESULTS AND DISCUSSION

Plant Growth and Biomass Accumulation

The combined application of compost and AMF significantly improved the growth performance of *C. annuum* under both normal and drought conditions (Figure 1). Plants treated with AMF + compost recorded the highest shoot and root lengths, leaf area, and dry biomass compared with control and individual treatments (Table 2). Under normal conditions, the combined compost + AMF treatment produced the greatest enhancement in all measured traits. Shoot length increased by 59% relative to the control (54.80 vs. 34.40 cm), while root length increased by 39%. Shoot and root dry weights rose by 114 and 146%, respectively, compared with untreated plants. Leaf area nearly doubled (978 vs. 435 cm² per plant), reflecting a substantial improvement in canopy development and photosynthetic surface.

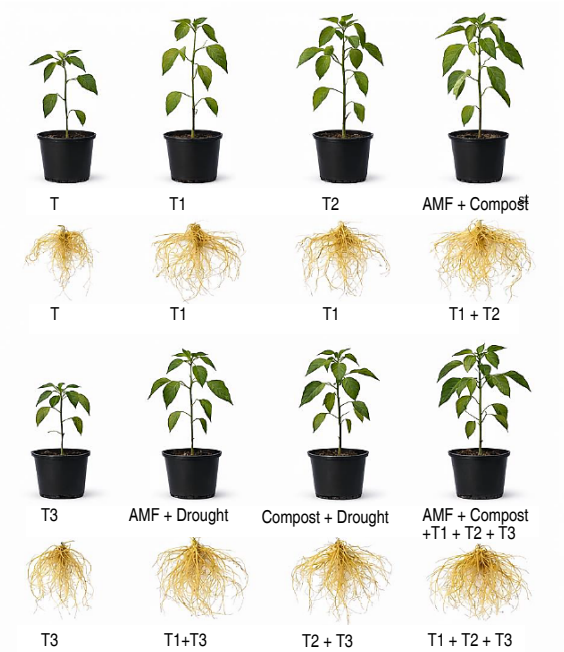


Figure 1. Representative images of *Capsicum annuum* plants grown under different combinations of arbuscular mycorrhizal fungi (AMF), compost, and drought treatments. Upper panels show shoot morphology, and lower panels display the corresponding root systems after harvest. Treatments include: control (T), AMF (T1), compost (T2), AMF + compost (T1+T2), drought (T3), AMF + drought (T1+T3), compost + drought (T2+T3), and AMF + compost + drought (T1+T2+T3).

Table 2. Influence of compost and AMF on growth characteristics of *Capsicum* under drought stress.

Treatments	Plant length (cm)		Plant dry weight (gm)		Leaf area (cm^2 per plant)
	Shoot	Root	Shoot	Root	
Control (T)	34.40±2.72^{d}	13.22±1.31^{d}	1.71±0.18^{c}	0.52±0.06^{e}	435±16.81^{de}
AMF (T1)	41.33±2.90^{c}	16.33±1.28^{bc}	2.56±0.44^{b}	0.88±0.03^{c}	680±14.14^{b}
Compost (T2)	45.92±2.62^{b}	15.44±1.08^{c}	2.43±0.35^{b}	0.74±0.04^{d}	850±18.44^{ab}
AMF + compost (T1 + T2)	54.80±2.22^{a}	18.32±1.25^{ba}	3.66±0.62^{a}	1.28±0.08^{a}	978±23.20^{a}
Drought (T3)	23.12±1.83^{e}	9.29±1.01^{ef}	0.81±0.10^{f}	0.35±0.04^{f}	312±13.21^{f}
AMF + drought (T1 + T3)	38.56±2.45^{c}	19.45±1.58^{ab}	2.28±0.38^{b}	0.92±0.06^{c}	560±20.25^{c}
Drought + compost (T2 + T3)	27.38±1.33^{e}	11.92±1.31^{e}	1.06±0.15^{e}	0.42±0.03^{f}	490±11.40^{dc}
AMF + compost + drought (T1 + T2 + T3)	46.62±2.85^{b}	22.32±1.52^{a}	3.45±0.72^{a}	1.32±0.12^{a}	820±26.08^{ba}

AMF = Arbuscular mycorrhizal fungi, Means sharing the same letter, for a parameter, do not differ significantly at $P \leq 0.05$.

Individual applications of AMF or compost also improved growth over the control, although the magnitude was lower than their combined use. Compost alone produced greater increases in shoot length and leaf area than AMF alone, whereas AMF had a comparatively stronger influence on root development, suggesting complementary functional roles.

Under drought stress, untreated plants exhibited a sharp decline in growth, whereas AMF + compost treatment effectively mitigated this reduction. Relative to the well-watered control, drought reduced shoot length by 33%, root length by 30%, shoot dry weight by 53%, root dry weight by 33%, and leaf area by 28%. However, AMF inoculation under drought (AMF + drought) substantially alleviated these reductions. Shoot dry weight increased by 181% and root dry weight by 163% compared with drought-stressed plants without amendments. Notably, root length under AMF + drought (19.45 cm) exceeded even the well-watered control, suggesting enhanced root system plasticity under symbiotic association.

The greatest mitigation of drought effects was observed in the drought + compost + AMF treatment. Compared with drought alone, shoot and root dry weights increased more than fourfold and nearly fourfold, respectively, while leaf area improved by 163%. Root length (22.32 cm) was the highest among all treatments, exceeding both drought and well-watered conditions, demonstrating a strong stimulatory effect on belowground growth under combined amendment.

These results confirm that compost and AMF interact synergistically to enhance plant growth through improved nutrient availability and water retention. Compost contributes to soil structure and moisture-holding capacity, facilitating better root development, while AMF extends the effective absorptive area of roots for water and nutrient acquisition. Similar synergistic effects of compost and AMF on growth enhancement under abiotic stress have been reported in tomato and wheat (Mekkaoui et al. 2024).

Mycorrhizal colonization and spore production

AMF inoculation established successful symbiosis across all inoculated treatments; however, spore density and colonization parameters varied with compost addition and irrigation regime (Table 3). Under normal irrigation, compost + AMF produced the highest spore density (424.4 spores), representing a 57% increase over AMF alone (270.6 spores). Root colonization also increased significantly from 81.80% (AMF) to 85.21% (compost + AMF), placing the combined treatment in a statistically superior group ($P \leq 0.05$). This response reflects enhanced propagule multiplication and more stable root–fungus interaction in the presence of organic amendment.

Drought stress altered sporulation dynamics. Spore density under drought + AMF (282.8 spores) remained comparable to AMF alone, suggesting that moderate water deficit did not drastically suppress sporulation. However, total spores declined relative to compost + AMF under well-watered conditions. When compost was included under drought

Table 3. Influence of compost and drought on total spores, Root colonization and intensity of AMF colonization in capsicum under drought stress.

Treatments	Total spores	Root colonization (%)	Intensity of mycorrhizal colonization (%)								
			Mycelium			Vesicles			Arbuscules		
			P	M	A	P	M	A	P	M	A
AMF (T1)	270.6^{d}±10.39	81.80^{b}±5.12	8.2	36.0	55.7	67.9	25.0	7.04	14.0	19.1	66.7
AMF + compost (T1 + T2)	424.4^{a}±17.19	85.21^{a}±4.16	2.8	24.1	72.9	47.8	27.1	25.0	3.88	10.6	85.4
AMF + drought (T2 + T3)	282.8^{d}±12.8	80.69^{b}±3.34	17.5	65.3	17.0	30.5	40.8	29.7	40.5	32.8	26.7
AMF + compost + drought (T1 + T2 + T3)	374.8^{b}±17.68	81.95^{b}±4.59	17.5	65.3	17.0	30.5	40.8	29.7	40.5	32.8	26.7

P = Poor, M = Moderate, A = Abundant, means sharing the same letter, for a parameter, do not differ significantly at $P \leq 0.05$.

(drought + compost + AMF), spore density increased to 374.8 spores, exceeding drought + AMF by 33%. This pattern demonstrates that compost partially offsets drought-induced constraints on fungal reproduction. Root colonization remained consistently high across inoculated treatments (80–85%), with only marginal reductions under drought. The drought + compost + AMF treatment maintained 81.95% colonization, closely comparable to AMF alone under normal irrigation. This stability under stress suggests that compost-supported soil structure and moisture buffering sustained hyphal viability and root penetration capacity.

The intensity of colonization components (mycelium, vesicles and arbuscules) showed treatment-specific shifts. Compost + AMF under normal irrigation exhibited a higher proportion of abundant arbuscules (85.4%), indicating active nutrient exchange structures. In contrast, drought treatments showed greater proportions of moderate to poor arbuscular abundance, reflecting functional adjustment of the symbiosis under water limitation. Increased mycelial presence under drought may represent a compensatory strategy to enhance soil exploration.

The improved colonization may be attributed to enhanced soil organic matter and microbial activity provided by compost, creating a more favorable environment for AMF proliferation. Previous studies have shown that organic amendments promote mycorrhizal spore germination and hyphal extension by improving aeration and carbon availability (Tahiri et al. 2022; Soussani et al. 2023). Therefore, compost not only complements AMF function but also enhances its persistence during adverse conditions.

Relative water content (RWC)

Drought stress significantly reduced relative water content (RWC) in *C. annuum* leaves compared with well-watered control plants (Figure 2). Control plants maintained an RWC of 86.4%, whereas drought treatment reduced RWC

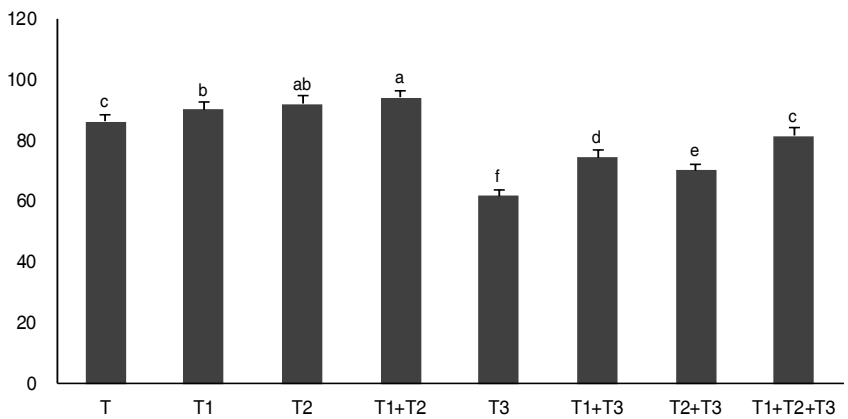


Figure 2. Effect of compost and AMF on relative water content (RWC, %) of *Capsicum annuum* under normal and drought conditions. Values are means ± SE (n=5). Means sharing the same letter do not differ significantly at $P \leq 0.05$ according to DMRT.

to 61.8%, representing a decline of approximately 28%. Application of AMF or compost individually improved plant water status under both irrigation regimes. Under normal conditions, AMF and compost treatments increased RWC to 90.2 and 92.1%, respectively. The combined AMF + compost treatment recorded the highest RWC (94.3%), indicating enhanced water retention and improved hydration status.

Under drought conditions, AMF inoculation (T1+T3) significantly mitigated water loss, increasing RWC to 74.6% compared with drought alone. Compost application under drought (T2+T3) resulted in a moderate improvement (70.3%). The combined drought + compost + AMF treatment (T1+T2+T3) showed the greatest recovery, with RWC reaching 81.7%, significantly higher than all other drought treatments ($P \leq 0.05$). These findings demonstrate that AMF and compost improve plant water relations under moisture deficit, likely through enhanced root development, improved soil moisture retention, and increased hydraulic conductivity associated with mycorrhizal symbiosis.

Drought stress markedly reduced leaf RWC in *C. annuum,* reflecting impaired plant water status under moisture limitation. The 28% decline in RWC under drought relative to well-watered controls confirms the sensitivity of this species to soil water deficit and aligns with established responses of Solanaceous crops to dehydration stress. The superior performance of the combined AMF + compost treatment under optimal irrigation indicates a synergistic interaction that promotes improved hydration status, likely associated with expanded root surface area, improved soil structure, and enhanced rhizospheric water availability (El-Maghraby et al. 2024; Soussani et al. 2025). Under drought, AMF inoculation produced a stronger ameliorative effect than compost alone, highlighting the role of mycorrhizal symbiosis in maintaining plant water balance through improved hydraulic conductivity, greater root absorptive efficiency, and possible osmotic adjustment.

Photosynthetic pigments

Drought stress markedly decreased chlorophyll a, chlorophyll b, and carotenoid concentrations, indicating reduced photosynthetic efficiency (Figure 3). Under drought alone (T3), Chl a declined to approximately 1.45 mg g^{-1} FW, representing a reduction of nearly 36% relative to the control (T). Similar decreases were observed for Chl b and carotenoids, confirming impairment of the photosynthetic apparatus under water deficit.

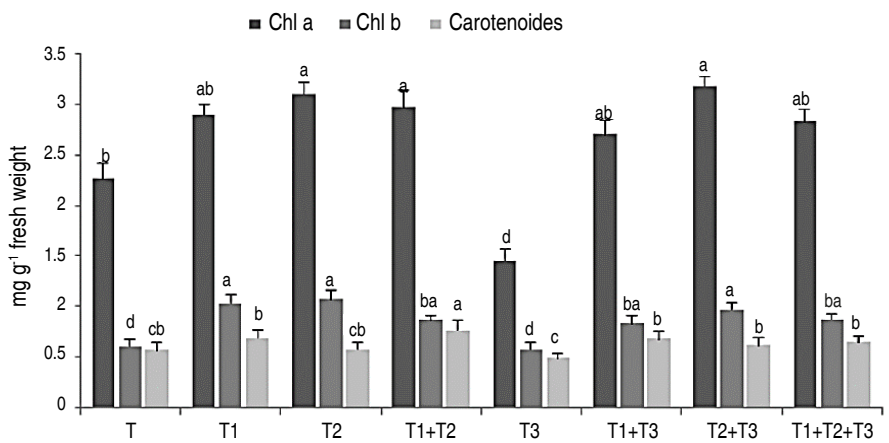


Figure 3. Influence of arbuscular mycorrhizal fungi (AMF) and compost on photosynthetic pigments (chlorophyll a, chlorophyll b, and carotenoids) in *Capsicum annuum* under drought stress. Treatments include control (T), AMF inoculation (T1), compost treatment (T2), AMF + compost treatments (T1+T2), drought treatment (T3), AMF + drought (T1+T3), compost + drought (T2+T3), and AMF + compost + drought (T1 + T2 + T3). Values are means ± SE (n=3). Different letters indicate significant differences (P<0.05) among treatments according to Duncan's multiple range test.

Under normal irrigation, compost (T2) and compost + AMF (T1+T2) recorded the highest pigment concentrations. Compost alone produced the greatest Chl a content ($\approx$3.1 mg g^{-1} FW), significantly exceeding the control and AMF-only treatments. Chl b and carotenoids followed a comparable trend, indicating improved pigment biosynthesis and stabilization in compost-amended plants.

AMF alone (T1) increased pigment concentrations relative to the control, although values remained slightly lower than compost-treated plants. The combined compost + AMF treatment under normal irrigation maintained high pigment levels comparable to compost alone, suggesting no antagonistic interaction.

Under drought conditions, AMF and compost moderated pigment loss. AMF + drought (T1+T3) and drought + compost (T2+T3) both improved Chl a and Chl b compared with drought alone. The most pronounced mitigation occurred in drought + compost + AMF (T1+T2+T3), where Chl a recovered to approximately 2.8–2.9 mg g^{-1} FW, nearly doubling the drought-only treatment. Carotenoids exhibited a similar recovery pattern, reflecting enhanced photoprotective capacity. However, AMF and compost treatments mitigated this decline, with compost-treated plants showing the highest pigment levels under normal conditions. The AMF + compost combination improved pigment stability under drought, though without exceeding the individual compost treatment in total chlorophyll content.

These findings suggest that both AMF and compost help maintain photosynthetic capacity by enhancing water and nutrient supply and by limiting oxidative pigment degradation. AMF symbiosis has been linked to improved stomatal regulation and antioxidant protection, which preserve chlorophyll integrity during stress (Zou et al. 2021). The observed increase in carotenoid concentration also supports enhanced photoprotection and reactive oxygen species (ROS) detoxification in inoculated plants (Tang et al. 2021).

Nutrient uptake

Nitrogen (N) and phosphorus (P) concentrations in both shoots and roots were strongly influenced by AMF inoculation, compost application, and irrigation regime (Table 4). Under normal irrigation, the compost + AMF treatment recorded the highest nutrient concentrations in shoots (4.01 mg g^{-1} dw N; 0.63 mg g^{-1} dw P) and roots (2.57 mg g^{-1} dw N; 0.47 mg g^{-1} dw P). Relative to the control, shoot N increased by approximately 30% and shoot P by 37%, while root N increased by 65%. These values were statistically superior (P<0.05) to individual treatments, confirming a positive interaction between AMF and compost. AMF alone enhanced nutrient accumulation more effectively than compost alone, particularly in roots, where N concentration increased from 1.56 to 2.14 mg g^{-1} dw. Compost alone produced moderate increases in shoot nutrients but exerted a comparatively smaller effect on root P, suggesting that its primary contribution may involve improved nutrient availability rather than enhanced uptake efficiency.

Table 4. Influence of compost and AMF on total nitrogen and phosphorus content in shoot and root of capsicum under drought stress.

Treatments	Shoot		Root	
	Total nitrogen (mg g^{-1} dw)	Total phosphorus (mg g^{-1} dw)	Total nitrogen (mg g^{-1} dw)	Total phosphorus (mg g^{-1} dw)
Control (T)	3.09±0.22^{cb}	0.46±0.03^{b}	1.56±0.12^{cd}	0.38±0.02^{c}
AMF (T1)	3.55±0.32^{b}	0.58±0.04^{a}	2.14±0.24^{b}	0.42±0.03^{c}
Compost (T1)	3.18±0.21^{cb}	0.51±0.04^{b}	1.72±0.20^{c}	0.42±0.03^{c}
AMF + Compost (T1 + T2)	4.01±0.38^{a}	0.63±0.04^{a}	2.57±0.20^{a}	0.47±0.03^{c}
Drought (T3)	1.23±0.18^{f}	0.25±0.02^{d}	0.78±0.10^{f}	0.19±0.02^{c}
AMF + drought (T1 + T2)	2.54±0.24^{d}	0.35±0.02^{c}	1.18±0.11^{d}	0.24±0.01^{c}
Compost + drought (T2 + T3)	1.97±0.20^{e}	0.37±0.02^{c}	0.99±0.08^{de}	0.27±0.02^{c}
AMF + compost + drought (T1+ T2 + T3)	2.79±0.26^{cd}	0.40±0.03^{c}	1.28±0.13^{d}	0.30±0.02^{c}

AMF = Arbuscular mycorrhizal fungi, Means sharing the same letter, for a parameter, do not differ significantly at P<0.05.

Drought markedly reduced nutrient concentrations. Shoot N declined from 3.09 mg g^{-1} dw in the control to 1.23 mg g^{-1} dw under drought ($\approx$60% reduction), while shoot P decreased by nearly 46%. Root N and P followed a similar downward trend, reflecting impaired nutrient mobility and restricted uptake under limited soil moisture.

Inoculated plants under drought showed substantial recovery. Drought + AMF increased shoot N by 106% and root N by 51% compared with drought alone. The drought + compost + AMF treatment produced the highest nutrient values under stress (2.79 mg g^{-1} dw shoot N; 0.40 mg g^{-1} dw shoot P), demonstrating partial restoration of nutrient status. Although these values did not reach well-watered compost + AMF levels, they significantly exceeded drought-only and single-treatment stress conditions.

AMF improve nutrient acquisition by expanding the soil volume explored through their extraradical hyphae, particularly enhancing phosphorus uptake. Compost further contributes by supplying slow-release nutrients and improving soil cation exchange capacity. The synergistic improvement in N and P uptake observed here aligns with earlier findings in tomato and maize (El Amerany et al. 2020). Enhanced nutrient uptake under AMF + compost application consequently supports higher biomass production and improved drought resilience.

Antioxidant enzyme activity

Activities of SOD, CAT, APX, GR, and DHAR were markedly influenced by irrigation regime and soil amendments (Figure 4). Drought alone elevated antioxidant enzyme activities relative to the well-watered control, reflecting induction of oxidative stress. However, enzyme activities were substantially higher in AMF- and compost-treated plants, particularly under combined application.

For SOD, drought increased activity compared with the control, but the greatest values were observed in T2+T3 and T1+T2+T3, in both leaves and roots. Root SOD activity under T2+T3 approached ~150 units mg^{-1} protein, exceeding drought alone by a wide margin. Similar patterns were recorded for CAT, where drought increased activity moderately, while combined AMF + compost under drought (T1+T2+T3) produced the highest values, especially in roots.

APX activity showed progressive enhancement from control to drought and further increases with AMF and compost supplementation. The combined treatment under drought consistently produced the highest APX activity in both organs, suggesting reinforcement of the ascorbate-dependent detoxification pathway.

GR and DHAR activities followed a comparable trend. Drought significantly stimulated GR relatives to control, but the strongest upregulation occurred in T1+T2+T3, where both leaf and root values were maximal. DHAR activity displayed a steady increase across treatments, with the combined AMF + compost under drought showing the highest enzyme levels (approaching 3.0 µmol min^{-1} mg^{-1} protein in leaves). Across all enzymes, roots frequently exhibited greater activity than leaves, indicating a strong belowground antioxidative response. Statistical grouping confirms that combined treatments, particularly under drought, formed the upper significance classes ($P \leq 0.05$).

These results demonstrate that while drought alone triggers oxidative defense, AMF and compost amplify the antioxidant system beyond stress-induced levels. The coordinated increase in SOD, CAT, APX, GR, and DHAR suggests strengthened ROS detoxification via both enzymatic dismutation of superoxide radicals and efficient regeneration of the ascorbate–glutathione cycle, contributing to improved stress tolerance in *C. annuum.*

This enhanced antioxidant response indicates that AMF and compost alleviate drought-induced oxidative stress by activating enzymatic defense mechanisms. SOD catalyzes the dismutation of superoxide radicals, while CAT and APX detoxify hydrogen peroxide, preventing cellular damage. The increased GR and DHAR activities reflect efficient regeneration of the ascorbate–glutathione cycle, maintaining redox balance in stressed tissues (Soussani et al. 2023). Thus, the co-application of AMF and compost not only supports physiological performance but also strengthens the plant's biochemical defense system (Singh et al. 2024b).

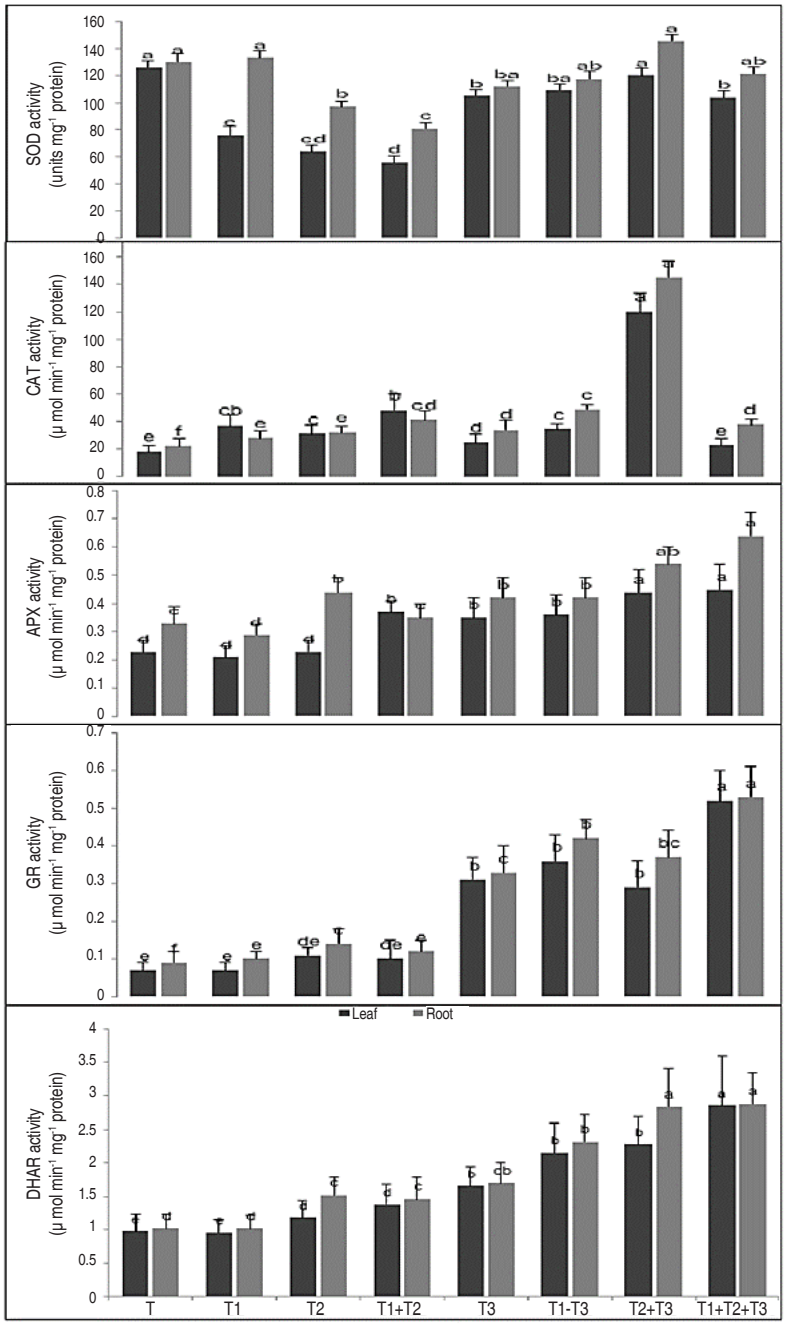


Figure 4. Effect of arbuscular mycorrhizal fungi (AMF) and compost on antioxidant enzyme activities in *Capsicum annuum* under drought stress. The activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), and dehydroascorbate reductase (DHAR) were measured in leaf and root tissues. Treatments include control (T), AMF inoculation (T1), compost treatment (T2), AMF + compost treatments (T1+T2), drought treatment (T3), AMF + drought (T1+T3), compost + drought (T2+T3), and AMF + compost + drought (T1 + T2 + T3). Values are means ± SE (n=3). Different letters indicate significant differences (P<0.05) among treatments according to Duncan's multiple range test.

Principal component analysis (PCA)

Principal Component Analysis extracted three components with eigenvalues greater than 1, together explaining 81.8% of the total variance. PC1 had the highest eigenvalue (8.72) and accounted for 58.1% of the total variance (Table 5). This component was strongly and positively associated with shoot and root length, shoot and root dry weight, leaf area, chlorophyll a, chlorophyll b, carotenoids, nitrogen and phosphorus concentrations (shoot and root), and antioxidant enzyme activities (SOD, CAT, APX, GR, and DHAR). PC1 therefore represents an integrated plant performance axis, combining growth, photosynthetic capacity, nutrient acquisition, and oxidative stress defense. PC2 exhibited an eigenvalue of 2.94 and explained an additional 16.9% of the variance, increasing cumulative variance to 75.0%. This component was primarily associated with spore density, root colonization percentage, arbuscular intensity, and relative water content (RWC). PC2 reflects the mycorrhizal–water status dimension, distinguishing treatments based on symbiotic development and plant hydration.

Table 5. Principal Component Analysis (PCA) summary showing eigenvalues, percentage variance explained, and major contributing variables in *Capsicum annuum* under compost, AMF, and drought treatments.

Principal Component	Eigenvalue	Variance Explained (%)	Cumulative Variance (%)	Major Positive Loadings
PC1	8.72	58.1	58.1	Shoot length, root length, shoot dry weight, root dry weight, leaf area, chlorophyll a, chlorophyll b, carotenoids, N, P (shoot and root), SOD, CAT, APX, GR and DHAR
PC2	2.94	16.9	75.0	Spore density, root colonization (%), arbuscular intensity and RWC
PC3	1.21	6.8	81.8	Minor contributions from vesicle intensity and root P

Only principal components with eigenvalues >1 are shown.

PC3 contributed 6.8% of the total variance (eigenvalue 1.21), raising cumulative variance to 81.8%. This component showed relatively minor contributions, mainly from vesicle intensity and root phosphorus content, suggesting limited additional explanatory power beyond the first two components. The PCA demonstrates that most variability among treatments is driven by coordinated changes in growth, nutrient status, pigment concentration, and antioxidant defense (PC1), followed by variation in mycorrhizal colonization and water relations (PC2).

PCA revealed that treatment-induced variability was largely structured along two dominant axes, with PC1 alone explaining 58.1% of the total variance and integrating morphological, physiological, nutritional, and biochemical attributes into a single performance gradient. PC2, accounting for an additional 16.9% of the variance, was primarily defined by mycorrhizal colonization traits and relative water content, separating treatments on the basis of symbiotic development and plant hydration status. The comparatively low explanatory value of PC3 indicates that vesicle intensity and root phosphorus content contributed marginally to overall variability once the major growth and symbiotic dimensions were accounted for. The PCA underscores that treatment effects are predominantly governed by coordinated enhancement of growth, nutrient status, pigment synthesis, and antioxidant defense, with mycorrhizal colonization and water balance representing a secondary but biologically meaningful dimension of response (Shalaby et al. 2024).

CONCLUSION

The present study demonstrates that the combined application of AMF and compost significantly enhanced drought tolerance in *C. annuum* by improving growth, nutrient acquisition, photosynthetic pigment stability, and antioxidant enzyme activity. The synergistic effect of AMF with compost were more effective than

individual treatments, underscoring the complementary roles of organic amendments and symbiotic fungi in mitigating drought stress. Enhanced nutrient uptake and antioxidant defense capacity contributed to greater physiological stability and reduced oxidative damage under water-limited conditions. These findings suggest that the integrated use of compost and AMF can serve as a sustainable and environmentally friendly strategy for improving crop resilience and productivity in drought-prone regions. Future research should focus on field-scale validation of these results and explore long-term impacts on soil health, microbial diversity, and yield stability to optimize the practical application of these bioresource-based interventions in sustainable agriculture.

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CONFLICT OF INTERESTS

There is no conflict of interest among the authors.

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