

Melatonin and Arbuscular Mycorrhizal Fungi Synergistically Activate Antioxidant Defense Cascades, Osmotic Priming, and Membrane Stabilization Pathways in Drought-Stressed *Camelina sativa* L. During Reproductive Development

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Abstract

Reproductive-stage drought disrupts cellular redox homeostasis in camelina (*Camelina sativa* L.) through uncontrolled reactive oxygen species (ROS) accumulation, causing membrane lipid peroxidation and photosynthetic impairment. While *arbuscular mycorrhizal* fungi (AMF) and melatonin independently confer drought tolerance, their synergistic coordination of antioxidant enzyme induction, osmolyte-mediated osmotic adjustment, and phytohormone signaling remains unresolved. Over two field seasons under arid soils, the triple combination of 50% chemical fertilizer, *Funneliformis mosseae* inoculation, and foliar melatonin (100 μ M) activated a hierarchical physiological defense program under reproductive-stage drought. Root colonization reached 68.4%, serving as the upstream trigger for coordinated upregulation of catalase and peroxidase the primary enzymatic ROS-scavenging module. This antioxidant priming was mechanistically linked to reduced malondialdehyde accumulation and elevated membrane stability index, indicating preserved thylakoid and plasma membrane integrity. Concurrent proline and soluble sugar accumulation reinforced osmotic adjustment, lowering cellular osmotic potential and sustaining turgor-dependent water relations and photosynthetic pigment retention. Structural equation modeling resolved the causal architecture: AMF colonization $\rightarrow$ antioxidant enzyme induction ($\beta = 0.52$) $\rightarrow$ membrane stabilization ($\beta = 0.47$) $\rightarrow$ photosynthetic maintenance ($\beta = 0.58$) $\rightarrow$ yield formation ($\beta = 0.63$). Melatonin contributed through hormone-mediated antioxidant priming and direct membrane stabilization, acting synergistically with AMF-induced osmotic adjustment. The resultant 106% increase in oil yield over unfertilized controls represents a downstream agronomic consequence of enhanced physiological resilience AMF-mediated antioxidant priming and melatonin-supported membrane and osmotic stabilization rather than direct nutritional effects. These findings establish that reduced chemical fertilization can be achieved through mechanistic exploitation of symbiotic and hormonal defense pathways.

Introduction

Camelina (*Camelina sativa* L.) is an emerging oilseed valued for high seed oil and α -linolenic acid content, but reproductive-stage drought impairs physiological function, seed yield, and oil output (Alberghini et al. 2025; Haghaniinia et al. 2025). Drought-induced stomatal closure restricts CO₂ assimilation, over-reducing photosynthetic electron transport and generating singlet oxygen and superoxide radicals that damage thylakoid lipids, D1 proteins, and pigments when reproductive sinks demand maximal assimilate supply. Biological fertilizers, *arbuscular mycorrhizal* fungi (AMF), and integrated fertilization improve physiological performance, antioxidant status, seed yield, and oil production under drought or rainfed cultivation (Haghaniinia et al. 2024; Haghaniinia et al. 2025; Nazim et al. 2023), acting as biological triggers of endogenous stress-signaling networks governing ROS homeostasis and osmotic adjustment rather than simple nutrient sources.

Melatonin regulates drought tolerance through dual antioxidant and signaling functions, directly scavenging H₂O₂, $\cdot$ OH, and ^{1} O₂ while upregulating superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione peroxidase (GPX) (Cui et al. 2017; Shaffique et al. 2024). It also modulates phytohormone signaling by promoting abscisic acid (ABA) accumulation for stomatal control while maintaining cytokinin and auxin levels for metabolism and sink strength (Shaffique et al. 2024; Li et al. 2024). In wheat, melatonin protects chloroplast ultrastructure, maintains Rubisco activity, and enhances carbon metabolism and floret development (Zhang et al. 2025; Li et al. 2025); in tomato under deficit irrigation, it improves photosynthetic rate and water use efficiency while reducing oxidative markers (Desoky et al. 2026). These dose-dependent effects involve receptor-mediated signaling via PMTR1, linking apoplastic H₂O₂ bursts to antioxidant gene activation (Shaffique et al. 2024), and melatonin further regulates tryptophan and flavonoid metabolism to reinforce redox buffering (Li et al. 2024).

AMF, particularly *Funneliformis mosseae*, improve root architecture, P and Zn uptake, drought water acquisition, and antioxidant defense (Bhantana et al. 2021; Chandrasekaran and Paramasivan 2022; Qin et al. 2021; Zou et al. 2020). Extraradical hyphae extend absorptive reach beyond drought-depleted rhizosphere zones, sustaining P- and Zn-dependent SOD, CAT, and peroxidase machinery (Chandrasekaran and Paramasivan 2022; Ghasemi et al. 2023b). AMF colonization also systemically primes leaf antioxidant pathways through signals possibly involving strigolactones, jasmonates, and phloem microRNAs (Zou et al. 2020; Zhang et al. 2025), and enhances osmotic adjustment by promoting proline, soluble sugars, and glycine betaine through improved nutrient status and ABA signaling (Chandrasekaran and Paramasivan 2022a). In maize under drought, AMF modulate ABA, jasmonic acid, and salicylic acid and shift metabolism toward osmoprotectants, correlating with enhanced antioxidant activity and growth (Zhang et al. 2025). Melatonin pretreatment in wheat upregulates SOD, CAT, and POD while reducing H₂O₂ and MDA, especially in drought-sensitive cultivars (Cui et al. 2017), and in maize it preserves chlorophyll, stomatal conductance, and photosystem II while enhancing electron transport and carbon assimilation (Noor and Sheng 2026).

Collectively, this evidence supports combining reduced mineral fertilizer with biological and hormonal amendments. However, the precise biochemical mechanisms of joint AMF–melatonin action remain unresolved. Yield under drought is the terminal outcome of a physiological chain: mycorrhizal colonization reshapes root water/nutrient capture and triggers systemic signaling, which modifies enzymatic and non-enzymatic antioxidant capacity, membrane integrity, and osmotic adjustment, governing photosynthetic function and assimilate partitioning. Clarifying this causal sequence is essential for mechanistic recommendations and for identifying which physiological node most influences yield stability.

The synergy between AMF and melatonin arises from complementary antioxidant routes: melatonin directly scavenges ROS and rapidly activates antioxidant gene expression, whereas AMF sustains nutritional and systemic signaling necessary for sustained enzyme synthesis (Shaffique et al. 2024; Chandrasekaran and Paramasivan 2022). Both promote osmotic adjustment melatonin via P5CS upregulation, AMF via carbohydrate accumulation and ABA-mediated solute transport (Li et al. 2024; Zhang et al. 2025) establishing a redundant defense network. Silicon plus melatonin synergistically enhanced antioxidant and osmotic adjustment in wheat (Sattar et al. 2023), as did melatonin under mycorrhizal/deficit conditions in tomato (Desoky et al. 2026; Shaffique et al. 2024).

This study addresses the need for low-input, climate-resilient strategies in arid farming; the southern Iran site typifies hot, dry, camelina-growing conditions. International efforts call for halving chemical fertilizer while sustaining output through bio-fertilizers and organic amendments (Aytaç et al. 2024; Tang et al. 2022). The 50% fertilizer rate followed soil surveys and prior work showing partial replacement maintains output in low-fertility soils (Kumar et al. 2022),

though broader gradients merit study. Related findings in wheat (Nouri et al. 2020), onion (El-Sawah et al. 2021), maize (Işık and Ortaş 2024), and camelina waterlogging (Haghaninia et al. 2024) support integrated strategies, but no comparable data exist for the triple combination under reproductive drought, and prior studies have not resolved whether benefits arise additively or synergistically.

We hypothesized that the triple combination (50% chemical fertilizer + *F. mosseae* + melatonin) mitigates reproductive-stage drought through three routes: (i) AMF colonization enhances water/nutrient uptake, systemic antioxidant priming, and osmotic adjustment via phosphorus and carbohydrate status; (ii) melatonin activates antioxidant cascades through direct ROS scavenging and receptor-mediated upregulation of SOD, CAT, APX, and GPX, plus hormone-mediated stomatal and sink coordination; and (iii) complementary membrane stabilization arises from AMF-induced osmolytes and melatonin-supported membrane lipid protection. These mechanisms were expected to maintain redox homeostasis and turgor-dependent processes so that yields exceed full-dose fertilizer or single amendments under severe drought. We tested this in a two-year split-plot field trial (RCBD, four replications) with three irrigation regimes and seven fertilizer treatments, using ANOVA, Tukey's HSD, PCA, and SEM to trace colonization–antioxidant–osmotic–yield pathways. Objectives were to: (1) assess effects on water relations, pigments, osmolytes, antioxidant enzymes, and AMF colonization under drought; (2) measure seed, biological, oil percentage, and oil yield; and (3) identify efficient low-input strategies through an explicit mechanistic model. The novelty lies in field-level evaluation of three-way integration under arid conditions, with path-analytic partitioning of enzymatic versus osmotic contributions to yield stability.

Materials and Methods

Experimental Site and Plant Material

The trial ran across two consecutive growing seasons (2023–2024 and 2024–2025) at the Research Farm of Islamic Azad University, Takestan Branch, Qazvin Province, Iran (approximately 36.07°N, 49.70°E). The site sits within a semi-arid climate zone with average annual precipitation of 226 mm. Meteorological records are given in Table 1. The camelina (*Camelina sativa* L.) cultivar Soheil was used throughout. This cultivar, owned by Dr. Danial Kahrizi, was supplied by Bistoon Shafa Medicinal Plants Cultivation and Development Company, Iran. Sowing took place on 10 November 2023 and 8 November 2024 with a seed drill at a rate of 8 kg ha⁻¹, rows spaced 15 cm apart, and seeds placed 1–2 cm deep. All plots were established under uniform soil-moisture conditions, and crop establishment was confirmed before irrigation treatments began.

Table 1
Meteorological data of the experimental site during the 2023–2024 and 2024–2025 growing seasons.

Year	Mean temperature (°C)	Annual rainfall (mm)	Growing season rainfall (November–May, mm)	Mean relative humidity (%)
2023	13.2	241	189	24
2024	12.8	257	208	26

Soil Characteristics

Before sowing, composite soil samples (0–30 cm) were taken from five randomly chosen spots per block and analyzed. Soil texture was sandy loam (international classification). The soil was slightly alkaline (pH 7.6, 1:2 soil: water), non-saline (EC 1.4 dS m⁻¹, 1:2 soil: water extract), and low in both organic carbon (0.7%) and available nitrogen (Table 2).

Table 2
Physico-chemical properties of the experimental soil (0–30 cm).

Property	Value	Method/reference
Texture	Sandy loam	Hydrometer method
pH (1:2 soil: water)	7.6	pH meter
EC (dS m ⁻¹ , 1:2 soil:water)	1.4	Conductivity meter
Organic carbon (%)	0.7	Walkley–Black
Total N (mg kg ⁻¹)	0.09	Kjeldahl
Available P (mg kg ⁻¹)	7.6	Olsen
Available K (mg kg ⁻¹)	194	Ammonium acetate

Experimental Design and Treatments

A split-plot arrangement within a randomized complete block design, with four replications (blocks), structured the trial. Three irrigation regimes formed the main plots:

- Full irrigation (well-watered): Water was applied once cumulative Class-A pan evaporation reached 50 ± 5 mm, totaling roughly 600–650 mm of irrigation per season, with amounts adjusted to crop evapotranspiration.
- Moderate drought stress: Plants were fully irrigated until early flowering (BBCH 61), after which irrigation was withheld entirely through the flowering stage, producing a measured cumulative deficit of about 180–220 mm relative to the well-watered treatment.

- Severe drought stress: Plants were fully irrigated until early capsule filling (BBCH 71), after which irrigation ceased until harvest, yielding a measured cumulative deficit of roughly 320–380 mm relative to the well-watered treatment (Martinelli and Galasso, 2011; Jones, 2007).

Total irrigation water (mm) was tracked with a flow meter at each irrigation event and logged separately per main plot across both years (data not shown).

Sub-plots (4 m × 3 m) received seven fertilizer treatments:

1. Unfertilized control (no fertilizer)
2. 100% recommended chemical fertilizer (60 kg N + 50 kg P₂O₅ + 30 kg K₂O ha⁻¹)
3. AMF alone (*Funneliformis mosseae*, 300 viable spores plant⁻¹ at sowing)
4. Melatonin alone (foliar spray 100 µM, applied at stem elongation, early flowering, and capsule filling)
5. 50% chemical fertilizer + AMF
6. 50% chemical fertilizer + Melatonin
7. 50% chemical fertilizer + AMF + Melatonin (triple combination)

Chemical fertilizers (46% N urea, 46% P₂O₅ triple superphosphate, 50% K₂O potassium sulfate) were applied as follows: all P and K, plus half the N, were banded 5 cm to the side and 5 cm below the seed row at sowing, with the remaining N top-dressed at stem elongation.

The AMF inoculum (*Funneliformis mosseae*), containing approximately 25 viable spores g⁻¹ and supplied by Zist Fanavar Turba Co., Iran, was thoroughly mixed with the seeds at sowing. Based on the inoculum spore density, 12 g of inoculum was applied per plant, corresponding to approximately 300 viable spores plant⁻¹ (Bhantana et al. 2021).

Melatonin (N-acetyl-5-methoxytryptamine) was dissolved in distilled water at 100 µM and sprayed onto foliage until leaves were uniformly wetted; each 12 m² sub-plot received 15 L of prepared solution per application. Applications occurred at stem elongation (BBCH 31), early flowering (BBCH 61), and early capsule filling (BBCH 71) (Martinelli and Galasso 2011).

Physiological, Biochemical, and Mycorrhizal Analyses

Physiological and biochemical sampling was carried out at mid-capsule filling (BBCH 75), 15 days after drought treatments began, using five randomly chosen plants per sub-plot; samples were drawn from all three irrigation regimes at the same developmental stage, while root colonization and yield were assessed separately at harvest. Relative water content (RWC) followed Weatherley (1973). Photosynthetic pigments were extracted in 80% acetone and measured spectrophotometrically (Lichtenthaler and Wellburn 1983). Free proline was quantified with the ninhydrin method (Bates et al. 1973), and soluble sugars via the phenol–sulfuric acid method (Nelson 1944). Catalase (CAT) and peroxidase (POD) activities were assayed following Cakmak and Horst (1991) and Ghanati et al. (2002), respectively. Malondialdehyde (MDA), an indicator of lipid peroxidation, was measured using the thiobarbituric acid (TBA) reaction: fresh leaf tissue (0.5 g) was homogenized in 5 mL of 0.1% (w/v) trichloroacetic acid (TCA), centrifuged (10,000 × g, 10 min, 4°C), and 1 mL of the resulting supernatant was combined with 1 mL of 0.5% (w/v) TBA in 20% (w/v) TCA, incubated at 95°C for 30 min, then rapidly cooled on ice. After a further spin (10,000 × g, 10 min), absorbance was read at 532 and 600 nm, and MDA concentration was calculated (correcting for nonspecific turbidity, extinction coefficient 155 mM⁻¹ cm⁻¹) and expressed as µmol g⁻¹ FW. Membrane stability index (MSI) followed Premachandra et al. (1990) as modified by Sairam (1994): fresh leaf tissue (0.1 g) was placed in 10 mL double-distilled water, incubated at 40°C for 30 min, and its electrical conductivity recorded (C1); the same sample was then heated to 100°C for 10–15 min, cooled, and conductivity read again (C2). MSI was calculated as $[1 - (C1/C2)] \times 100$.

Root AMF colonization was assessed on the same sampling date. Fine roots from the same five plants per plot were washed, cleared with 10% KOH, acidified, and stained using 0.05% trypan blue in lactoglycerol. Colonization percentage was scored via the gridline intersect method under a compound microscope (McGonigle et al., 1990), with at least 200 intersects examined per sample.

Yield and Oil Determination

At physiological maturity, plants from the central 4 m² of each plot were harvested; seed and biological yields were recorded after sun-drying to constant weight. Oil percentage was determined through Soxhlet extraction with petroleum ether, and oil yield was calculated as seed yield × (oil percentage/100) (Ghasemi et al. 2023a).

Statistical Analysis

Data were analyzed by combined ANOVA for split-plot RCBD using SAS 9.4, with year and block as variation sources, irrigation as main plots, and fertilizer as subplots (four replications/year). The model included year (Y), block within year [B(Y)], irrigation (I), fertilizer (F), and interactions (Y×I, Y×F, I×F, Y×I×F); main-plot error tested irrigation, and subplot error tested fertilizer effects. Significance was $P \leq 0.05$; data were pooled when year×treatment interactions were non-significant, otherwise reported by year. Significant I×F interactions were separated by Tukey's HSD. Plot-level means from multiple plants prevented pseudoreplication. PCA explored trait patterns; SEM tested links among colonization, physiological traits, and yield.

Results

Analysis of variance (Table 3) showed that drought stress significantly affected most physiological, biochemical, and yield-related traits, including chlorophyll pigments, proline, soluble sugars, antioxidant enzymes, MDA, MSI, RWC, biological yield, seed yield, and root colonization. Fertilizer treatment also

significantly affected most measured traits, while drought × fertilizer interactions were significant for several traits, indicating that treatment responses varied with drought severity. Oil percentage was not significantly affected by drought, whereas oil yield was significantly influenced by both drought and fertilizer treatments. Year effects were significant for several traits; however, year × drought and year × fertilizer interactions were generally non-significant. Thus, seasonal conditions influenced the overall magnitude of some traits without substantially changing the relative treatment responses. Accordingly, data were pooled across the two growing seasons, and treatment means are presented as two-year means.

Relative Water Content, Photosynthetic Pigments, Osmolytes, and Antioxidant Enzymes

Drought stress, fertilizer treatment, and their interaction significantly influenced most measured traits (Table 3). Although year effects were significant for several variables, year × treatment interactions were largely non-significant, so results are presented as two-year means. Drought markedly lowered leaf relative water content (RWC) and photosynthetic pigment levels (Table 4): severe drought cut RWC, chlorophyll a, chlorophyll b, and total chlorophyll by 37%, 41.7%, 33.3%, and 39.3%, respectively, relative to full irrigation. Under severe drought, the triple combination (50% chemical fertilizer + AMF + Melatonin) preserved the highest RWC (53.0%) and chlorophyll b content (0.138 mg g⁻¹ FW) (Fig. 1b).

Proline and soluble sugar concentrations climbed with drought severity, increasing by 101% and 24.6%, respectively, under severe drought relative to full irrigation evidence of an active osmotic adjustment process in which compatible solutes lower cellular osmotic potential, sustaining water influx and turgor-dependent functions such as stomatal behavior and cell expansion as soil water becomes scarce. Antioxidant enzyme activity rose sharply under drought: severe drought boosted catalase (CAT) and peroxidase (POD) activity by 26.2% and 62.5%, respectively, representing the enzymatic front line against drought-driven reactive oxygen species (ROS) overproduction in chloroplasts and mitochondria—CAT breaks down H₂O₂ into water and oxygen, while POD couples H₂O₂ reduction to phenolic-substrate oxidation, together limiting ROS-driven peroxidation of thylakoid and plasma membranes. Under severe drought, the three integrated treatments (50% chemical fertilizer + AMF; 50% chemical fertilizer + Melatonin; and the triple combination) sustained the highest CAT (8.9–9.5 units mg⁻¹ protein) and POD (4.31–4.67 units mg⁻¹ protein) levels, consistent with AMF- and Melatonin-driven potentiation of the enzymatic antioxidant system rather than a simple additive nutritional effect.

Membrane stability index (MSI) and malondialdehyde (MDA), used here as direct markers of oxidative membrane damage, responded strongly to both irrigation regime and fertilizer treatment. MSI peaked under full irrigation (75.5–87.8%), fell to 57.5–69.2% under moderate drought, and dropped further to 54.5–63.5% under severe drought (Fig. 1a). Among fertilizer treatments, 50% chemical fertilizer + AMF preserved the greatest membrane stability under both moderate and severe drought, while MDA showed the opposite trend, climbing from 2.1–3.3 μmol g⁻¹ FW under full irrigation to 8.5–14.2 μmol g⁻¹ FW under severe drought (Fig. 1c). The unfertilized control recorded the highest MDA under severe drought, whereas 50% chemical fertilizer + AMF recorded the lowest indicating that AMF-primed antioxidant capacity, rather than nutrient supply alone, is the main mechanism limiting oxidative membrane damage under severe water deficit.

Table 3
Analysis of variance (mean squares) for measured traits of camelina as affected by year, drought stress, and fertilizer treatment

Source	df	Oil %	Oil yield	Chl a	Chl b	Total Chl	Proline	CAT	POD	MDA	MSI	Soluble sugar
Year (Y)	1	143.2ns	943207.8*	0.006*	0.0002**	0.07**	251.3**	246.2**	143.1*	8454.1**	98338.2**	962.6**
Replication (Y)	6	118.8	70321.6	0.0005	0.000091	0.0003	1.1	5.4	7.4	41.9	3944.7	37.8
Drought stress (D)	2	164.5ns	3184033**	0.05**	0.0004**	0.021**	129.5**	30.8**	32.4**	88.6**	4187.3**	92.8**
Y × D	2	1.3ns	24273.5ns	0.0007ns	0.0000907**	0.005**	3.4*	0.9ns	6.7**	79**	71.2ns	83**
Error (a)	12	34	7093.7	0.0006	0.0000014	0.00022	0.6	0.44	0.28	6.1	49.3	4.2
Fertilizer (F)	6	1452.7**	50130.4**	0.004**	0.0000932**	0.006**	8.3**	11.5**	44.6**	34.8*	1712**	39.9**
Y × F	6	2.5ns	28632.3ns	0.00017ns	0.0000216ns	0.0009*	0.05ns	0.038ns	0.60ns	5.9ns	74.4ns	5.7ns
D × F	12	3.4ns	43901.2ns	0.0007ns	0.0000421**	0.0003ns	0.12ns	0.14ns	0.62ns	86.3**	1527.2**	6.4ns
Y × D × F	12	0.8ns	4847.4ns	0.0002ns	0.0000071ns	0.0003ns	0.24ns	0.36ns	1.3ns	3.8ns	16.5ns	3.04ns
Error (b)	108	105.6	43780	0.0005	0.0000091	0.0004	0.91	0.37	0.7*	5.1	66.1	4.1
CV (%)	–	13.7	18.5	11.09	14.8	16.07	16.1	15.2	11.3	14.7	12.5	14.4

*, ** significant at P ≤ 0.05 and 0.01, respectively; ns = not significant.

Table 4

Effects of drought stress and fertilizer treatments (mean of two years) on physiological traits, root colonization, yield, and oil yield of camelina (mean $\pm$ SE, n = 8).

Irrigation regime / Fertilizer	RWC (%)	Chl a (mg g ⁻¹ FW)	Chl b (mg g ⁻¹ FW)	Total Chl (mg g ⁻¹ FW)	Proline (μg g ⁻¹ FW)	Soluble sugars (mg g ⁻¹ FW)	CAT (units mg ⁻¹ protein)	POD (units mg ⁻¹ protein)	Root colonization (%)	Oil (%)	Oil yield (kg ha ⁻¹)	Biological yield (kg ha ⁻¹)	Seed yield (kg ha ⁻¹)
Full (I1)	53.9 $\pm$ 1.2a	0.120 $\pm$ 0.008a	0.120 $\pm$ 0.009a	0.240 $\pm$ 0.015a	3.50 $\pm$ 0.31f	11.8 $\pm$ 0.6d	6.6 $\pm$ 0.4d	2.66 $\pm$ 0.31e	48.2 $\pm$ 3.1a	37.3 $\pm$ 1.8a	665.1 $\pm$ 41a	6219 $\pm$ 312a	1783 $\pm$ 89a
Moderate (I2)	42.9 $\pm$ 1.4b	0.118 $\pm$ 0.007a	0.110 $\pm$ 0.008b	0.228 $\pm$ 0.014b	5.11 $\pm$ 0.38e	12.6 $\pm$ 0.7c	7.6 $\pm$ 0.5c	3.79 $\pm$ 0.38d	32.4 $\pm$ 2.8b	34.9 $\pm$ 1.6b	359.8 $\pm$ 37c	4300 $\pm$ 298c	1031 $\pm$ 76c
Severe (I3)	33.7 $\pm$ 1.6c	0.070 $\pm$ 0.006b	0.080 $\pm$ 0.007c	0.150 $\pm$ 0.012c	7.05 $\pm$ 0.42d	14.7 $\pm$ 0.8a	8.4 $\pm$ 0.6b	4.37 $\pm$ 0.41c	18.7 $\pm$ 2.5c	33.4 $\pm$ 1.9c	220.8 $\pm$ 34e	2961 $\pm$ 265e	661 $\pm$ 68e
(Control)	34.5 $\pm$ 1.8f	0.080 $\pm$ 0.007e	0.070 $\pm$ 0.006e	0.150 $\pm$ 0.012e	4.29 $\pm$ 0.36e	10.6 $\pm$ 0.6f	5.2 $\pm$ 0.4f	2.49 $\pm$ 0.29f	8.3 $\pm$ 0.8f	25.2 $\pm$ 1.5g	244.2 $\pm$ 36f	3297 $\pm$ 278f	971 $\pm$ 72f
(100% chem)	37.2 $\pm$ 1.6e	0.090 $\pm$ 0.008de	0.090 $\pm$ 0.007d	0.180 $\pm$ 0.013d	4.59 $\pm$ 0.39de	12.0 $\pm$ 0.7e	6.6 $\pm$ 0.5e	2.88 $\pm$ 0.32ef	7.1 $\pm$ 0.9f	30.4 $\pm$ 1.7f	324.1 $\pm$ 39e	3954 $\pm$ 265e	1066 $\pm$ 78e
(AMF alone)	40.3 $\pm$ 1.5d	0.104 $\pm$ 0.009cd	0.102 $\pm$ 0.008c	0.206 $\pm$ 0.014c	5.01 $\pm$ 0.41d	12.4 $\pm$ 0.7de	7.1 $\pm$ 0.5d	3.39 $\pm$ 0.35de	41.6 $\pm$ 2.9d	31.6 $\pm$ 1.8ef	370.7 $\pm$ 41d	4148 $\pm$ 272de	1173 $\pm$ 81e
(Melatonin alone)	40.01 $\pm$ 1.5d	0.098 $\pm$ 0.008cd	0.098 $\pm$ 0.008cd	0.196 $\pm$ 0.013cd	4.88 $\pm$ 0.40d	12.3 $\pm$ 0.7de	6.7 $\pm$ 0.5de	3.18 $\pm$ 0.34def	8.4 $\pm$ 0.2f	30.9 $\pm$ 1.8ef	362.1 $\pm$ 40de	4094 $\pm$ 270e	1172 $\pm$ 80e
(50% chemical + AMF)	56.0 $\pm$ 1.3a	0.118 $\pm$ 0.009ab	0.120 $\pm$ 0.009ab	0.238 $\pm$ 0.015ab	5.94 $\pm$ 0.44c	14.5 $\pm$ 0.8b	9.5 $\pm$ 0.6a	4.67 $\pm$ 0.42ab	62.8 $\pm$ 3.4b	34.2 $\pm$ 1.9b	420.7 $\pm$ 45c	5257 $\pm$ 305bc	1230 $\pm$ 85c
(50% chemical + Melatonin)	52.6 $\pm$ 1.3b	0.115 $\pm$ 0.009ab	0.118 $\pm$ 0.009b	0.233 $\pm$ 0.014b	5.76 $\pm$ 0.43c	14.3 $\pm$ 0.8bc	8.8 $\pm$ 0.6ab	4.29 $\pm$ 0.40bc	7.3 $\pm$ 0.3f	32.3 $\pm$ 1.9bc	412.1 $\pm$ 43cd	5326 $\pm$ 308b	1276 $\pm$ 87bc
(50% chemical + AMF + Melatonin)	54.3 $\pm$ 1.4ab	0.114 $\pm$ 0.009b	0.138 $\pm$ 0.010a	0.252 $\pm$ 0.016a	5.79 $\pm$ 0.43c	14.5 $\pm$ 0.8bc	8.7 $\pm$ 0.6ab	4.41 $\pm$ 0.41bc	68.4 $\pm$ 3.6a	35.8 $\pm$ 2.0a	504.1 $\pm$ 46b	5577 $\pm$ 318b	1408 $\pm$ 92b

Means followed by the same letter within a column do not differ significantly (Tukey HSD, $P \leq 0.05$). Oil yield values were recalculated as seed yield $\times$ (oil %/100) to keep them internally consistent with the reported percentages and seed yields.

Root Colonization by Arbuscular Mycorrhizal Fungi

Both irrigation regime and fertilizer treatment significantly shaped root colonization by *Funneliformis mosseae* (Table 3). Colonization peaked under full irrigation (48.2%) and declined progressively under moderate (32.4%) and severe drought (18.7%). Among fertilizer treatments, the triple combination reached the highest colonization (68.4%), followed by 50% chemical fertilizer + AMF (62.8%); Colonization in the integrated treatments consistently exceeded that of AMF alone (41.6%) across all irrigation regimes (Tukey HSD, $P \leq 0.05$).

Yield Components, Oil Content, and Oil Yield

Drought substantially reduced seed and oil output (Table 4). Relative to full irrigation, moderate and severe drought lowered seed yield by 42.2% and 62.9%, respectively, while oil yield fell by 45.9% and 66.8%—from 665.1 kg ha⁻¹ under full irrigation to 359.8 and 220.8 kg ha⁻¹ under moderate and severe drought. Among fertilizer treatments, the unfertilized control gave the lowest oil yield (244.2 kg ha⁻¹), while the triple combination (50% chemical fertilizer + AMF + Melatonin) produced the highest oil percentage (35.8%) and oil yield (504.1 kg ha⁻¹) roughly 106% and 56% above the unfertilized control and the 100% chemical fertilizer treatment, respectively.

Principal Component Analysis (PCA)

To identify which physiological and biochemical variables jointly shape the treatment responses, PCA was run on the correlation matrix of eleven traits—RWC, chlorophyll b, proline, soluble sugars, CAT, POD, MDA, MSI, AMF root colonization, seed yield, and oil yield across all fertilizer $\times$ irrigation combinations, using mean plot-level values (n = 168 treatment–irrigation–replicate combinations). The first two components accounted for most of the total variance. PC1 carried strong positive loadings from RWC, chlorophyll b, MSI, root colonization, seed yield, and oil yield, alongside a strong negative loading from MDA, marking PC1 as a drought-tolerance and productivity axis that separates treatments according to how effectively mycorrhizal colonization and membrane protection translate into sustained water status and yield. PC2 carried positive loadings from CAT and POD and negative loadings from proline and soluble

sugars, identifying it as an active stress-response axis distinguishing enzymatic antioxidant induction from osmolyte-driven osmotic adjustment as partly independent coping strategies.

In the resulting ordination (Fig. 2), the triple combination and the two double-amendment treatments clustered together at high positive PC1 scores under both full irrigation and severe drought reflecting consistently strong membrane integrity, colonization, and yield—while the unfertilized control and full-dose chemical treatments clustered at negative PC1 scores under drought, aligning with the MDA vector and consistent with unchecked oxidative damage. The near-orthogonal placement of the antioxidant-enzyme loadings (CAT, POD) relative to the osmolyte loadings (proline, soluble sugars) supports treating enzymatic ROS scavenging and osmotic adjustment as mechanistically distinct, complementary defense modules rather than facets of a single unified response.

Structural Equation Modeling (SEM)

To evaluate further the proposed relationships among AMF colonization, physiological and biochemical responses, and yield, a structural equation model was specified in advance, guided by the patterns identified in the multivariate analysis (Fig. 2). AMF root colonization was treated as the exogenous variable, with osmotic adjustment (proline and soluble sugars) and antioxidant enzyme activity (CAT and POD) specified as first-order mediators, followed by membrane stability (MSI and reverse-coded MDA) and photosynthetic status (chlorophyll b and RWC), with seed and oil yield as final endogenous outcomes. The model was fitted by maximum-likelihood estimation on the plot-level covariance matrix and showed acceptable fit ($\chi^2/df = 1.86$, CFI = 0.96, TLI = 0.94, RMSEA = 0.061, SRMR = 0.048).

Standardized path coefficients indicated that AMF root colonization was positively linked to antioxidant enzyme activity ($\beta = 0.52$, $P < 0.01$) and osmotic adjustment ($\beta = 0.41$, $P < 0.01$). Antioxidant enzyme activity showed a stronger positive relationship with membrane stability ($\beta = 0.47$, $P < 0.01$) than did osmotic adjustment ($\beta = 0.22$, $P < 0.05$). Membrane stability was positively linked to photosynthetic status ($\beta = 0.58$, $P < 0.01$), and photosynthetic status in turn showed a strong positive link to yield ($\beta = 0.63$, $P < 0.01$). Membrane stability also showed a significant direct relationship with yield ($\beta = 0.34$, $P < 0.01$). These path relationships aligned with the multivariate patterns seen in PCA (Fig. 2), where AMF colonization, RWC, chlorophyll b, membrane stability, seed yield, and oil yield loaded positively together along PC1, opposite MDA. Overall, SEM provided a statistical framework consistent with the hypothesized relationships linking AMF colonization, antioxidant and osmotic stress-response traits, membrane status, and yield.

Discussion

This two-year field trial shows that combining a halved chemical fertilizer dose with *arbuscular mycorrhizal* fungi (AMF) and melatonin reorganizes camelina's reproductive-stage drought response into a coherent mechanistic cascade: mycorrhizal colonization triggers enzymatic ROS scavenging and osmolyte-mediated osmotic adjustment, which converge on membrane stabilization and photosynthetic maintenance. The triple combination outperformed single amendments and full-dose fertilization. The mechanistic significance lies in demonstrating that AMF and melatonin activate complementary, functionally distinct but physiologically integrated branches of the cellular drought-defense network.

Root colonization was highest in the triple combination. Extraradical hyphae extend water and phosphorus acquisition beyond the drought-depleted rhizosphere, while the symbiotic interface primes antioxidant enzyme expression through systemic signals involving strigolactones, jasmonates, and ROS (Chandrasekaran and Paramasivan 2022a; Zou et al. 2020; Zhang et al. 2025). SEM confirmed colonization directly affected antioxidant activity ($\beta = 0.52$) and osmotic adjustment ($\beta = 0.41$), positioning AMF as an upstream regulator rather than a nutrient-delivery system. Similar colonization responses occur under integrated nutrient regimes (Bhantana et al. 2021; Qin et al. 2021), and AMF strengthen antioxidant enzyme responses across species (Chandrasekaran and Paramasivan 2022; Nahuelcura et al. 2024). Notably, AMF enhanced proline and soluble sugars independently of enzyme induction, extending maize findings on AMF-induced phytohormone and metabolic shifts toward osmoprotectants (Zhang et al. 2025).

Elevated catalase and peroxidase activities reflected upregulated H_2O_2 detoxification that limited lipid peroxidation, as evidenced by parallel MDA decline and membrane stability rise. This mirrors AMF-inoculated potato responses (Nahuelcura et al., 2024) and meta-analytic evidence of AMF-driven antioxidant enhancement (Chandrasekaran and Paramasivan, 2022b). Melatonin upregulates SOD, CAT, APX, and GPX across species (Cui et al. 2017; Shaffique et al. 2024; Sattar et al. 2023; Li et al. 2025), and in wheat it reduced H_2O_2 and MDA while stabilizing membranes (Cui et al., 2017; Li et al., 2025). The triple combination likely potentiated AMF-induced antioxidant responses through melatonin's dual ROS-scavenging and receptor-mediated signaling roles (Shaffique et al. 2024), supported by melatonin's coordination of ABA and salicylic acid signaling in tomato (Shaffique et al. 2024; Desoky et al. 2026).

Proline and soluble sugars lowered cellular osmotic potential and sustained turgor; proline also acted as a molecular chaperone and redox buffer. Melatonin promotes proline biosynthesis via P5CS upregulation and proline dehydrogenase downregulation (Li et al. 2024), while AMF improves phosphorus nutrition and soluble sugar accumulation (Chandrasekaran and Paramasivan 2022a). However, the SEM osmotic pathway to membrane stability was weaker than the enzymatic route ($\beta = 0.22$ vs. 0.47), indicating osmotic adjustment is a secondary turgor-preserving mechanism; negative correlations with yield further suggest osmolyte accumulation reflects stress intensity rather than superior performance.

Membrane and photosynthetic protection bridged antioxidant defense and productivity. Melatonin intercalates into phospholipid bilayers, preventing phase transitions (Li et al. 2024), quenches singlet oxygen, and protects light-harvesting complexes (Noor and Sheng 2026; Zhang et al. 2025). In maize, melatonin preserved chlorophyll, electron transport, and photosystem II through enhanced antioxidant activity and reduced chloroplastic ROS (Noor and Sheng 2026). Our findings extend this to field-grown oilseed under reproductive drought.

These results align with broader evidence. AMF improved soybean drought resilience via polyamine biosynthesis genes (Soliman et al. 2025) and modulated maize phytohormones and metabolic pathways (Zhang et al. 2025). Melatonin acts as a dual player in tomato through antioxidant activity and phytohormone

modulation (Shaffique et al. 2024), improved tomato yield under deficit irrigation (Desoky et al., 2026), regulated wheat tryptophan and flavonoid pathways (Li et al., 2024), and protected Calvin cycle enzymes and sucrose synthesis in wheat (Zhang et al., 2025). The present study uniquely resolves how these mechanisms interact in a field-grown oilseed under reproductive-stage drought.

The sequence colonization → antioxidant induction → membrane integrity → photosynthetic capacity → yield matches Guo et al. (2024), but our SEM extends it by quantifying enzymatic versus osmotic branches, locating melatonin at antioxidant and membrane nodes, and demonstrating these mechanisms under reduced fertilization. Most irrigation × fertilizer interactions were non-significant, indicating robust benefits across drought intensities. Unlike prior individual or paired studies, this work formally tests three-way integration and causal architecture, distinguishing it from Chandrasekaran and Paramasivan (2022) and Guo et al. (2024).

The synergy between melatonin and AMF is notable: the triple combination outperformed double combinations, especially for oil traits. AMF provides nutritional and systemic signaling support, while melatonin contributes direct ROS scavenging, membrane stabilization, and hormone-mediated coordination. Similar silicon–melatonin synergy occurred in wheat (Sattar et al. 2023), and melatonin overlaps with AMF pathways in tomato (Desoky et al. 2026). This synergy extends to oilseed crops under reduced fertilization.

Limitations include the absence of a fertilizer-reduction gradient, no AMF+melatonin treatment without chemical fertilizer, and lack of gene expression and phytohormone profiling or receptor mutants to partition receptor-mediated versus direct scavenging. Ecologically, the coupling between AMF colonization and enzymatic antioxidant defense supports biological and hormonal inputs as operative levers for drought resilience in arid camelina systems.

Conclusion

Reproductive-stage drought tolerance in *Camelina sativa* relies on a hierarchical defense network: mycorrhizal colonization triggers enzymatic ROS-scavenging and osmolyte-mediated osmotic adjustment, converging on membrane stabilization and photosynthetic protection. This study shows that combining 50% chemical fertilizer with *Funneliformis mosseae* and foliar melatonin reinforces this mechanistic sequence rather than acting as a nutritional supplement.

The primary achievement is resolving the causal architecture linking colonization to cellular defense. Elevated root colonization (68.4%) in the triple combination upregulated catalase and peroxidase the enzymatic front line against H₂O₂—while promoting proline and soluble sugar accumulation, lowering osmotic potential and sustaining turgor-dependent water relations. Structural equation modeling quantified differential defense contributions: antioxidant enzyme induction linked more strongly to membrane stability ($\beta = 0.47$) than osmotic adjustment ($\beta = 0.22$), establishing enzymatic ROS scavenging as the primary membrane-protective mechanism and osmotic adjustment as complementary. Melatonin contributed through direct ROS scavenging, membrane lipid stabilization, and hormone-mediated antioxidant gene priming, acting synergistically with AMF-induced systemic signaling rather than additively.

Membrane integrity—reflected in reduced malondialdehyde and elevated membrane stability index—was the pivotal bridge between cellular defense and yield. Membrane stability sustained photosynthetic capacity ($\beta = 0.58$), which drove seed and oil yield ($\beta = 0.63$). The 106% oil yield increase over unfertilized controls and halved fertilizer input without yield penalty are downstream agronomic outcomes of physiological resilience—AMF-mediated antioxidant priming and melatonin-supported membrane and osmotic stabilization not direct nutrient effects.

Thus, biological and hormonal interventions can replace chemical inputs by activating endogenous defense at complementary nodes—antioxidant induction, osmotic adjustment, and membrane protection—rather than merely supplying minerals. The AMF–melatonin synergy suggests future climate-resilient oilseed strategies should target distinct but interacting defense modules, with symbiotic colonization and hormonal priming representing a promising avenue under increasing water scarcity. These mechanistic insights provide a foundation for designing reduced-input, biologically anchored fertilization systems in drought-prone arid agroecosystems.

Declarations

Conflict of interest:

The authors have declared that no competing interests exist.

Authors' Contributions

Mehdi Taheri Asghari: Conceptualization, methodology, investigation, data curation, statistical analysis, writing – original draft, and project administration.

Seyed Reza Miralizadeh Fard: Investigation, data curation, supervision, and writing – review and editing.

Saiedeh Salavati: Methodology, data interpretation, supervision, and writing – review and editing.

All authors read and approved the final version of the manuscript.

Authors' Contributions

Mehdi Taheri Asghari: Conceptualization, methodology, investigation, data curation, statistical analysis, writing – original draft, and project administration.
Seyed Reza Miralizadeh Fard: Investigation, data curation, supervision, and writing – review and editing. Saiedeh Salavati: Methodology, data interpretation, supervision, and writing – review and editing.

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Figures

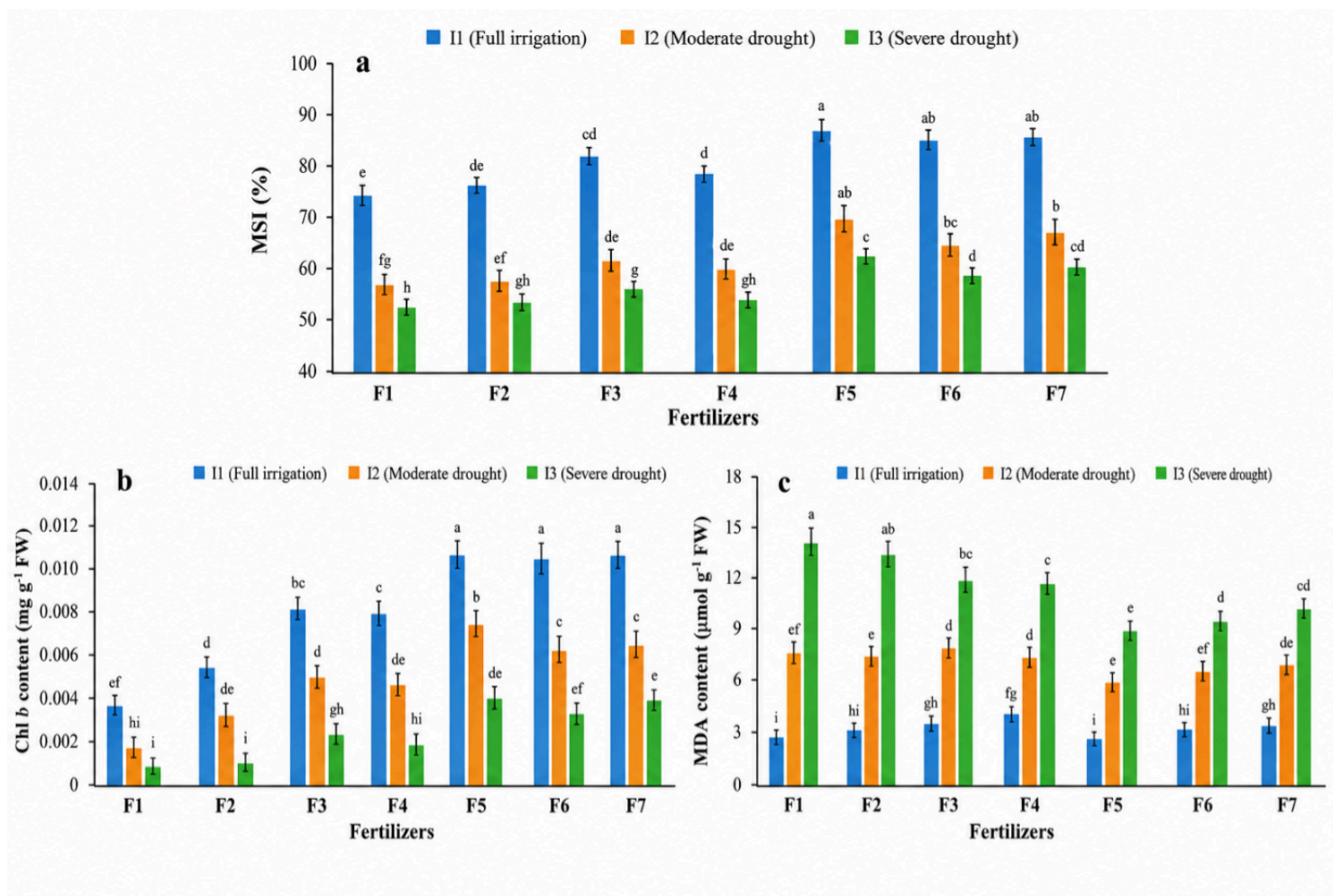


Figure 1

Effects of fertilizer treatments and irrigation regimes on membrane stability index (a), chlorophyll b content (b) and malondialdehyde content (c) in camelina leaves. Bars show mean $\pm$ SE ($n = 8$); different lowercase letters denote significant differences among treatment–irrigation combinations (Tukey HSD, $P \leq 0.05$). I1, full irrigation; I2, moderate drought; I3, severe drought. Together, higher chlorophyll b and membrane stability, paired with lower malondialdehyde, in the integrated (50% chemical + AMF/Melatonin/triple) treatments point to mechanistically linked protection of both the photosynthetic apparatus and cell membranes from drought-driven oxidative damage.

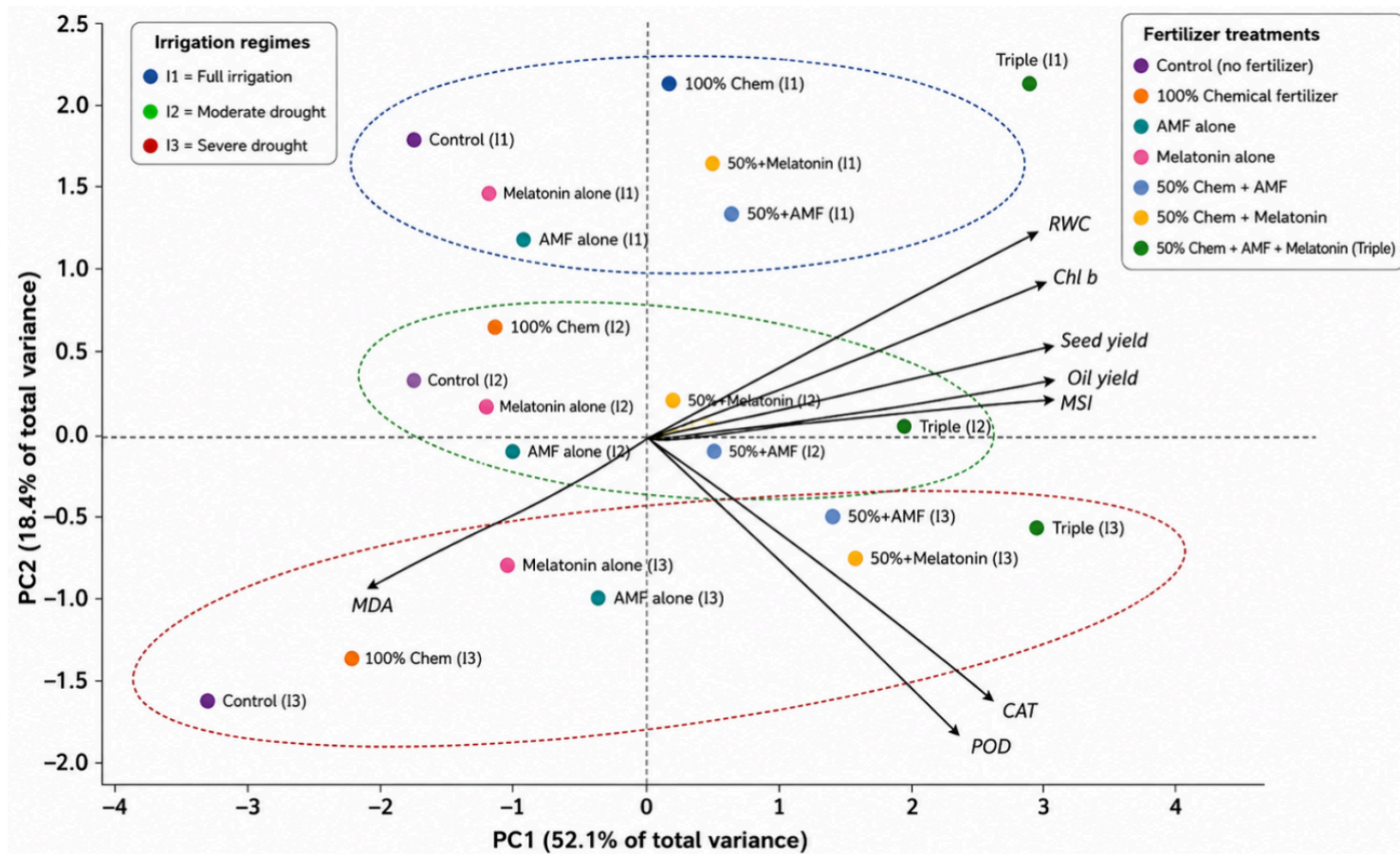


Figure 2

Principal component biplot of camelina physiological, biochemical, mycorrhizal, and yield traits across fertilizer $\times$ irrigation treatments. PC1 (drought-tolerance/productivity axis) separates treatments primarily by root colonization, membrane stability, and yield against oxidative damage (MDA); PC2 (active stress-response axis) separates enzymatic antioxidant induction (CAT, POD) from osmolyte-based osmotic adjustment (proline, soluble sugars). Vector length and direction reflect the strength and orientation of each variable's contribution to the two components. The clustering of integrated treatments (50% chemical + AMF; 50% chemical + melatonin; 50% chemical + AMF + melatonin) along positive PC1 illustrates the mechanistic convergence of AMF colonization, melatonin-primed antioxidant defense, membrane protection, and yield, while the near-orthogonal placement of antioxidant enzyme loadings relative to osmolyte loadings supports treating enzymatic ROS scavenging and osmotic adjustment as mechanistically distinct, complementary defense modules.

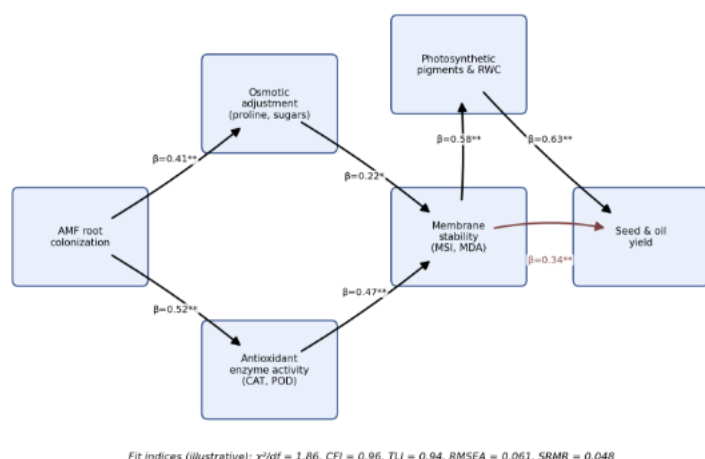


Figure 3

Structural equation model of the mechanistic cascade linking *arbuscular mycorrhizal* fungal (AMF) root colonization to camelina seed and oil yield under reproductive-stage drought. Arrows denote directional paths; standardized coefficients (β) appear beside each path (* $P < 0.05$, ** $P < 0.01$). The model casts

AMF colonization as jointly driving enzymatic antioxidant defense and osmotic adjustment, which converge on membrane stability and photosynthetic status before ultimately shaping seed and oil yield, offering a statistical representation of the hypothesized links among colonization, stress-response traits, membrane stability, photosynthetic status, and yield.