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Magnetic Seed Treatment Modulates Phenolic and Fatty Acid Metabolism of Sunflower under Water Scarcity

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Abstract: Environmental and anthropogenic activities are pushing the earth towards warmer years, which is reducing agricultural land and causing water scarcity. It is well documented that sunflower (being drought tolerant) crops can be grown under water deficit conditions with some additional supportive priming applications to compensate for drought-induced challenges. However, finding the most efficient and eco-friendly priming tools is always a top priority among researchers to improve plant growth, adaptive traits, and productivity. In this study, an experiment was performed on oil-producing crops (sunflower) using seed magnetic treatment. The seeds were subjected to 0.1, 0.2, and 0.3 Tesla for 20 min, respectively. Plants were exposed to water limitation (100 and 50%) after 30 days of germination. Sunflower showed its tolerance to water limitation by maintaining the majority of growth parameters, nutritive value, metabolizable energy, and higher proline content. Nevertheless, a reduction in the achene number per capitulum, capitulum weight, chlorophyll, catalase activity, unsaturated to saturated fatty acids, anthocyanin, and hydroxyl derivatives of cinnamic acid supported our hypothesis about the need for some supportive techniques. Better metabolic adjustment and percentage of oil yield were manifested by 0.3 T magnetic seed treatment, which was used for phenolic and fatty acid profiling. To conclude, magnetic treatment of seeds may improve their primary metabolic capacity and antioxidation potential, which in turn may activate their secondary metabolism as evidenced by an excess of gallic acid, quercetin, benzoic acid, curcumin acid, sinapic acid, and chlorogenic acid.

Keywords: magnetic treatment; limited water; antioxidants; phenolics; fatty acid; profile

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1. Introduction

Sunflower is one of the important oil seed crops with high nutraceutical importance. Its leaves are traditionally used as herbal remedies due to the presence of nutraceutical secondary metabolites, including alkaloid, tannin, steroid, and sesquiterpenoid compounds [1]. Sunflower oil used in the food industry can also be reused as CI engine fuel [2]. The husks of sunflower seeds after oil consumption are utilized for electricity production [1].

Sunflower has an additional benefit due to its moderate drought tolerance. This has made it possible to grow in areas with water deficit conditions. Water scarcity is a universal problem for the flora and fauna of the ecosystem. It is reducing the agricultural area day by day. In addition to environmental calamities, anthropogenic contributions are making the situation worse, which further poses a serious threat to sustainable agriculture [3–6]. The most practiced and simplest strategy to resolve this issue at the agronomic level is to grow drought-tolerant crops like sunflowers. Despite being tolerant, plants experience disturbed metabolism under stressful conditions. Photosynthesis, the efficiency of electron transport [7], the genomes of the hormonal system, the redox system, secondary metabolism, and hampered signaling are some of these phenomena [8].

A search for strategic applications is needed to alleviate drought-induced metabolic disturbances in sensitive and tolerant plants. In agriculture, the static magnetic field (SMF) is an environmentally friendly physical method of seed priming. It supports growth, metabolism, and development in normal [9], biotic, and abiotic [10] stressed environments. However, the efficacy of magnetic seed priming varies from species to species/variety to variety and magneto-reception (μT to 200 SMF) [9]. SMF activates cell magneto-receptor cryptochromes. It involves differences in electron spinning that vary with cell type, energy, and the dose of SMF [11]. Cryptochrome-1 can mediate stress tolerance [11,12].

Sunflowers with improved growth proved their potential to respond to applied SMF [13]. It is well studied at the agronomic and physiological levels. The proteomic, transcriptomic, and metabolomic levels of studies of SMF-treated sunflowers are scarce in the literature. A series of experiments using these approaches for SMF-treated sunflowers in normal and stressed environments are needed. As SMF is dose- and species-specific, we aimed to optimize the best dose for sunflower seeds, followed by an assessment of its effect on metabolic manipulation in sunflowers under limited water conditions using a metabolomics approach.

2. Materials and Methods

2.1. Seed Source and Selection of Levels

The present work was performed in an experimental field at Govt College University, Faisalabad. Sunflower seeds (FH-129) were collected from Ayub Agricultural Research Institute. Seeds were subjected to magnetic treatment following Mahajan and Pandey's [14] method, with minor changes. With the help of magnetic field generators, a variable horizontal magnetic field was applied at 0.0, 0.1, 0.2, and 0.3 T for 20 min. A steady DC supply source (0–45 v/0–7.5 A) was supplied to the electromagnet of the magnetic field. The distance between magnetic poles was maintained at 5 mm. Magnetic strength was checked thoroughly using a gauss meter. Three sets of samples (each comprising 25 seeds) were organized in plastic bags to be exposed to the magnetic field between the pole pieces of the electromagnet.

2.2. Experimental Plan

Seeds were planted in two equal-sized plots: one plot was irrigated with regular irrigation, and the other was irrigated with 50% irrigation. Suggested doses (150-60-60 NPK kg ha^{-1}) of fertilizer were applied. At sowing time, 1/3 of nitrogen was added to the soil as urea. The leftover dose was divided into two doses: one was applied at the vegetative stage and one was applied later at the flowering stage. Phosphorus (P) and potassium (K) were applied as triple super phosphate and sulphate of potash (K_2SO_4) during seedbed preparation. All regular practices such as irrigation, weed management, hoeing, plant protection, etc., were kept normal for the crop during the experiment. The growth and biochemical aspects were evaluated during the flowering stages. The final harvest was taken when the backs of the heads turned yellow and the bracts were brown and dried for 4 to 5 days. Five capitula were selected for the determination of different yield components. After measuring the capitulum weight, achenes were separated. The number of achenes

per capitulum was counted and the weight of 100 achenes was measured. The individual parameters were quantified by averaging five readings.

2.3. Estimation of Photosynthetic Pigments

Leaf total chlorophyll and carotenoid contents were estimated following the protocol of Davies and Goodwin [15]. The absorbance of the leaves extracted with 80% acetone was recorded at 645, 663, and 480 nm.

2.4. Determination of Total Phenolics, Flavonoids, and Total Protein Contents

Leaf total phenolics were determined following Julkunen-Tiitto [16] using FolinCiocalteu's reagent. Sample extraction was prepared as described in the procedure. After 90 min of incubation, the absorbance was measured at 765 nm by spectrophotometer. Total flavonoid contents were determined following the detailed method of Marinova et al. [17], whereas total protein contents were estimated using the method explained by Bradford [18]. The reaction mixture was prepared and the absorbance of the samples was determined using a spectrophotometer at 595 nm.

2.5. Estimation of Reducing Sugar Contents and Total Soluble Sugars

The conten of reducing sugars was determined as described by Miller [19]. The reaction mixture was vortexed and absorbance was measured at 540 nm. The total soluble sugars were estimated using the method of Yoshida et al. [20]. After the vortex, the reaction mixture was heated at 95 °C for 15 min. The absorbance of the samples was measured at 625 nm.

2.6. Determination of Total Anthocyanin Content

The quantitative analysis of anthocyanin was performed spectrophotometrically using the method of Nozzolillo [21]. Acidified methanolic leaf extract of 3 mL was taken, and the absorbance was recorded by using a spectrophotometer at 536 and 600 nm.

2.7. Determination of % Oil Content

The percent oil content was determined by following Horwitz [22]. In a 50 mL capacity plastic test tube, three grams of the plant sample was used and 30 mL n-hexane was added to it. At 100 rpm, the shaker was adjusted and the tubes were left for over 24 h after the samples were centrifuged and the supernatant was collected. Two successive repetitions of extractions were performed on the residue via vortexing and centrifugation. Clear extracts from all three collections were preserved for oil estimation. The residues were dried as fat-free samples with a difference in weight after extraction was recorded as the fat content.

2.8. Estimation of Nutritional Value and Metabolizable Energy

This value was determined using the method of Indrayan et al. [23] as

$$Nutritivevalue = (4 \times \%ageofprotein) + (9 \times \%ageoffats) + (4 \times \%ageofcarbohydrates) \quad (1)$$

The metabolizable energy of samples was determined using the following expressions by Macrae et al. [24]

$$Energy\ (\text{kJ}) = (4\ \text{kcal}/g \times gprotein \times 4.186) + (4\ \text{kcal}/g \times gfat \times 4.186) + (9\ \text{kcal}/g \times gcarbohydrate \times 4.186)$$

$$Energy\ (\text{kJ}) = \frac{4\ \text{kcal}}{g} \times gprotein \times 4.186 + (4\ \text{kcal}/g \times gfat \times 4.186) + (9\ \text{kcal}/g \times gcarbohydrate \times 4.186) \quad (2)$$

2.9. Determination of Growth and Yield Attributes

Plant growth in terms of leaf fresh weight, dry weight, and yield attributes like cake weight and achene weight were determined in grams using an electronic balance. Achenes per cake were counted and noted. For growth and yield, five plants were used as a single replicate.

2.10. Quantification of Free Proline

Proline content was measured following the method of Bates et al. [25]. Frozen leaves (0.2 g) were homogenized in 5 mL 3% sulpho-salicylic acid using a pestle and mortar. After centrifugation, the supernatant was collected. One mL of the samples ninhydrin and glacial acetic acid was taken, respectively. The solution was kept in a water bath for one hour at 100 °C and then shifted to an ice bath. Two mL of toluene was mixed and kept at room temperature for 30 min until two layers were formed. The absorbance was measured at 520 nm wavelength. The same sequence was run with a blank (2 mL of toluene).

2.11. Determination of Catalase (CAT) Activity

Fresh frozen leaves were taken (0.2 g) and 50 mM 5 mL of cooled phosphate buffer (pH 7.8) was ground using a tissue grinder mortar and pestle, which was placed in an ice bath for the antioxidant enzyme extraction. The mixture solution was centrifuged at 4 °C at 14,000× *g* for 10 min. The supernatants from each sample were stored in Eppendorf tubes at −20 °C and utilized for antioxidant activities on CAT. Catalase activity was determined by calculating the conversion rate of H_2O_2 to H_2O and oxygen molecules using the protocol explained by Chance and Maehly [26]. The reaction mixture was prepared by using t 300 µL of 30% H_2O_2 mixed in 200 mL of phosphate buffer (pH 7.0). The reaction solution (3 mL) and 0.1 mL of supernatant were added to it later. Then, the reaction was started by the addition of enzyme extract. A change in absorbance of the reaction solution was interpreted every 60 s at 240 nm. CAT activity was described as an absorbance change of 0.01 units per min.

2.12. HPLC Based Phenolic Profiling

The internal standards used were as follows: Quercetin, gallic acid, caffeic acid, benzoic acid, vanillic acid, cinnamic acid, syringic acid, p-coumaric acid, m-coumaric acid, ferulic acid, sinapic acid, and chlorogenic acid. Phenolics were extracted with minor modifications using the method of Stalikas [27]. Powdered leaves were extracted in 100% methanol (1:10) and filtered through a 0.45 µm cellulose acetate filter (EMD Millipore, Billerica, MA, USA). An aqueous suspension of the extract was then prepared using double distilled water, the pH was adjusted to 2 with 6 M HCl, and the mixture was kept at 100 °C for 3 h. All of the standards and extracts were filtered through a 0.451 m syringe membrane filter (Type Millipore) and sonicated for 15 min in a micro clean 109 bath prior to HPLC analysis. Phenolic compounds were analyzed using Gradient HPLC (LC-10AT, SCTL, Shimadzu, Kyoto, Japan), and elution was performed for 60 min at a flow rate of 1 mL/min in a gradient system of two mobile phases A (H_2O_2:AA-94:6, pH 2.27) and B (ACN100%).

2.13. GC-Based Fatty Acid Profiling

Fatty acid methyl esters (FAMEs) were organized by IUPAC, 1987 standard method 2.301 and determined using a Perkin Elmer gas chromatograph model Clarus 500 fitted with an Rt-2340 NB (RESTEK, Corp., 800-356-1638, Centre County, PA, USA), methyl-lignocerate-coated (film thickness 0.20 µM), polar capillary column (60 m × 0.25 mm), and an FID detector. At a flow rate of 67.4 Psi, oxygen-free nitrogen was used as the carrier gas. Other conditions were as follows: initial oven temperature, 80 °C; final temperature, 210 °C; ramp rate, 3 °C/min; injector temperature, 210 °C; detector temperature, 220 °C; and FAMEs were determined by analyzing their relative and absolute retention times with authentic standards bought from Sigma-Aldrich (Buchs, Switzerland). Fatty acid methyl esters (FAMEs) were prepared following the IUPAC official method. Paquot's [28] standard protocol was followed for the preparation of FAMEs, which was carried out by deriving samples from fatty acid methyl esters of triglycerides via saponification of the glycosides liberation, and esterification using methanol from the fatty acids. The oil sample (0.2 g) was weighed into a 100 mL round neck round bottom flask. One pellet of KOH and 30 mL of methanol were then mixed in the flask, and the flask was then refluxed for 25 min until the droplets of fats disappeared. The reaction mixture was cooled and then gently

shifted to a separating funnel, and a small amount of n-hexane was mixed. The separating funnel was stirred gently by rotating it many times, and the upper layer of hexane was separated and washed several times with 10 mL of deionized water. The hexane solution was dried over anhydrous sodium sulfate, filtered, and used for GC analysis. The dry and solvent-free methyl esters were preserved in a sealed sample tube in a deep freezer and used for further analysis.

2.14. Statistical Analysis

All collected data were subjected to statistical analysis using the software CoStat CoHort 6.4. Analysis of variance and LSD were used to evaluate the effects of factors and comparison of means.

3. Results

Statistical analysis (analysis of variance) of the data showed a non-significant effect of water reduction and SMF on leaf area, leaf dry weight, and plant height (Table 1). With water deficit, no reduction was observed in leaf number. The SMF increased this attribute under limited water conditions (Table 1). There was negligible effect of water deficit on leaf fresh weight. The comparison of means (LSD @ 0.1 level) showed that SMF of 0.2 T enhanced leaf fresh weight under stressed and non-stressed conditions.

The yield experienced a marked reduction at 50% irrigation in terms of achenes per capitulum ($p \leq 0.001$) and capitulum weight ($p \leq 0.001$), whereas the % of oil yield, 50 achenes weight, and achene weight/plant did not show significant differences with variation in irrigation level. The yield experienced a marked reduction ($p \leq 0.001$) with reduced irrigation (50%) in terms of achenes per capitulum. All doses of SMF increased achenes number per capitulum in stressed plants. The capitulum weight notably reduced ($p \leq 0.001$) under water deficit (50% irrigation) conditions. Seed treatment with 0.1 T improved this attribute in stress (Table 1).

The percentage of oil yield remained invariable in both irrigation regimes (100 and 50%). SMF seed treatment at the highest dose (0.3 T) markedly improved the percent of oil yield under limited water conditions. Fifty achene weight and achene weight per plant remained similar for both factors (irrigation and SMF) (Table 1)

On the other hand, chlorophyll a levels in untreated plants were reduced by 50% via irrigation reduction (Figure 1A). For plants grown under regular irrigation, none of the treatments had a discernible impact on this trait. However, magnetic treatment (0.1, 0.2, and 0.3 T) significantly improved this attribute in plants grown under 50% irrigation. For chlorophyll b, neither factor (irrigation and priming) was significant (Figure 1A). With 50% less irrigation, there was a noticeable reduction in the overall chlorophyll content. The total chlorophyll content in sunflower leaf samples varied with the treatment doses. In 100% irrigation, plants grown after 0.1 T magneto-priming showed a marked decrease in the chlorophyll level. However, other levels (0.2 and 0.3 T) were not significantly different from the control (unprimed plants). Fifty % irrigation in plants raised from SMF (0.1, 0.2, and 0.3 T) equally improved this attribute in sunflower leaves (Figure 1C).

Table 1. Growth and yield of sunflower raised using magnetic seed treatment (0.1. 0.2 and 0.3 T), grown under two irrigation conditions (100 and 50%). Where similar alphabets present non-significant differences in means at 0.01 level (LSD by CoStat CoHort 6.4).

Stress Level	Manetic Treatments	Leaf Area	Leaf Number	Leaf Fresh Weight	Leaf Dry Weight	Plant Height	Capitulum Weight	% Oil Yield	No. of Achenes/Capitulum	50 Achenes Weight	Achene Weight/Plant
100% Irrigation	Control	242.3 ^{ab}	19.0 ^{ab}	2.00 ^{c}	0.38 ^{a}	99.66 ^{ab}	129.3 ^{a}	14.8 ^{b}	533 ^{b}	2.55 ^{abc}	16.66 ^{cd}
	0.1 T	272.6 ^{a}	24.3 ^{a}	3.18 ^{bc}	0.47 ^{a}	118.6 ^{a}	85.3 ^{b}	15.6 ^{b}	296 ^{b}	2.78 ^{a}	43 ^{a}
	0.2 T	216.5 ^{ab}	20.3 ^{ab}	4.11 ^{ab}	0.54 ^{a}	105.3 ^{ab}	110.3 ^{ab}	17.8 ^{ab}	142 ^{e}	2.91 ^{a}	28.33 ^{b}
	0.3 T	125.3 ^{b}	18.3 ^{ab}	2.89 ^{bc}	0.39 ^{a}	81.66 ^{b}	104.3 ^{ab}	16.5.^{ab}	118 ^{e}	1.97 ^{c}	20 ^{c}
50% Irrigation	Control	139.7 ^{b}	14.3 ^{b}	3.32 ^{bc}	0.54 ^{a}	72.66 ^{b}	036.0 ^{c}	15.2 ^{b}	194 ^{de}	2.04 ^{c}	11.00 ^{de}
	0.1 T	156.3 ^{b}	26 ^{a}	2.13 ^{c}	0.32 ^{a}	88.00 ^{b}	103.6 ^{ab}	5.7 ^{a}	583 ^{b}	2.44 ^{abc}	12.00 ^{de}
	0.2 T	167.8 ^{a}	25.5 ^{a}	4.76 ^{a}	0.61 ^{a}	97.66 ^{b}	38 ^{c}	9.6 ^{c}	733 ^{a}	1.91 ^{c}	6.33 ^{f}
	0.3 T	243.0 ^{ab}	25.6 ^{a}	3.13 ^{bc}	0.43 ^{a}	84.33 ^{b}	41 ^{c}	20.4 ^{d}	350 ^{c}	2.19 ^{abc}	6.66 ^{ef}

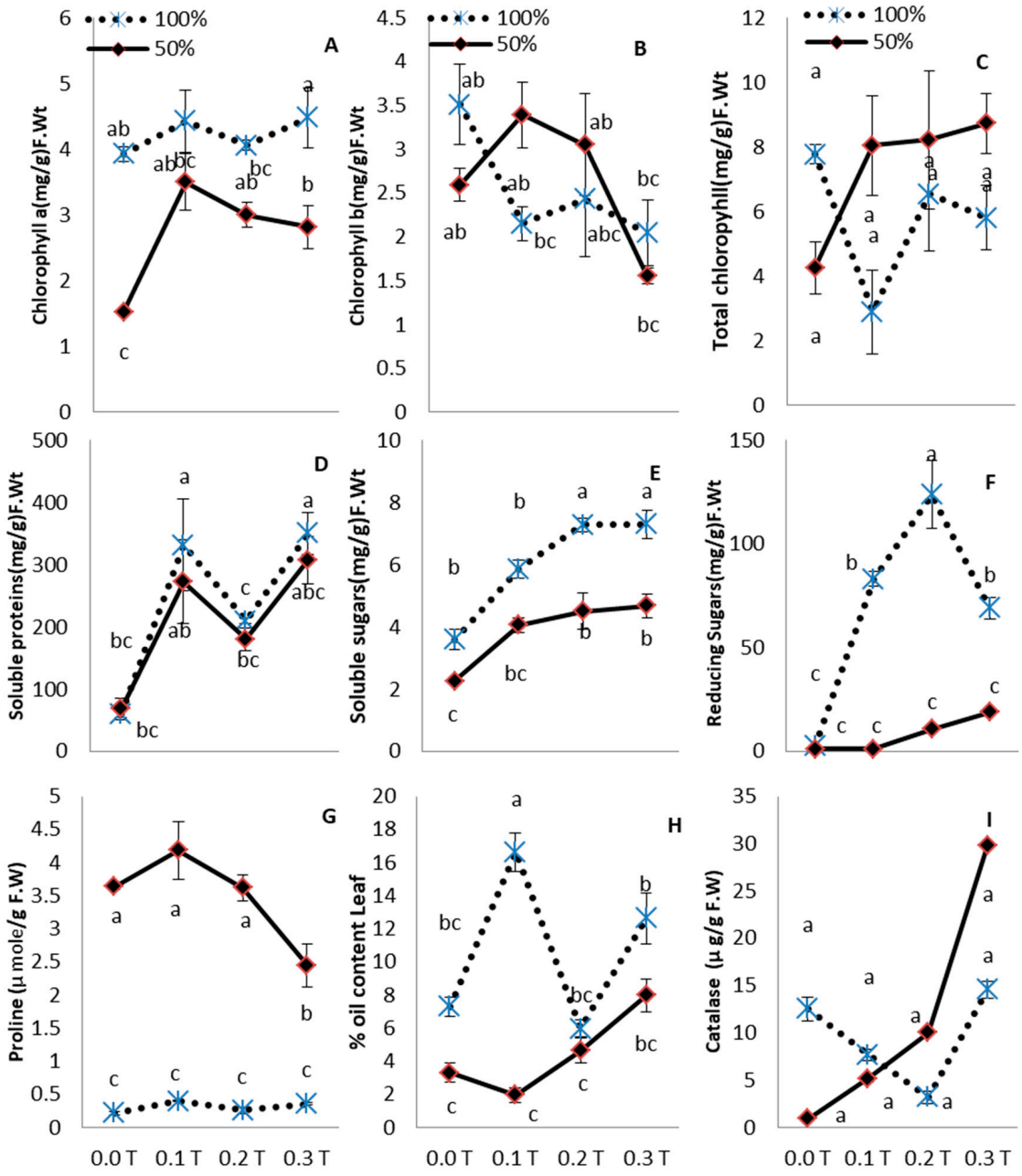

Figure 1. Biochemical attributes (primary metabolites) of sunflower plants grown under 100% versus 50% irrigation after magnetic seed treatment (0, 0.1, 0.2, and 0.3 T) at the flowering stage. Similar alphabets present non-significant differences in means at 0.01 level (LSD by CoStat CoHort 6.4).

Water deficiency in the growth medium had a non-significant effect on the total leaf-soluble proteins. However, seed treatment (0.1 T and 0.3 T) had a positive effect ($p < 0.001$) in improving total leaf soluble proteins in both water regimes (Figure 1D). Total soluble sugars experienced a decline ($p < 0.01$) with water deficit (50% irrigation). Higher doses (0.2 and 0.3 T) of magnetic application were successful in improving ($p < 0.001$) this attribute in normal and stressed plants (100 and 50% irrigations) (Figure 1E). The leaf-reducing sugars were ineffective to stress. In the case of untreated plants, the results were similar under both irrigation conditions. Plants grown after magnetic treatment showed significantly enhanced (Figure 1F) accumulation in plants grown under 100% irrigation.

All plants grown under 50% irrigation had notably ($p < 0.001$) higher levels of proline in comparison to 100% irrigation. None of the magnetic applications was statistically effective for this attribute (Figure 1G). The statistical analysis of the data recorded for leaf

percent oil content indicated a significant difference between treatments ($p \leq 0.001$). All untreated and treated plants remained indifferent under both water conditions, except for the plants grown under normal irrigation after the 0.1 T pre-sowing treatment (Figure 1H). The activity of catalase experienced a marked decline with a reduction in water in the growth medium. SMF enhanced its activity with an increase in treatment intensity (0.1 to 0.3 T), and there was a linear increase in activity (Figure 1I).

Total leaf phenolics did not experience any deterioration with water reduction (50% irrigation). Magneto-priming of 0.1 and 0.3 T further enhanced ($p < 0.001$) its accumulation in both water environments (100 and 50%). Magnetic application of 0.1 and 0.3 T enhanced its accumulation under 100% irrigation, whereas under 50% condition, this enhancement was observed in plants grown after 0.2 and 0.3 T magnetic treatment only (Figure 2A). Statistical analysis of leaf flavonoid data was not significant for stress and priming (Figure 2B). Leaf anthocyanin content experienced a marked (Figure 2C) decline with reduced irrigation (50% irrigation) in untreated plants. Under 100% irrigation, 0.1 T decreased its level but other higher levels remained non-significant to unprimed plants. After performing 50% irrigation, 0.1, 0.2, and 0.3 T equally enhanced their level in sunflower leaves. Data observed for leaf carotenoids varied with treatment levels and growing conditions in terms of irrigation percentage. Under normal irrigation, 0.1 treated (magnetic application) seeds had reduced content, whereas the other two levels were insignificant. Under 50% irrigation, lower levels of improved leaf carotenoids were observed (Figure 2D).

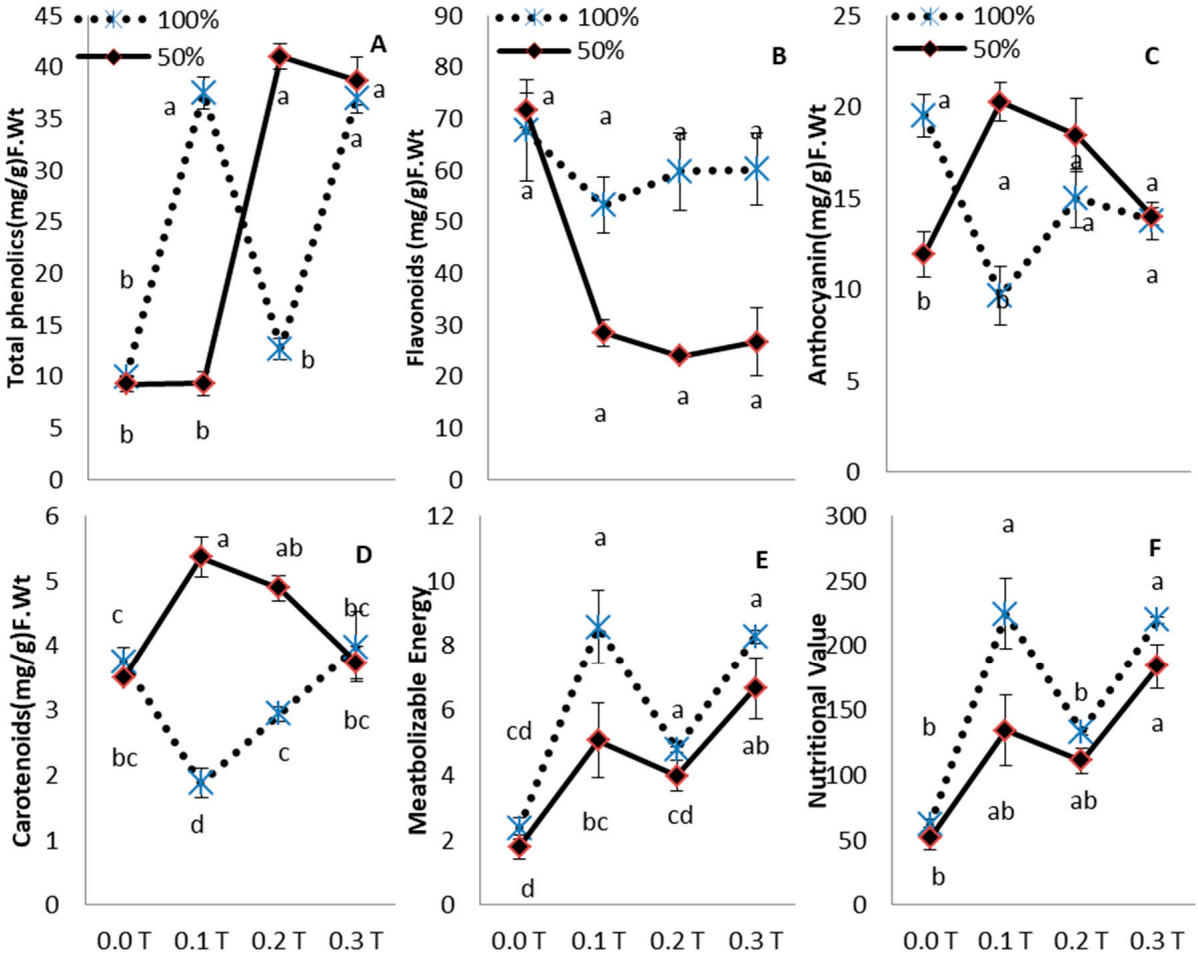

Figure 2. Secondary metabolites, metabolized energy, and nutritive values of sunflower plants grown under 100% versus 50% irrigation after magnetic seed treatment (0, 0.1, 0.2, and 0.3 T) at the flowering stage. Similar alphabets present non-significant differences in means at 0.01 level (LSD by CoStat CoHort 6.4).

The metabolizable energy of the leaf samples remained stable under water scarcity conditions. SMF improved ($p < 0.001$) its level under both growing conditions at 0.1 T and 0.3 T radiation doses (Figure 2E). Water limits (under 50% irrigation) failed to affect leaf nutritional values (Figure 2F). The priming of 0.1 T and 0.3 T treatment levels significantly increased, whereas under 50% irrigation, the highest level (0.3 T magnetic seed treatment) significantly enhanced this characteristic (Figure 2A). The metabolomics profiling of selected plants grown from 0.3 T treated seeds showed better oil and achene production.

HPLC chromatograms for phenolics showed variable patterns for irrigation levels and treatment levels. In untreated plants, 50% irrigation reduced the hydroxy derivative of cinnamic acid (26.6%) compared to untreated plants. With water reduction (50% irrigation), there was a 26.6% reduction in leaf hydroxy derivatives, as described by the HPLC chromatogram (Figure 3A,B). Fifty percent irrigation in comparison to 100% irrigation showed a percent increase in quercitin (34.80), gallic acid (100), vanillic acid (62.2577), chlorogenic acid (100), syringic acid (100), p-coumaric acid (5.59), m-coumaric acid (6.29), cinnamic acid (81.15), and sinapic acid (100). Caffiec acid (121.08), benzoic acid (146.51), and ferulic acid (0.04) underwent a percent decrease in untreated plants (Figure 3). Magneto-primed plants grown under 100% irrigation had greater accumulation of gallic acid (8.2 ppm), caffiec acid (5.5 ppm), and cinnamic acid (9.5 ppm), with a reduction in caffiec acid, chlorogenic acid, and syringic acid (7.53, 291.25, 229.53, and 55.43%, respectively). Plants grown under 50% irrigation responded positively towards seed treatment in terms of enhanced accumulation of benzoic acid, p-coumaric acid, chlorogenic acid, and ferulic acid (16.87, 32.3, 10.7, and 9.72, respectively) (Figure 3C,D).

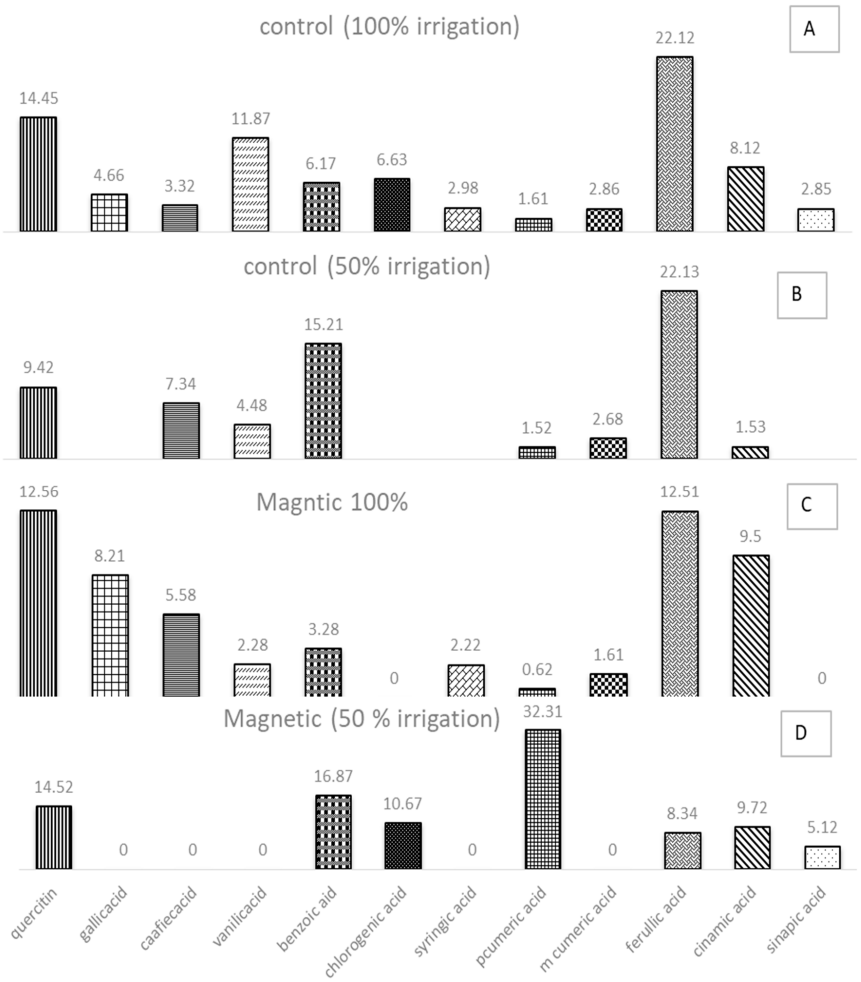

Figure 3. Phenolic profile of untreated ((**A**) 100%/(**B**) 50%) and magnetic (0.3 T) ((**C**) 100%/(**D**) 50%) sunflower leaves at the flowering stage under 100% irrigation.

The majority of fatty acids that were determined in sunflower achenes included palmitic acid (8.01%), stearic acid (4.11%), oleic acid (36.5%), and linoleic acid (50.76%). The unsaturation-to-saturation ratio was 46.96. Water deficit reduced the unsaturation-to-saturation ratio by up to 43.63. Magnetic treatment of 0.3 T under 50% irrigation enhanced unsaturated fatty acids and decreased the level of saturated fatty acids. Ultimately, there was a marked improvement in the unsaturation-to-saturation ratio (50.81) (Table 2).

Table 2. Fatty acid composition of oil yield obtained from sunflower grown under control (100%) and limited water (50% irrigation) conditions after presowing magnetic (0.3 T) irradiation. S indicates saturated fatty acid.

Irrigations	Seed Treatment	Palmitic Acid (%)	Stearic Acid (%)	Oleic Acid (%)	Linoleic Acid (%)	Sum	U/SRatio
100%	Control	8.01	4.11	36.51	50.76	99.39	46.957
	Magnetic	8.9	4.11	36.69	50.86	100.56	46.51
50%	Control	8.41	6.58	31.82	44.02	90.83	43.634
	Magnetic	6.13	2.98	75.28	12.57	96.96	80.31

4. Discussion

As a biotechnological tool, the static magnetic field (SMF) has gained an eminent position in the literature in recent years. This is due to the successful outcomes in the number of species, including wheat [29], alafa [30], almond seeds [31], and soybean [10]. The dose and timing of positive results varies from specie to specie. In the present study, sunflower plants were treated with SMF (0.1, 0.2, and 0.3 T) and varied in growth and metabolism regimes. SMF with activated meristematic cell division and auxin enhancement modulate metabolic activities and chloroplast growth, as per previous reports. Its genomic response is linked to cryptochrome and auxin signaling [32]. In previous findings, high doses have been reported to induce fragmentation, bond rupturing, and ROS production [33]. In the current study, the SMF range used (0.1–0.3 T) was found to be safe for sunflower seeds, even when 0.3 TSMF provided the highest oil yield. In agreement with the literature, the SMF-treated sunflower plants in the present study experienced better oil and capitulum yield under 50% irrigation.

In the present and previous investigations, it was shown that when there was a water shortage, sunflower plants had lower levels of photosynthetic pigments, chlorophyll a, and total chlorophyll. This was ascribed to stress-induced photoinhibition, with restricted light harvesting and electron transport mechanisms [7]. Magnetic field treatment of seeds was employed for different crops with positive outcomes [30,31]. Calcium-dependent strategic mechanisms behind plants' stress tolerance are associated with SMF treatment, which enhances photosynthesis [34–36]. SMF has recently been known to activate key enzymes of carbon metabolism, thus facilitating CO_2 provision to Rubisco for direct promotion of carbon fixation [10,37,38]. Our findings seem in line with the literature; the treated plants had better photosynthetic pigments and better accumulation of products of photosynthesis, which may be related to the possible activation of Ca^{2+} signaling pathways and carbonic anhydrase, although these parameters were not part of the determinations of this research.

Osmoregulation is a kinase cascade-mediated phenomenon accredited with a number of enzymatic and non-enzymatic proteins [39]. In addition to this, recent advancements in proteomic studies [40] have revealed a reconnoitered link between protein concentration in plants and their photosynthetic efficiency, defense system, folding, and degradation. Therefore, lowered amino acid metabolism and ROS scavenging proteins show the sensitivity of crops under different abiotic and biotic stresses. In the present study, no reduction in total soluble proteins was observed with water limits, indicating tolerance of the variety under 50% irrigation in terms of total soluble proteins. SMF further enhanced protein content under both irrigation conditions. Possible protein-mediated improvement in ROS scavenging under drought was observed under UV-B stress in soybean [10]. There is no direct evidence supporting SMF-induced molecular activation for protein overaccumulation. However, there is evidence of variation in protein expression in different cryptochrome-1

and 2 mutants. Therefore, external SMF with the potential to affect cryptochrome may be justified for protein enhancement [41].

Carbohydrate accumulation in plants plays a crucial role in osmoregulation under drought stress, directly or by producing amino acids and organic acid-like products to maintain osmotic balance [42]. In the present study, total soluble sugars were markedly reduced with stress. Recent molecular studies have revealed decreased carbohydrate levels under drought stress with the expression of autophagy-related genes. Stress enhances their expression. Leaf glucose and fructose experience a decline in drought, which resulted in a break in metabolic balance. Such a disorder of intracellular nutrients has a positive correlation with the expression of autophagy-related genes [43]. Uncontrolled autophagy in stressed cells may lead to the formation of bubble-like autophagic vesicles. Under extreme conditions, fragmentation of cytoplasmic components may occur. SMF seed pre-treatment enhanced carbohydrates in sunflowers, which is in agreement with the results of Zareei et al. [44]. Enhancement of the sugar content in plant leaves can support plants with active regulation of osmotic pressure. This plays a crucial role in drought stress tolerance [45,46]. There are possibilities of some suppression of autophagy-related genes too. Stressed plants of sunflowers with SMF showed better photosynthesis, soluble sugars, and yield, which is in line with the existing literature.

Proline, along with other osmolytes, can regulate osmotic pressure with consequential drought tolerance [45,46]. The higher proline content in stressed sunflower plants in the present study is in line with the literature [45]. Better accumulation of proline lessens osmotic potential and water evaporation results in the instability of the intracellular environment. In contrast to the literature regarding SMF-induced enhancement of proline, there was a negligible effect of treatment on sunflowers. In the present study, untreated plants experienced a marked decline in catalase, indicating a negative impact of drought. Sunflower leaves showed enhanced activity of anthocyanin in plants grown in limited water after treatment, in accordance with previous work [31]. SMF treatment is known to alter cryptochrome-mediated protein expression [41]. Catalase protein was enhanced with SMF in the present study linearly with the applied doses ($0.1 < 0.2 < 0.3$). Another justification from the literature may be a possible link between the magnetic field and Ca^{2+} ion pumps [35], which is in turn linked to enzyme production [47].

Phenolics and flavonoids showed resistance against stress [48] and nutraceutical value. SMF enhances the phenolics in the current and earlier studies [31]. When a magnetic solution is applied to plants, it activates key enzymes of phenolic metabolism. Currently, the positive effect of SMF on the quantitative and qualitative values of phenolics may be dependent upon the possible modulation of the PAL enzyme by SMF seed treatment. This was depicted by the variable phenolic profiles in SMF-treated and -untreated plants under both water regimes (Figure 3). Anthocyanin content was reduced with lowered irrigation. There was a positive correlation between plant anthocyanin content and drought tolerance [49,50]. The decline in antioxidant anthocyanin in drought was compensated by all doses of SMF in the present study. Anthocyanin biosynthesis in plant cells was regulated by cryptochromes [51]. In magnetically treated plants, the cryptochromes act as receptors [11] and regulate the expression of genes encoding for chalcone synthase, which is a key enzyme in anthocyanin metabolism [52,53]. Cryptochrome-1 regulation is also associated with drought [54] and UV-B [11] stress tolerance. Enhanced anthocyanin content in magnetically treated stressed sunflowers is in accordance with the literature [31,44].

The variable effect of applied SMF doses could also be justified by cryptochrome-mediated magneto-perception. Its modulation and activation generally rely on electron spinning of radical pairs, spin physics, and the chemistry behind this process. The external magnetic field splits the energy of the target atom (in terms of the Zeeman Effect). The coupling between the orbital momentum and spin angular momentum needs to be cancelled for a positive effect. The intensity of the magnetic field that successfully disrupts the coupling between the orbital momentum and spin angular momentum of cryptochrome could show positive results with a sufficient Paschen–Back effect. On the other hand, a

magnetic field weaker than that level may be useless or less beneficial due to the dominant coupling of spin and orbit [11,55]. In our study, the strongest SMF (0.3 T) was successful. The present results of magnetically treated sunflower leaves with enhanced carotenoids under stress are in line with the findings of Hasan et al. [56] and de Souza-Torres et al. [57]. Carotenoid accumulation supports stable photosynthesis under drought [50] and acts as an antioxidant [45].

For the use of edible oil in the food industry, WHO, 1985, and the United Nations Food and Agriculture Organization (FAO) have recommended saturated fatty acid limits up to 10% of the total caloric intake. The expert opinions of WHO and FAO have been coined to attain better prevention of chronic diseases with a healthy use of nutrition [58]. Biodiesel production from sunflowers is also correlated with a better composition of unsaturated fatty acid in oil [59]. In the present study, sunflower seeds with 87.27% unsaturated fatty acids confirmed previous findings regarding the healthy use of sunflower as edible oil. Irrigation at 50% reduced its level up to 75.84% and enhanced the saturated fatty acid composition from 12.12 to 14.99%. This decline in nutritive value induced by water deficit supported our hypothesis that a moderately tolerant variety, if grown in a water-limited environment, could experience reduced nutritive value and may need an additional supportive strategy to cope with it.

Therefore, any method that increases the amount of unsaturated fatty acids, like oleic and linoleic acid, would be a potential strategy for future sunflower oil production. Water reduction lowered oleic acid and linoleic acid in the present study. Magnetic seed treatment (0.3 T) improved the oleic acid content and total unsaturated-to-saturated fatty acid ratio (87.85%) of sunflower achene oil. Previous reports indicated the potential of magnetically treated water to increase the essential fatty acids [60]. Oleic acid and linoleic acid increased in the current study, which has some association with plant stress tolerance as well [61]. Therefore, SMF with enhanced oleic acid improved the drought tolerance of sunflowers along with their nutritive value.

5. Conclusions

In conclusion, SMF at 0.3 T could manipulate sunflower metabolism to produce better nutraceutical metabolites with enhanced drought tolerance in sunflower.

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