

# Evaluation of the phenolic content and antioxidant activity of sunflower seeds under deficit irrigation conditions

Sıla Barut Gök<sup>1,✉</sup>  
Yasemin Erdoğan<sup>2</sup>  
Erhan Göçmen<sup>3</sup>  
Buse Salbaş<sup>3</sup>  
Tolga Erdem<sup>3</sup>

<sup>1</sup>Department of Food Technology  
Çorlu Vocational School  
Tekirdağ Namık Kemal University  
Çorlu, Tekirdağ, Turkey

<sup>2</sup>Department of Field Crops  
Faculty of Agriculture  
Tekirdağ Namık Kemal University  
Tekirdağ, Turkey

<sup>3</sup>Department of Biosystem Engineering  
Faculty of Agriculture  
Tekirdağ Namık Kemal University  
Tekirdağ, Turkey

✉ CORRESPONDING AUTHOR:  
S. Barut Gök  
E-mail address: sbgok@nku.edu.tr  
Tel: + 902822503512

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Abiotic stress like deficient irrigation might affect the synthesis of secondary metabolites in plants, which has several pharmacological effects. In this study, the effect of water deficit was determined on sunflower (*Helianthus annuus* L.) seed protein content, antioxidant activity, phenolic content and flavonoid and phenolic compounds of defatted seed extract for industrial usage. One sunflower genotype was planted in a field with six irrigation regimes (IR) under water deficit conditions that were set according to evaporation values measured during the 7-day irrigation interval (Kcp: 0, 0.25, 0.50, 0.75, 1.00 and 1.25) in a randomised complete block design. Results indicated that total phenolic content of seed extracts varied between 1.29-1.96 times higher than that under drought stress conditions (IR<sub>1</sub>). The antioxidant capacity of seed extracts was 7.82 (IR<sub>2</sub>) and 9.87% (IR<sub>4</sub>) higher than under drought stress conditions. The most abundant phenolic compounds that accumulated due to low water deficit (75%) were gallic acid and catechin. Based on principal component analysis, the biplot generated from PC1 and PC2 indicated that phenolic compounds in different irrigation regimes were collected under four subgroups. The drought stress conditions separated higher caffeic acid in a subgroup distant from the other irrigation regimes.

**Keywords:** Sunflower; Drought stress; Irrigation; Phenolic compounds; Antioxidant activity

## 1. INTRODUCTION

Sunflower (*Helianthus annuus* L.) is the third most important oilseed crop cultivated worldwide after soybean and rapeseed with a production of 56 million tons [1]. Turkey is an important country for sunflower production, ranking 7th in the world, and the Thrace region, the largest sunflower production area in Turkey, accounts for 50% of production.

Sunflower, to some extent more drought resistant than other oilseed crops, is highly sensitive to drought stress from flowering to seed filling stage [2]. In sunflower agriculture, especially in dry conditions where hybrid cultivars are used, if the precipitation is irregular and low, the yield is low. Among the various types of stress, water deficit has gained prominence in the literature due to its implications for the cultivation of plants, especially in arid and semi-arid regions where the decrease in precipitation is persistent [3, 4]. Fresh water is a resource that needs to be carefully managed for global food security, and optimisation of irrigation is essential to increase yield per unit of water in these regions [5]. Major changes have occurred in global and regional precipitation regimes due to global warming in recent years [6].

Sunflower seeds are an important nutrient due to their high oil (44%) and protein content (16%). Besides being an important oil plant, it is the third oilseed meal, comprising 5.6% of the global production [7]. Although many plants are cultivated for their economically important primary metabolites, the

secondary metabolites obtained from these plants are also extremely important for human nutrition because environmental, economic and social difficulties experienced in the world have made it necessary to use resources effectively. The pulp remaining after oil extraction from sunflower seeds is rich in secondary metabolites and can be used as a food additive.

Recent studies about the pharmacological effects of sunflower extract indicated that it is antioxidant [8], antibacterial [9], antifungal [10], anti-inflammatory [11], anticancer [12], cardioprotective [13], and dermo protective [14]. Pharmacological characteristics of sunflower are mainly due to its ability to accumulate some active secondary metabolites, mainly phenolic (caffeic acid, chlorogenic acid, caffeoylquinic acid, gallic acid, protocatechuic,  $\beta$ -coumaric, ferulic acid, sinapic acid) and flavonoid compounds (heliannone, quercetin, kaempferol, luteolin, apigenin) [15-16].

Secondary metabolite components in plants are greatly affected by abiotic stress factors such as drought stress [17]. Drought stress changes a plant's physiological and biochemical characteristics [18-19]. Phenols are involved in plant adaptations to biotic and abiotic stresses [20], and these compounds may contribute to the reduction of environmental stress effects [21]. Flavonoids are an important defensive secondary metabolite that protects plants from water stress [22]. The literature shows that plant flavonoid and phenolic contents could be affected by drought stress, and this response could be different among

and within species and plant parts. In some studies, water stress increased plant total phenolic content, flavonoid content, and antioxidant activity [23-25]. In addition to this, it was reported that oilseed plant total phenolic content [26], antioxidant capacity [27], phenolic and flavonoid components [28-29] can be affected by drought stress. There is little information about the changes in secondary metabolites of plant seeds in response to different irrigation treatments in semi-arid regions. The aim of this study was to determine the phenolic content antioxidant scavenging activity, and protein content, and to measure phenolic and flavonoid compounds in defatted sunflower seed extract from a semi-arid region.

## 2. MATERIALS AND METHODS

### 2.1 PLANT MATERIAL AND GROWTH CONDITIONS

In the study, sunflower seeds of the Sanay MR variety were used as plant material. This study was carried out in Tekirdağ Viticultural Research Institute, Tekirdağ, Turkey. The experimental area was located 4 m above sea level between 40° 59' north latitude and 27° 29' east longitude. The region has a semi-arid climate. The climate data for the locations are given in Table I.

Soil samples taken from three different depths in the experimental area were analysed in the laboratory (Tab. II). According to these results for soil analysis, clay loam, slightly salty, low calcareous and low or-

**Table I** - Climate data of location for 2019.

Month	Avg. temperature, °C	Avg. relative humidity (%)	Avg. wind speed	Sunshine duration (h)	Evaporation rate (mm/d)	Precipitation (mm)
April (30)	17.50	67.80	3.10	11.5	4.00	-
May (1-10)	15.29	69.88	2.45	4.62	3.88	5.40
May (11-20)	17.75	74.26	2.41	5.26	3.27	22.0
May (21-31)	20.39	67.73	2.09	8.45	4.81	3.80
June (1-10)	22.17	68.16	2.45	8.65	5.82	2.30
June (11-20)	24.33	64.68	2.66	7.04	5.89	4.90
June (21-30)	25.69	61.44	3.30	8.02	7.22	0.30
July (1-10)	24.77	64.27	2.54	10.11	7.00	2.60
July (11-20)	22.23	65.10	3.19	7.75	5.24	16.20
July (21-31)	24.61	64.06	2.82	10.40	7.29	-
August (1-10)	24.92	63.81	2.91	10.23	7.05	-
August (11-20)	24.84	62.47	4.09	8.96	6.98	-
August (21-31)	25.92	60.48	3.97	9.22	7.30	-
September (1-5)	24.68	63.40	4.32	9.18	6.54	-

(Turkish State Meteorological Service, 2019).

\* Measured at 2m height; \*\* These are the total values measured from a class A evaporation pan.

**Table II** - Soil properties of the experimental area.

Soil depth (cm)	pH	EC (ds m <sup>-1</sup> )	CaCO <sub>3</sub> (%)	Field capacity (%)	Wilting point (%)	Bulk density (g cm <sup>-3</sup> )
0-30	7.19	0.62	1.00	23.01	15.91	1.49
30-60	6.71	0.49	1.00	27.05	17.71	1.58
60-90	6.95	0.55	1.50	31.76	20.96	1.61

ganic matter content were determined as the soil parameters. The quality class of the irrigation water was C2S1. According to the United States Salinity Laboratory classification system, the electrical conductivity (EC) was  $0.72 \text{ dS m}^{-1}$  and the sodium absorption ratio (SAR) was 0.62.

The field trial was conducted in randomised block design with 3 replications. Plants were sown on April 30, 2019 by sowing machine. Each plot consisted of  $25.20 \text{ m}^2$  ( $4.20 \text{ m} \times 6.00 \text{ m}$ ) and there were 120 plants cultivated at  $0.70 \text{ m} \times 0.30 \text{ m}$  intervals in the plot area. A space of 3 m was created between all parcels in order to prevent the effects of the treatments on each other. A total of  $250 \text{ kg ha}^{-1}$   $23(\text{N})12(\text{P})9(\text{K})+10(\text{SO}_3) + 2(\text{MgO})+\text{B}+\text{Zn}$  fertilisation was applied during sowing, and this fertilisation was specially prepared for sunflowers. Mechanical control was applied against weeds.

## 2.2 IRRIGATION TREATMENT

In this study, a drip irrigation method was used. The irrigation water in the storage pool was transferred to the parcels by means of a pump. In the irrigation system, a 50 mm polyethylene (PE) pipe was used for the main pipe, 32 mm for the manifold, and 16 mm for the laterals. In the experiment, a lateral pipe was laid for every two rows of plants. The dripper flow rate was  $4 \text{ L h}^{-1}$  under 1 atm operating pressure. Dripper intervals were selected as 0.50 m according to soil properties. The percentage of wetted area (P) was calculated as 36% [30].

In the study, irrigation treatments were created based on different rates of evaporation measured from class A evaporation pans during the 7-day irrigation interval. Treatments are shown as 0 ( $\text{IR}_1$ ), 0.25 ( $\text{IR}_2$ ), 0.50 ( $\text{IR}_3$ ), 0.75 ( $\text{IR}_4$ ), 1.00 ( $\text{IR}_5$ ) and 1.25 ( $\text{IR}_6$ ), respectively. The amount of irrigation water was determined using the equation below:

$$\text{IR} = E_p \times K_{cp} \times P \quad (1)$$

Where:

$\text{IR}$  = Irrigation amount (mm)

$E_p$  = Cumulative pan evaporation (mm) for 7 days interval

$K_{cp}$  = Crop-pan coefficient

$P$  = Percentage of wetted area.

The soil water content in the plots was measured gravimetrically every 30 cm to a depth of 90 cm before irrigation. Evapotranspiration was determined using the soil water balance equation [31]. The soil water balance equation is as follows;

$$\text{ET} = \text{IR} + P + \Delta\text{SW} - \text{DP} - \text{RO} \quad (2)$$

Where:

$\text{ET}$  = Evapotranspiration (mm)

$\text{IR}$  = Irrigation water (mm)

$P$  = Precipitation (mm)

$\Delta\text{SW}$  = Change in soil water storage in 0.90 m soil profile (mm)

$\text{DP}$  = Deep percolation (mm)

$\text{RO}$  = Runoff (mm).

The runoff was assumed to be zero, as the amount of irrigation water was controlled.

## 2.3 TOTAL PROTEIN CONTENT OF THE SEED

Total protein content in the samples was investigated in accordance with the Kjeldahl method procedures described in the AOAC method 991.20 (1995). For this purpose, 1 g of solid sample was mineralised with 10 mL of 96%  $\text{H}_2\text{SO}_4$  (v/v) and approximately 10 mg of catalyst at  $450^\circ\text{C}$  during 150 min. Subsequently, the sample was distilled with 32% NaOH (w/v) and after reaction with 4% boric acid (w/v) solution, it was titrated against 0.1 N HCl. The blank consisted of a non-protein sample. To convert total nitrogen content into sunflower protein concentration, a conversion factor of  $\text{N} \times 5.6$  was used, as previously described [32, 33]. All analyses were performed in triplicate and average values were calculated with standard deviation.

## 2.4 PROCEDURE FOR EXTRACTION OF PHENOLIC CONTENT OF DEFATTED SEEDS

Methanol extraction was carried out according to the method of Khattak et al. [34]. The sunflower seeds were ground in a laboratory mill (Analysenmühle A10, IKA-Werke GmbH, Germany) and defatted with hexane using a magnetic stirrer for 24 hours. A 5-g portion of the defatted flour was weighed into screw-capped dark glass bottles and extracted at room temperature for 24 hours at 140 rpm by shaking (Edmund Bühler GmbH KS-15) with 200 mL of 80% (v/v) aqueous methanol. Extractions were carried out three times. The extracts were centrifuged at 10,000 rpm for 30 min at  $15^\circ\text{C}$  and the supernatant was collected. The extraction procedure was carried out in triplicate.

## 2.5 DETERMINATION OF ANTIOXIDANT CAPACITY (AC) BY DPPH RADICAL SCAVENGING METHOD

Antioxidant activities of extracts were determined according to Brand-Williams et al. [35]. Different concentrations of seed extracts were taken into tubes and 600  $\mu\text{l}$  of molar DPPH\* radical solutions were added to each tube; the total volume was completed to 6 ml with methanol. After mixing and incubating the tubes for 30 min at room temperature in a dark environment, absorbance was read at 517 nm wavelength against the control. By using the absorbance value, the % inhibition of DPPH radicals (I %) for each of the samples was calculated by using equation (3). In equation (3), the absorption of control (methanol instead of seed extract) is expressed as  $A_{\text{control}}$  and the absorption of the analysed sample is expressed as  $A_{\text{sample}}$ .

$$\text{Inhibition \%} = ((A_{\text{control}}) - A_{\text{sample}})/(A_{\text{control}}) \times 100 \quad (3)$$

Inhibition values were graphed versus different concentrations for each seed and linear regression analysis was applied in order to obtain the equation defining the curve. By using the equation, the  $\text{EC}_{50}$

value was calculated. The EC<sub>50</sub> value is the amount of antioxidant necessary to decrease the initial DPPH<sup>•</sup> concentration by 50%.

## 2.6 DETERMINATION OF TOTAL PHENOLIC MATTER CONTENT

Total phenolic content of methanolic extracts, as stated Sadasivam and Manickam [36], was carried out by modifying the method based on the reaction occurring between phenolic compounds in the seed and Folin-Ciocalteu reagent. A 500 µl aliquot of methanolic extract was diluted with 2.0 ml of deionised water, and the obtained solution was mixed with 2.5 ml of Folin-Ciocalteu reagent. Then, 2 ml of sodium carbonate solution was added after an incubation time of 3 minutes. The absorption of the blue-coloured solution after an incubation time of 60 minutes was read at 720 nm wavelength and evaluated with the standard curve for gallic acid [37]. The results are given as mg gallic acid equivalent/g dry matter (mg GAE/g dry matter) of seeds.

## 2.7 DETERMINATION OF THE COMPOSITION OF PHENOLIC COMPOUNDS

Analyses of the flavonoids and phenolic acids were quantified by high-performance liquid chromatography (HPLC; SHIMADZU LC-20A Series, Japan), coupled with a diode array detector (SPD-M20A, spectra from 300 to 800 nm, Shimadzu), according to the method described by Uysal Seçkin et al. [38]. An Inertsil-ODS3 C18 column (GL Science, Tokyo, Japan) with size 4.6 mm × 250 mm (5 µm) was used as the stationary phase and maintained at 40°C.

Phenolic acids, such as ferulic, chlorogenic, p-coumaric and caffeic acids, were detected at 320 nm and gallic and vanillic acids were detected at 280 nm. Flavonoids such as quercetin, kaempferol and rutin-3-hydrate were monitored at 360 nm and epicatechin was detected at 280 nm. All the standards for flavonoids and phenolics were provided by Sigma Company. The standards were dissolved using HPLC-grade methanol. Chromatographic separation was performed with gradient elution at a flow rate of 1.5 ml/min using two solvents: Eluent A -2% (v/v) acetic acid in water and Eluent B – and acetonitrile (100, v/v; eluent B), as mobile phases. The gradient program was identical to Meng et al. [39] and Uysal

Seçkin [38]. Solutions were injected into the column with an injection volume of 20 µm and the flavonoid and phenolic content of the seed extracts were calculated by comparison of the peak area and retention times with the pure standards according to the method described by Yeloojeh et al. [29]. The results are shown as mg per 100 g of dried seed weight.

## 2.8 STATISTICAL METHODS

Data were statistically analysed using analysis of variance (ANOVA) by JMP 7 (SAS Institute Inc.) statistical software. LSD (least significant difference) test ( $P \leq 0.05$ ) was used to determine differences among means [40]. Principal component analysis (PCA) was used for the metabolite profiles and the entire transcriptome dataset, respectively, using R software.

# 3. RESULTS AND DISCUSSION

## 3.1 IRRIGATION WATER AMOUNTS AND EVAPOTRANSPIRATION

The total sunflower growing period was recorded as 129 days for the year 2019. The amounts of applied irrigation water, precipitation, measured soil water depletion and measured seasonal evapotranspiration data are shown in Table III.

In 2019, all treatments were irrigated 12 times. The total amount of irrigation water applied varied between 133.7 and 668.1 mm in the treatments. During the total growing season, seasonal evapotranspiration measured from the treatments ranged from 278.2 mm to 801.3 mm [41]. Seasonal evapotranspiration increased as the amount of water applied increased. The total seasonal evapotranspiration values for sunflowers obtained from the study are consistent with the values obtained from previous studies in the Thrace region, Turkey and the world [42-44].

## 3.2 TOTAL PROTEIN CONTENT OF THE SEEDS

The total protein content of the sunflower seed is shown in Table V. Although there was a difference between irrigation applications in terms of protein content of the samples, this difference was not found to be statistically significant. In irrigation studies of sunflowers, stress reduced the seed filling stage, resulting in decreased oil content and increased protein content [45-50]. The protein content and amino acid

**Table III** - Applied irrigation water and measured seasonal evapotranspiration for treatments.

Irrigation Treatment	Soil water depletion (mm)	Precipitation (mm)	Total applied irrigation water (mm)	Measured seasonal evapotranspiration (mm)
IR <sub>1</sub>	220.7	57.5	-	278.2
IR <sub>2</sub>	176.0		133.7	367.2
IR <sub>3</sub>	156.0		267.4	480.9
IR <sub>4</sub>	120.9		401.1	579.5
IR <sub>5</sub>	90.7		534.4	682.6
IR <sub>6</sub>	75.7		668.1	801.3

**Table IV** - Analysis of variance (mean squares) for the traits in seed of sunflower genotype evaluated at six levels of irrigation regimes (IR).

Source of variation	DF	TPC	EC <sub>50</sub>	Protein
IR	5	182.80**	78.20**	1.59 <sup>ns</sup>
Rep (IR)	2	25.16 <sup>ns</sup>	1.62 <sup>ns</sup>	1.11 <sup>ns</sup>
Error	10	69	1.28	0.55
Total	17	53.80	23.94	0.92
CV %		0.77	1.37	4.99

ns: non-significant. DF: Degrees of freedom. TPC: Total phenolic content; EC<sub>50</sub>: the effective concentration at which the absorbance was 0.5; CV: Coefficient of variation

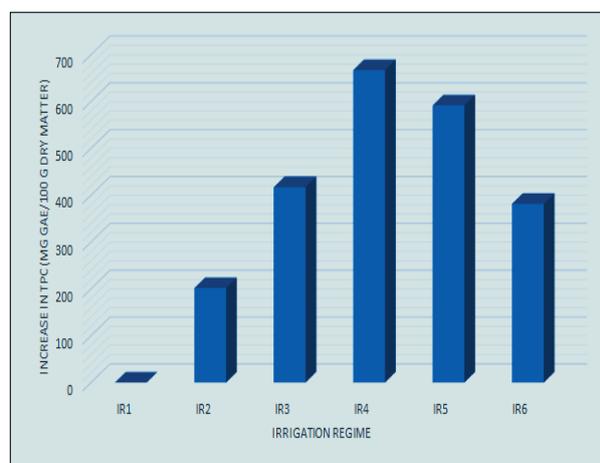
\*\*Significant at 5 and 1% levels of probability.

composition of sunflowers are not only affected by factors such as irrigation and fertilisation, but also by genetic variation, environmental factors and other agronomic practices [51]. Therefore, it is important to use drought tolerant genotypes suitable for sunflower production in drought stress conditions. The Sanay MR genotype used in the study may have been stable under stress conditions in terms of quality characteristics such as oil and protein, which are important for sunflower breeding. At the same time, there was no significant difference in oil content in different irrigation regimes for the Sanay MR genotype used in a previous investigation related to this study [41].

### 3.3 TOTAL PHENOLIC CONTENT (TPC)

The analysis of variance results for the total phenolic content (TPC) of seeds under the six irrigation regimes are presented in Table IV. TPC of non-irrigation regime extract estimated by the Folin-Ciocalteu method was 694.90 mg of GAE/ g DW. The phenolic content of sunflower seed significantly increased when plants were subjected to different irrigation regimes compared to drought stress conditions. Thus, the total phenolic contents of seed extracts were increased significantly by 1.29-, 1.60-, 1.96-, 1.85- and 1.55-fold under irrigation regimes from IR<sub>2</sub> to IR<sub>6</sub>, respectively, compared to the phenolic content observed under drought stress conditions (IR<sub>1</sub>). This increase was, however, marginally greater under a low water deficit

than under severe drought (Fig. 1). The irrigation regime of 75% recorded the highest and 25% irrigation had the lowest increase in phenolic contents (Fig. 1). Thus, under irrigation treatments of 25, 50, 75, 100 and 125%, TPC were about 29.02%, 60.01%, 95.99%, 85.14% and 54.92% higher than the non-irrigation regime (IR<sub>1</sub>), respectively. Phenolic acids occur in plants in different forms, such as aglycones (free phenolic acids), esters, glycosides, and/or bound complexes [52-53]. Phenolic acids are natural hydrophilic antioxidants, which occur ubiquitously in fruits, vegetables, spices and aromatic herbs [54]. Predominant phenolics in sunflower seeds are chlorogenic, quinic and caffeic acids [55, 16]. In addition to these, caffeoylquinic, sinapic, ferulic, gallic, coumaric, and protocatechuic acids, glucoside, glucopyranoside, and cynarine are the sunflower seed polyphenols which have high antioxidant potential. Similarly, the results for the phenolic content correlated with the findings of Alinian et al. [56], who reported a significant increase in the total phenolic content of both seeds and aerial parts of cumin under water stress. Consistent with our findings, Rasheed et al. [57] reported that the total leaf phenolic content of sunflowers increased under water deficit stress; however, the effect of the stress on the phenolics of seeds was not investigated. Furthermore, the results agree with



**Figure 1** - Phenolic response to water stress in sunflower seed.

**Table V** - Mean comparisons of the irrigation regimes for TPC, EC<sub>50</sub> and protein content of sunflower seed extract.

Treatments	TPC (mg GAE/g DW)	EC <sub>50</sub> (µg/ml) on DPPH	Protein (%)
IR <sub>1</sub>	694.90±5.57 <sup>f</sup>	83.73±0.34 <sup>c</sup>	13.51±1.84
IR <sub>2</sub>	896.56±4.16 <sup>e</sup>	77.18±2.20 <sup>d</sup>	15.05±1.22
IR <sub>3</sub>	1111.90±7.21 <sup>c</sup>	85.89±0.32 <sup>b</sup>	14.68±0.48
IR <sub>4</sub>	1361.90±8.54 <sup>a</sup>	75.46±1.48 <sup>d</sup>	14.84±0.48
IR <sub>5</sub>	1286.56±5.51 <sup>b</sup>	88.64±0.92 <sup>a</sup>	15.53±0.01
IR <sub>6</sub>	1076.56±12.90 <sup>d</sup>	83.88±0.18 <sup>bc</sup>	15.42±0.70
Mean	1071.4	82.45	14.84
LSD	15.10	2.05	-

those reported by Rebey et al. [26] who found that phenolic content of cumin seeds increased by 43.7% and 15.3% under moderate and severe drought. Indeed, as reported by Jaafar et al. [58] and Rebey et al. [26], the total phenol content of the plant is also dependent on the cultivar used and affected by the origin of varieties. The increase in phenolic contents under drought stress was ascribed to the accumulation of soluble carbohydrates in plants because of reduced soluble sugar transport [59, 56]. Furthermore, the increase could be attributed to the enhanced phenylalanine ammonia-lyase enzyme activity under water stress conditions [20]. There is a lack of studies addressing metabolic response to water stress in different varieties of seeds. Moreover, Weisz et al. [55] reported that defatted sunflower meal had high total phenolic content (4200 mg/100g) and it could be used as a natural antioxidant. Furthermore, the relationship between secondary metabolites and irrigation regime is important in relation to sunflower proteins. Although phenolic compounds have various beneficial health roles, they might reduce the quality of sunflower proteins by inhibiting digestibility, causing undesirable browning and structural modifications, and altering protein functional properties and behaviour in various food matrices [53].

### 3.4 ANTIOXIDANT CAPACITY

The analysis of variance results for the antioxidant activity of seeds under six irrigation regimes are presented in Table IV. The DPPH radical scavenging activities of methanol extracts obtained from the sunflower seeds irrigated under high and low stress conditions of 25% and 75% showed the highest activity to neutralise the DPPH radical compared to the extract procured under drought stress conditions (Table V). The antioxidant capacities of seed extracts were 7.82 and 9.87% higher than under drought stress conditions. In a similar way, the highest total phenolic content of seed extracts was obtained in the low water deficit condition. The results showed that irrigation regimes had a significant ( $P < 0.01$ ) impact on antioxidant activity of the sunflower seeds, except for 125%, compared to drought stress conditions. The

highest antioxidant activity was obtained with the 75% treatment, while there was no change in antioxidant activity of the over-irrigation treatment of 125% compared to drought stress conditions. Similarly, Rezaei-Chiyaneh et al. [27] reported that antioxidant enzyme activity increased with increasing water deficit stress. The behaviour of antioxidant activity and phenolic components of defatted extracts are quite different from each other. The reason for this could be due to the substantially different contributions of individual phenolic compounds to antioxidant capacity [60-55]. In addition to this, Pajak et al. [16] suggested that non-phenolic compounds such as tocopherols or L-ascorbic acid might be potential scavengers of DPPH radicals.

### 3.5 PHENOLIC AND FLAVONOID COMPOUNDS

The phenolic and flavonoid compound concentrations in the extracts of seeds studied under different irrigation regimes are shown in Table VI for a clear understanding of phenolic responses under different irrigation regimes. Five of eleven phenolic and flavonoid compounds quantified were phenolic acids, including ferulic, gallic, caffeic, chlorogenic, vanillic and p-coumaric acids and the other 5 were flavonoids of rutin-3-hydrate, quercetin, catechin, epicatechin and kaempferol-3-glucoside. Concerning phenolic and flavonoid compounds in seed extracts, water deficit stress reduced vanillic acid, epicatechin, caffeic acid and rutin-3-hydrate, while it increased gallic acid, catechin, chlorogenic acid coumaric and ferulic acids, quercetin. It was also reported by Krol et al. [28] and Yeloojeh et al. [29] that water deficit stress significantly reduced caffeic acid in grapevine and rutin in safflower extracts, respectively. Water deficit stress was identified to increase gallic and chlorogenic acid in seed extracts [29]. The highest amount of phenolics and flavonoids in seed extracts was detected low water stress of 75% treatment. In addition to this, the second highest phenolic compounds were detected under high stress of 25% treatment. Moreover, an increase in all irrigation regimes conditions was detected compared to full irrigation treatment (100%). High water deficit was found to enhance the biosynthesis of

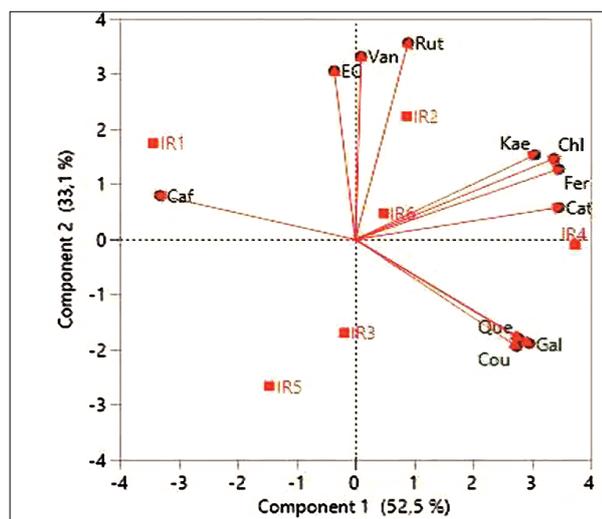
**Table VI - Contents of individual phenolic compounds in non-oilseed sunflower kernels and shells (mg/100 g of DW).**

Compound	IR <sub>1</sub>	IR <sub>2</sub>	IR <sub>3</sub>	IR <sub>4</sub>	IR <sub>5</sub>	IR <sub>6</sub>
Gallic acid	0.5	0.6	0.9	1.2	0.8	0.8
Catechin	181.1	266.0	216.3	271.8	216.8	233.6
Vanillic acid	683.5	701.4	522.2	643.4	576.0	616.5
Epicatechin	20.0	22.0	18.8	17.0	15.8	20.8
Chlorogenic acid	5956.3	6850.4	6101.5	7444.3	5803.4	6762.5
Caffeic acid	37.4	33.3	29.8	15.9	32.7	30.4
Coumaric acid	7.0	13.0	13.1	14.0	13.6	11.9
Ferulic acid	3.3	4.1	3.5	4.3	3.4	3.8
Rutin 3-hidrat	9.7	10.4	8.2	9.4	6.9	9.1
Kaempferol-3-glucoside	110.8	120.5	117.8	130.7	100.4	121.8
Quercetin	7.0	8.6	9.0	8.9	8.6	8.8
Total amount	7016.8	8030.2	7041.1	8560.9	6778.6	7820.0

phenolic compounds such as vanillic acid, epicatechin and rutin-3-hydrate, while the low water deficit (75%) induced the accumulation of phenolic compounds such as catechin, gallic, ferulic, chlorogenic and coumaric acids. The increase in phenolic acids seems to be a biochemical response to stress conditions and could be related to lignin biosynthesis in the cell wall to prevent water loss and the synthesis of certain amino acids maintaining osmotic adjustment in plant cells [61]. Furthermore, coumaric acid is one of the starting substances for lignin biosynthesis [61-64]. Although the increase in total protein content of sunflower seed extracts was insignificant, the increasing levels of amino acids were related to triggering of the production of phenolic acids (via cinnamic acid pathway) leading to lignin biosynthesis. Furthermore, a spectacular increase in free amino acid content was also determined in some plants subjected to water stress [65, 66]. In conclusion, the increase in the synthesis of phenolic compounds could be attributed to the stress induced in the plant because of an abiotic stress factor like water deficit or over-irrigation regimes.

### 3.6 PRINCIPAL COMPONENTS ANALYSIS (PCA)

The eleven phenolic compounds from the six irrigation regimes of sunflowers were analysed using principal component analysis (PCA) (Fig. 2). The first PC (PC1) explained 52.5% of the total variance with positive correlations with flavonoid compounds including kaempferol-3-glucoside, catechin, quercetin and phenolic acids including ferulic, coumaric and gallic acid, but with negative correlations to caffeic acid. The second PC (PC2) explained 33.1% of the total variance and had positive correlations with vanillic acid, rutin-3-hydrate, epicatechin and no negative



**Figure 2** - Principal component analysis of sunflower phenolic components (Gal: Gallic acid, Cat: Catechin, Van:Vanillic acid, EC: Epicatechin, Chl: Chlorogenic acid, Caf: Caffeic acid, Cou: Coumaric acid; Fer: Ferulic acid, Rut: Rutin-3-Hidrat, Kae: Kaempferol-3-glucoside, Que: Quercetin).

correlation. The first two principal components (PC1 and PC2) explained most of the variation (85.6%) (Fig. 2). The biplot generated from PC1 and PC2 indicated that phenolic compounds were collected under four subgroups in different irrigation regimes. The low water stress conditions (75%) were characterised by quercetin, gallic acid and coumaric acid, kaempferol-3-glucoside, chlorogenic-acid, ferulic acid and catechin, situated in subgroups 1 and 2. The second subgroup in the over-irrigated regime (I6) was characterised by kaempferol-3-glucoside, chlorogenic-acid, ferulic acid and catechin and was situated in the top right quadrant of the plot. The third subgroup, which was the low water stress conditions (25%), constituted rutin 3-hydrate and vanillic acid. The drought stress condition (fourth subgroup) was separated from the other irrigation regimes by higher caffeic acid. Moderate (50%) and full irrigation (100%) regimes were not characterised as subgroups by phenolic compounds.

## 4. CONCLUSION

Plant secondary metabolites, which mostly act as antimicrobials and antioxidants, have an important role in plant defence. These metabolites also have economical value due to their roles such as pharmaceuticals, food flavourings and agrochemicals. In this study, total phenolic content in seed extracts of sunflower increased under water deficit conditions. In addition to this, the induced water deficit stress affected bioactive components such as phenolic acids and flavonoids in sunflower seed extracts. Moreover, low and high-water stress conditions positively influenced the antioxidant potential of seed extracts. Therefore, water deficit stress increased seed quality in terms of phenolic and antioxidant content. Although the change in the amount of phenolic and flavonoid compounds in seed extracts due to water deficit stress was distinctive, chlorogenic acid and vanillic acid were detected as the most abundant polyphenol compounds in seed extracts under all irrigation regimes. Therefore, the antioxidant activity of defatted sunflower seed extracts could be attributed to the presence of these compounds. Sunflower is an important feedstock especially in the production of seed oil. This research ascertains that residue in sunflower oil production emerge as a natural source of phenolic compounds and might be used as natural antioxidants in diet and implementations such as pharmaceuticals due to the presence of components with high antioxidant activity. The irrigation regime should be kept at an economical point to enhance the phenolic content of the plant. The residue of sunflower processing, which has high polyphenolic content, could be converted to a polyphenol-enrichment agent in different food systems. There is a lack of studies addressing metabolic response to water stress in different varieties of seeds. In addition to this, although the increase in protein content was not sig-

nificant, based on common knowledge about protein digestibility being affected by phenolic compounds, further investigations regarding the interaction between the phenolics and sunflower protein digestibility in different irrigation regimes are planned.

### Author contributions

SBG: Conceptualisation; data curation; formal analysis; investigation; methodology; software; supervision; validation; visualization; writing – original draft; writing. YE: Data curation; formal analysis; investigation; methodology; validation; software; writing – original draft; writing. EG: Data curation; Formal analysis; methodology; writing – original draft; writing. BS: Formal analysis; writing. TE: Conceptualisation; methodology; writing. All authors have read and approved the final manuscript.

### Conflict of interest

The authors declare they have no conflict of interest.

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