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# The effect of irrigation treatments at different development stages on the bioactive components of sunflower cake

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**Abstract:** The aim of the study was to determine the effect of water deficiency at different development stages on the bioactive content and phenolic compounds in sunflower (*Helianthus annuus* L.) cake, the residue left after oil is extracted from sunflower seeds. A sunflower genotype was randomly planted in a complete block design with eight different combinations of irrigation ($T_1$–$T_8$) by increasing the available soil moisture measured at different plant growth stages (vegetative, flowering and grain formation). Results indicated that the total phenolics of extracts varied between 1.03–2.03 times more than under drought stress ($T_8$). The antioxidant capacity response of seed cake was 14–39% lower than under drought stress. Irrigation treatment, except in the grain formation stage, was found to enhance the biosynthesis of phenolic compounds such as vanillic and caffeic acids. Irrigation only in the grain formation stage induced the accumulation of phenolic compounds such as coumaric acid and rutin hydrate. The present study established that residues resulting from oil extraction could be converted to a polyphenol-enrichment agent for food systems by manipulating the irrigation treatments.

**Keywords:** oilseed; bioactive compound; drip irrigation, semi-arid region; climate change, phytonutrients

By-products of agricultural and industrial resources are a disposal problem for the industry, but they also represent sources for promising compounds of technological and nutritional interest (Sarkis et al. 2014). The extraction process of oilseeds results in oil cake. These residues are a source of bioactive compounds with beneficial properties for health that can be used in foods, cosmetics, and pharmaceutical industries (Ancuta and Sonia 2020). Sunflower seed kernels and hulls, as well as the seed oil-pressing by-product (cakes), are an important source of secondary metabolites (De Leonardis et al. 2005).

Phenolic compounds like simple phenols, flavonoids, and phenolic acids possess positive attributes such as anticarcinogenic, antioxidant, antimicrobial, and antimutagenic activity (Lule and Xia 2005). The antioxidant potential of sunflower was higher than that reported for other common oilseeds and nuts (Sarkis et al. 2014). Sunflower seeds also contain significant amounts of phenolic compounds (Zoumpoulakis et al. 2017). Sunflower seed kernels and hulls, as well as the seed oil pressing by-product (cake), have antioxidant properties (De Leonardis et al. 2005). Gou et al. (2017) reported that the antioxidant activity of sunflower seeds is due to enzymatic antioxidants, phenolic compounds, carotenoids, L-ascorbic acid and peptides.

Water is one of the most important inputs used in agricultural production. It has become even more

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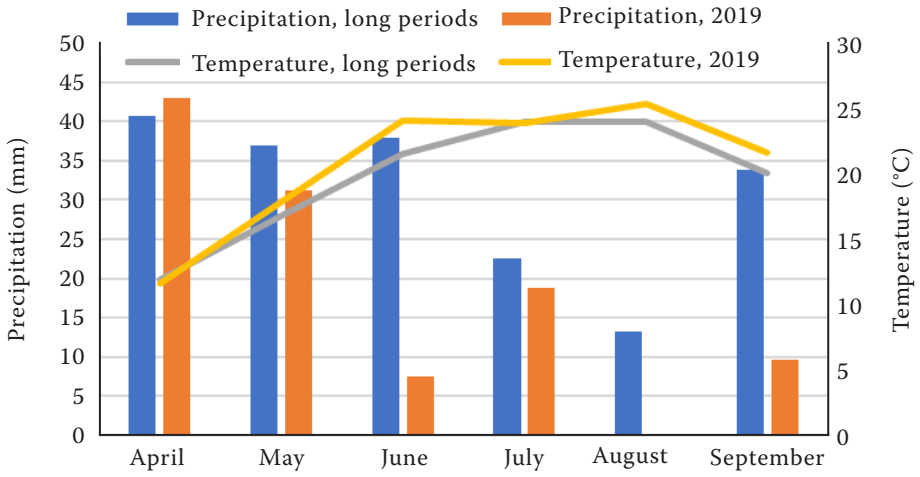


Figure 1. Monthly mean temperature and monthly total precipitation in 2019 and long periods (1960–2019)

important in arid and semi-arid regions due to climate change. Drought stress initiates the biosynthesis of different types of protective secondary metabolites, and these secondary metabolites provide multi-stress tolerance, including abiotic and biotic stresses (Yadav et al. 2021). Sunflower is a moderately drought-tolerant plant, but severe drought causes a reduction in seed and oil production (Hussein et al. 2018). Drought stress significantly enhanced total phenolics, as well as the activities of antioxidant enzymes in sunflower plants (Kosar et al. 2021). The number of studies describing the effect of drought stress on secondary metabolite components obtained from sunflower meal, which is a by-product of sunflower, is insufficient.

The research aimed to evaluate the effect of irrigation applied at different growth stages on sunflowers' phenolic and antioxidant responses and to determine the phenolic and flavonoid compounds in defatted seed kernels (by-product cake) from a semi-arid region.

## MATERIAL AND METHODS

**Growth conditions and plant material.** This study was carried out at Tekirdağ Viticultural Research Institute, Tekirdağ, Turkiye. The experimental area is located 44 m a.s.l. between 40°59' north latitude and 27°29' east longitude. The region has a semi-arid climate. According to the meteorologic data of 2019, the highest temperature was 25.3 °C in August. The highest total precipitation was obtained in April, with 42.9 mm, and there was no precipitation in August. The monthly average temperature and precipitation values for 2019 and long periods (1960–2019) are shown in Figure 1.

According to the analysis of soil samples (Table 1), the soils are clay loam, slightly salty, less calcareous and low in organic matter. The quality class of irrigation water has been determined as C2S1.

Sunflower seeds of cv. LG 5542 CL were used as plant material. A field trial was conducted with a randomised block design, including 3 replicates. Plants were sown on April 30, 2019, by a sowing machine. Each plot consisted of 8.40 $m^2$ (3.00 m × 2.80 m) and there were 40 plants cultivated at 0.70 m × 0.30 m intervals in the plot area. A gap of 3 m has been left between blocks and parcels. Sunflowers were harvested on September 4, 2019.

**Irrigation treatment.** In the study, the drip irrigation method was used. One lateral pipe with a diameter of 16 mm was laid for each plant row.

Table 1. Soil properties of the experimental area

| Soil depth (cm) | pH | Electrical conductivity (dS/m) | $CaCO_3$ | Field capacity | Wilting point | Bulk density ($g/cm^3$) |
|---|---|---|---|---|---|---|
| | | | (%) | | | |
| 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 |

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The dripper spacing on the lateral was 0.50 m. The dripper flow was 4 L/h under 100 kPa operating pressure. The wet area percentage (P) was calculated as 71% (Keller and Bliesner 1990).

Irrigation was applied in the middle of each growth period and one time. The amount of irrigation water was calculated by subtracting the amount of moisture measured from the $T_1$ treatment before irrigation from the field capacity value obtained before the experiment during the development periods of the plant at an effective root depth of 90 cm.

Treatments are: $T_1$ – vegetative, flowering, grain formation (3-irrigations); $T_2$ – vegetative, flowering (2-irrigations); $T_3$ – vegetative, grain formation (2-irrigations); $T_4$ – vegetative (1-irrigation); $T_5$ – flowering, grain formation (2-irrigations); $T_6$ – flowering (1-irrigation); $T_7$ – grain formation (1-irrigation); $T_8$ – no irrigation.

The amount of irrigation water was determined using the following equation:

$$I = (FC - SMCpi) \times A \times P \quad (1)$$

Where: I – irrigation amount (mm); FC – field capacity (mm); SMCpi – pre-irrigation soil moisture content (mm); A – plot area, and P – percentage of wetted area.

The soil water content in the plots was measured gravimetrically every 30 cm for a depth of 0–90 cm before irrigation. Evapotranspiration was determined using the soil water balance equation (James 1988). The soil water balance equation is as follows:

$$ET = I + P + \Delta S - DP - RO \quad (2)$$

Where: ET – evapotranspiration (mm); I – irrigation water (mm); PSW – change in soil water storage in 90 cm soil profile (mm); DP – deep percolation (mm) and RO – runoff (mm). The runoff was assumed to be zero, as the amount of irrigation water was controlled.

**Extraction of the phenolic content of seeds.** The extraction procedure was performed according to the method of Khattak et al. (2007). The sunflower seeds were ground in a laboratory mill (IKA-Werke GmbH & Co., Staufen, Germany) and defatted with hexane. A 5-g portion of the defatted flour was weighed into dark glass bottles and extracted at room temperature for 24 h at 140 rpm by shaking (Edmund Bühler GmbH, Bodelshausen, Germany) with 200 mL of 80% (*v*/*v*) aqueous MeOH. The extracts were centrifuged, and the supernatant was collected. The extraction was carried out in triplicate.

**Radical scavenging activity assay by DPPH.** Radical scavenging activity assay (RSA) was performed according to Brand-Williams et al. (1995). Different concentrations of seed extracts were placed into tubes, and 0.6 mL of molar DPPH* (diphenylpicrylhydrazyl) radical solutions were added to each tube. The total volume was completed to 6 mL with MeOH. Absorbance was read at 517 nm against the control after incubating the tubes for 30 min. RSA was estimated (I%) by using Eq. 3:

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

In Eq. 3, the absorptions of the sample and the control are expressed as $A_{sample}$ and $A_{control}$, respectively. Inhibition values were graphed against different concentrations for each extract, and linear regression analysis was applied in order to obtain the equation defining the curve. By using the equation, the $EC_{50}$ value, which is the number of antioxidants necessary to decrease the initial DPPH* concentration by 50%, was calculated.

**Determination of total phenolic content.** The total phenolic content (TPC) of extracts was examined by modifying the method based on the reaction occurring between phenolic compounds and the Folin-Ciocalteu reagent (Sadasivam and Manickam 1992). The absorption was read at 720 nm after 1-h incubation and calculated with the standard curve for gallic acid (Singleton 1985). The results are given as mg gallic acid equivalent/g dry matter (mg GAE/g dry matter) of seeds.

**Determination of the composition of phenolic compounds.** The flavonoids and phenolic acids were quantified by high-performance liquid chromatography (HPLC; SHIMADZU LC-20A Series, Kyoto, Japan), coupled with a diode array detector (SPD-M20A, Shimadzu, Kyoto, Japan), as stated by Uysal Seçkin (2019). 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 (Sigma Co., Tokyo, Japan), such as chlorogenic, ferulic, *p*-coumaric and caffeic acids, were detected at 320 nm and gallic and vanillic acids were monitored at 280 nm. Flavonoids (Sigma Co., Tokyo, Japan) were detected at 360 nm and epicatechin was monitored at 280 nm. 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 – 100% (*v*/*v*) acetonitrile, as mobile phases. The flavonoid and phenolic contents of the seed extracts were calculated by comparing the peak area and retention times with the pure standards, as stated

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Table 2. Applied irrigation water (mm) and measured seasonal evapotranspiration (mm) for treatments

| Irrigation treatment | Soil water depletion | Precipitation | Total applied irrigation water | | | Measured seasonal evapotranspiration |
|---|---|---|---|---|---|---|
| | | | late vegetative | flowering | grain formation | |
| $T_1$ | 189.4 | 57.5 | 100.0 | 105.0 | 90.0 | 541.9 |
| $T_2$ | 202.5 | | 100.0 | 105.0 | – | 465.0 |
| $T_3$ | 192.7 | | 100.0 | – | 90.0 | 440.2 |
| $T_4$ | 209.8 | | 100.0 | – | – | 367.3 |
| $T_5$ | 187.4 | | – | 105.0 | 90.0 | 439.9 |
| $T_6$ | 203.4 | | – | 105.0 | – | 365.9 |
| $T_7$ | 193.2 | | – | – | 90.0 | 340.7 |
| $T_8$ | 211.8 | | – | – | – | 269.3 |

by Yeloojeh et al. (2020). The results are shown as mg/100 g of dried seed.

**Statistical methods.** Data were statistically analysed using analysis of variance with the JMP Pro-16 (SAS Institute Inc., North Carolina, USA) statistical software. The differences among means were analysed by the least significant difference (*LSD*) test ($P \leq 0.05$). Principal component analysis (PCA) was used for the metabolite profiles and the entire transcriptome dataset using R software.

## RESULTS AND DISCUSSION

**Irrigation water amounts and evapotranspiration.** The amount of irrigation water applied in the experiment, amount of precipitation, decrease in moisture in the soil and measured seasonal evapotranspiration data are shown in Table 2.

The total amount of irrigation water applied in the treatments varied between 90 mm and 295 mm. During the total growing season, seasonal evapotranspiration measured from the treatments ranged from 269.3 mm to 541.9 mm. The total seasonal evapotranspiration values for sunflowers are consistent with those obtained from previous studies (Göksoy et al. 2004).

**Total phenolic content.** The results for the total phenolic content of seeds under the eight irrigation treatments are presented in Table 3. The TPC of the non-irrigation regime extract was 566.90 mg of GAE/g DW (dry weight). The highest phenolic content was detected in $T_2$, which was irrigated at vegetative and flowering stages, as 1 153 mg of GAE/g DW. $T_4$ irrigated only in the vegetative stage, was detected to have high phenolic content similar to $T_2$. This increase was, however, marginally greater with irrigation only at the vegetative stage and irrigation at both vegetative and flowering stages than under severe drought ($T_8$). However, all treatments had a significant effect on the TPC of the defatted seeds. The lowest TPC was obtained with the non-irrigated treatment ($T_8$). In addition, the irrigation treatment at the grain formation stage decreased the TPC of seeds ($T_5 < T_3 < T_1$). Therefore, the lower TPC of these treatments could be attributed to the intersection of irrigation performed at the grain formation stage. The late vegetative stage irrigation enhanced the TPC of seeds ($T_4 < T_2$). Under irrigation treatments at different development stages, TPC was about 2.7–103.5% higher than for the non-irrigation regime ($T_8$). Thus, TPCs of seed extracts increased

Table 3. Mean comparisons of irrigation regimes on total phenolic content (TPC) and $EC_{50}$ values of sunflower seed extracts

| Irrigation treatment | RSA | TPC |
|---|---|---|
| | $EC_{50}$ (µg/mL) of DPPH | (mg GAE/g DW) |
| $T_1$ | 85.97 ± 0.79$^{d}$ | 663.57 ± 4.04$^{e}$ |
| $T_2$ | 75.60 ± 0.33$^{e}$ | 1 153.57 ± 5.86$^{a}$ |
| $T_3$ | 86.77 ± 1.06$^{cd}$ | 631.57 ± 2.52$^{f}$ |
| $T_4$ | 89.45 ± 0.38$^{b}$ | 1 023.90 ± 7.94$^{b}$ |
| $T_5$ | 92.19 ± 0.55$^{a}$ | 582.23 ± 1.15$^{g}$ |
| $T_6$ | 87.70 ± 0.61$^{c}$ | 690.90 ± 3.61$^{d}$ |
| $T_7$ | 85.97 ± 1.19$^{d}$ | 798.23 ± 2.08$^{c}$ |
| $T_8$ | 66.22 ± 0.57$^{f}$ | 566.90 ± 4.00$^{h}$ |
| *LSD* | 1.47 | 7.25 |
| Prob-*F* | < 0.0001** | < 0.0001** |
| *CV* | 1.00 | 0.54 |

RSA – radical scavenging activity; DPPH – diphenylpicrylhydrazyl; GAE – gallic acid; DW – dry weight; *LSD* – least significant difference; *CV* – coefficient of variation

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significantly by 1.03-, 1.11-, 1.17-, 1.22-, 1.41-, 1.81- and 2.03-fold under irrigation regimes $T_5$, $T_3$, $T_1$, $T_6$, $T_7$, $T_4$ and $T_2$, respectively, compared to the phenolic content observed under drought stress ($T_8$).

Unlike these results, Tonkaz et al. (2019) and Lipan et al. (2019) detected an insignificant difference in total phenolics in defatted hazelnut kernels and almonds, respectively, related to irrigation application. However, Uslu and Özcan (2022) reported that the effect of the irrigation process on the phenolic content of the plant showed differences regarding variety and collection time. In addition, as reported by Rebey et al. (2012), the TPC of the plant is also dependent on the cultivar used and is affected by the origin of the varieties. Accumulation of anthocyanins and other phenolic compounds in the tissue of fruit species is associated with high phenylalanine ammonia-lyase (PAL) enzyme activity (Tovar et al. 2002), which catalyses a reaction that includes the formation of flavonoids, lignin and hydroxycinnamic acids. Furthermore, the increase could be attributed to the enhanced PAL activity under water stress (Oh et al. 2009). In addition to variety and harvest time reported previously, the plant's development stage affects the accumulation of phenolics due to the alteration in PAL activity. Tovar et al. (2002) reported that PAL activity significantly decreased with fruit ripening under all the irrigation treatments for olives. There is a lack of studies addressing the metabolic response to water stress in different varieties of seeds and water stress at different developmental stages. However, there are several studies on the alteration of phenolics in different parts of plants with different irrigation treatments. The results for the phenolic content are correlated with the findings of Rebey et al. (2012) and Rasheed et al. (2020), who reported significant increases in the TPC of cumin seeds under moderate and severe drought, the TPC of both seeds and aerial parts of cumin under water stress and the total leaf phenolic content of sunflowers, respectively. The results established that residues resulting from sunflower oil extraction are a natural source of phenolic compounds and could be converted to a polyphenol-enrichment agent for food systems.

**Radical scavenging activity assay.** The analysis of variance results for the radical scavenging activity assay (RSA) of seeds under eight irrigation treatments is presented in Table 3. The RSA of extracts irrigated at all growth stages, irrigated only at the grain formation stage and irrigated except at the flowering stage, had no significant difference compared to each other. Unlike phenolic accumulation, the response of the seeds grown in drought conditions was significantly higher than all other treatments. The antioxidant capacities of all seed extracts were 14–39% lower than those under drought stress. The results showed that irrigation treatments had a significant ($P < 0.01$) impact on the RSA of the sunflower seeds compared to drought stress. This response could be attributed due to the plant stress level under drought conditions. The antioxidant activity responses were affected negatively by irrigation only at the vegetative stage and no irrigation at the vegetative stage. Similar to the results, Rezaei-Chiyaneh et al. (2018) found that the antioxidant enzyme activity of black cumin increased with increasing water deficit stress. Unlike this, the lowest antioxidant capacity of defatted hazelnut kernels was detected in drought conditions. In contrast, the highest antioxidant activity was observed in extracts irrigated at all (1$^{st}$, 2$^{nd}$ and 3$^{rd}$) growth stages (Tonkaz et al. 2019). By our data, the total antioxidant capacity increased with irrigation at the two growth stages of vegetation and kernel development compared to irrigation only at the vegetative stage (Tonkaz et al. 2019). On the other hand, the antioxidant capacity of almonds was not affected by irrigation (Lipan et al. 2019). Uslu and Özcan (2022) found that irrigation decreased the antioxidant capacity of olive leaves from one of the varieties. The irrigation process caused minor differences in the antioxidant activity of olive leaves in the rest of the studied varieties.

As can be seen from the literature, the response of the plants to drought in terms of antioxidant and phenolic content might be affected by the stage of irrigation treatment. The emphasis in the results is that a distinctive effect could occur in terms of antioxidant activity and phenolic components of defatted extracts according to irrigation treatments (Weisz et al. 2009). The different responses between the antioxidants and phenolics could be attributed due to the nature of the phenolic compounds. The antioxidant ability of the phenolic acids is strictly related to the phenolic hydroxyl group (Reis Giada 2013). In addition to this, the position and the number of the phenolic hydroxyl group directly affect the antioxidant capacity of the phenolic compounds. Moreover, the carboxylic acid and methoxy groups contribute to the antioxidant capacity of phenolic acids (Reis Giada 2013, Lorigooini et al. 2020). Therefore, hydroxybenzoic acids, such as gallic and vanillic acids, and hydroxycinnamic acids, such as

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caffeic, chlorogenic, coumaric and ferulic acids, act as powerful antioxidants (Abramovič 2015, Lu et al. 2020). The results obtained in the RSA (Table 3) were found in accordance with the results in the individual phenolic compounds (Table 4) obtained from the drought stress conditions ($T_8$). Non-phenolic compounds such as tocopherols (Mohanan et al. 2018) or L-ascorbic acid might be potential scavengers of DPPH radicals, which could be one reason for seasons different responses in terms of the antioxidants and phenolics (Foti and Amorati 2010). It was also known that di-alkyl nitroxides and γ-terpinene, a non-phenolic monoterpene, act as radical-trapping antioxidants (Mollica et al. 2022). In addition, Uslu and Özcan (2022) reported that not only cultivar and harvest time, but also irrigation significantly affected olive leaves' TPC and antioxidant activities. The results established that the residues from the sunflower r oil extraction process have a possibility to be converted to a valuable input for another process as a natural additive. Therefore, enhancing the antioxidant capacity of the plant can be kept economical by manipulating the irrigation treatments.

**Phenolic and flavonoid compounds.** The phenolic and flavonoid compounds in the extracts from eight irrigation treatments are shown in Table 4 for a clear understanding of phenolic responses to different irrigation treatments at different development stages. Predominant phenolics in sunflower seeds are caffeic, quinic and chlorogenic acids (Weisz et al. 2009). In addition to predominant phenolics, gallic, ferulic, coumaric, caffeoylquinic, sinapic and protocatechuic acids are sunflower seed polyphenols with high antioxidant potential. Concerning phenolic and flavonoid compounds in seed extracts, drought stress enhanced catechin, quercetin, kaempferol-3-glucoside, ferulic and chlorogenic acids, while it reduced gallic acid and epicatechin. Similar to our results in terms of drought, Salem et al. (2014) found that severe water deficit decreased gallic acid and epicatechin in safflower extracts. Yeloojeh et al. (2020) reported that water deficit stress significantly reduced chlorogenic acid in safflower extracts while ferulic and vanillic acid increased. Drought stress was identified to increase ferulic and chlorogenic acid in sesame seed extracts (Ghotbzadeh Kermani et al. 2019). Results indicated that changes in phenolic and flavonoid compounds under different irrigation treatments depend on species, different plant parts, and the drought stress level (Yeloojeh et al. 2020). Moreover, weather, cultivation, genotype, maturity at harvest, and storage conditions are significant (Stagnari et al. 2016). The highest total amount of phenolics and flavonoids in seed extracts was detected with drought stress ($T_8$). In addition, the second-highest phenolic compounds were detected in seeds irrigated at both vegetative and flowering stages of development ($T_2$). Irrigation treatment, except in the grain formation stage, was found to enhance the biosynthesis of phenolic compounds such as vanillic and caffeic acids and catechin. At the same time, the seeds irrigated only at the grain

Table 4. Contents of individual phenolic compounds in non-oilseed sunflower kernels (mg/100 g of dry weight)

| Compound | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ | $T_6$ | $T_7$ | $T_8$ |
|---|---|---|---|---|---|---|---|---|
| **Phenolic acids** | | | | | | | | |
| Caffeic acid | 37.2 | 38.9 | 37.2 | 37.2 | 33.4 | 31.2 | 33.6 | 37.8 |
| Chlorogenic acid | 5 875.2 | 6 057.4 | 6 143.8 | 5 887.7 | 5 851.3 | 5 622.9 | 6 215.3 | 6 567.4 |
| Coumaric acid | 14.0 | 16.1 | 16.3 | 14.4 | 16.3 | 14.6 | 17.9 | 15.6 |
| Ferulic acid | 3.2 | 3.2 | 3.3 | 3.2 | 3.4 | 3.3 | 3.4 | 5.2 |
| Gallic acid | 1.2 | 2.3 | 2.7 | 2.9 | 2.9 | 2.8 | 2.7 | 1.3 |
| Vanillic acid | 185.9 | 780.6 | 653.7 | 777.3 | 729.8 | 586.6 | 675.2 | 719.3 |
| **Flavonoids** | | | | | | | | |
| Catechin | 357.1 | 433.7 | 332.7 | 348.7 | 311.0 | 354.0 | 341.1 | 429.0 |
| Epicatechin | 51.9 | 45.3 | 32.4 | 28.5 | 31.9 | 34.1 | 24.1 | 19.8 |
| Kaempferol-3-glucoside | 138.9 | 136.6 | 131.4 | 140.0 | 130.9 | 134.9 | 141.8 | 167.4 |
| Rutin hydrate | 8.3 | 8.8 | 9.1 | 8.4 | 9.2 | 8.3 | 10.1 | 9.2 |
| Quercetin | 9.2 | 11.0 | 9.5 | 10.6 | 9.1 | 10.2 | 9.3 | 11.7 |
| Total amount | 6 682.1 | 7 534.0 | 7 372.3 | 7 259.0 | 7 129.2 | 6 802.7 | 7 474.3 | 7 983.6 |

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formation stage of development ($T_7$) accumulated phenolic compounds such as coumaric acid and rutin hydrate. However, the lowest phenolic and flavonoid compounds detected in the treatment irrigated at all development stages were 6 682.1 mg/100 g of DW. The increase in phenolic acids could be a biochemical response to stress conditions, especially the increase in the non-irrigation conditions ($T_1$). It 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 (Ayaz et al. 1999). Consequently, the increase in the synthesis of phenolic compounds would be key for protection against damage caused by an abiotic stress factor like water deficit or over-irrigation regimes (Salem et al. 2014). Terzi et al. (2013) revealed that the accumulation of polyphenols in cell walls is a significant part of tolerance/defence responses related to lignification. Stefanelli et al. (2010) proposed that flavonoid biosynthesis was a favoured pathway under drought conditions. The result of the present study is in accordance with this fact, with the highest flavonoid content of 637.02 mg/100 g of DW in non-irrigation conditions. However, there is a lack of studies addressing the metabolic response to water stress according to the stage of irrigation in different varieties of seeds. Thus, Stagnari et al. (2016) reported that the influence of water deficit on phenolic content is strictly related to the time at which the stress condition occurs for Brassicaceae species.

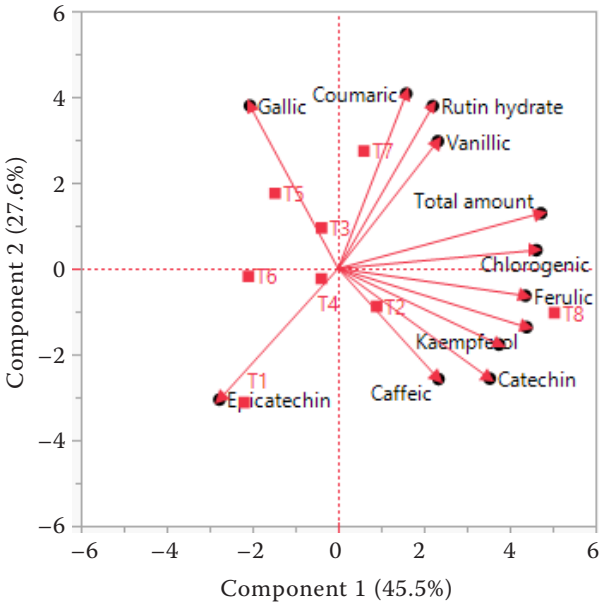


Figure 2. Principal component analysis (PCA) of sunflower phenolic components

**Principal component analysis of different irrigation patterns and phenolic compounds in sunflower.** The principal component analysis model was applied to all data to determine the most important variables that explain the relationships between the eight irrigation implementations for sunflowers to identify any group patterns (Figure 2). Two principal components explaining 73.1% of the overall variance (45.5% and 27.6% for PC1 and PC2, respectively) divided the analysed irrigation patterns into four distinct clusters. PC1 positively correlated with caffeic acid, catechin, quercetin, kaempferol-3-glucoside, ferulic acid, chlorogenic acid, vanillic acid, rutin hydrate and coumaric acid but negative correlations with epicatechin and gallic acid. The biplot generated from PC1 and PC2 indicates that phenolic compounds in different irrigation regimes were collected under four subgroups. The highest total phenolic compounds were obtained from drought stress ($T_8$). The first subgroup involved drought stress ($T_8$) and was characterised by caffeic acid, catechin, quercetin, kaempferol-3-glucoside, ferulic acid, and chlorogenic acid. The second subgroup of samples irrigated only at the grain formation stage of development ($T_7$) was characterised by vanillic acid, rutin hydrate and coumaric acid. The third subgroup of samples irrigated at flowering and grain formation stages of development ($T_5$) was characterised by gallic acid. The fourth subgroup of sunflowers irrigated at the vegetative, flowering and grain formation stages ($T_1$) was characterised by epicatechin.

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