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QUANTITATIVE ANALYSIS OF NUTRITIONAL BIOACTIVE COMPOUNDS IN IRRIGATED AND NON-IRRIGATED SAFFLOWER (*CARTHAMUS TINCTORIUS* L.) CULTIVAR CW 4440 ACHENES

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ABSTRACT

Safflower cultivar CW 4440 was grown on two different water regimes: non-irrigated, and full-dose irrigated. Achenes were harvested and taken for biochemical analyses.

They were found to contain low amounts of carotenoids, 2.38-4.38 mg/kg, with notable amounts of lutein and minor concentrations of xanthins. All carotenoids, except for cryptoxanthin, reached their highest values in irrigated plants, while β-carotene was found in detectable amounts only in irrigated plants. Phenolic inventory, on the other hands, was high compared to known data in literature: 40,627-44,574 mg/kg. Of these, hydroxycinnamic acids were dominant, with 26,023-28,233 mg/kg (63-64%). Flavonoids reached 11,854-13,201 mg/kg (29-30%), including a major fraction of flavanols (1,469-1,595 mg/kg). Stilbenes were another major component, with 2,516-2,750 mg/kg. All the highest values were found in irrigated plants.

Oil content ranged from 6.1% in non-irrigated plants to over 11.7% in irrigated ones. Ascorbic acid content was low (349-756 mg/kg), while soluble sugars were 1,731-2,508 mg/kg.

The concentrations of all these compounds, except for cryptoxanthin, were positively correlated to water dosage. The most affected by irrigation regime were carotenoids, ascorbic acids and oil. The least influenced were phenolic compounds.

INTRODUCTION

Safflower (*Carthamus tinctorius* L.) is a member of Asteraceae family, tribe Cardueae, subtribe Centaureinae. Is a native of the Middle East and has been cultivated for around 4,000 years. It is a herbaceous, annual plant, resembling thistles, with an erect, branched stem, a well-developed and branched taproot, basal leaf rosette in early stages, oval-lanceolate and sessile leaves. Stems and leaves are covered by spines. Inflorescences are orange pseudanthia, sometimes with red or yellow spots. Fruits are achenes.

While it is still a minor crop, its high adaptability allows its cultivation in wide variety of habitats. It is mainly grown in temperate areas of North America and Europe, the Middle East, south and East Asia, Australia. Russia, Kazakhstan, Mexico and the USA are the top four producers. The main usage is for its edible oil, valuable for cooking and margarine production. Other usages include ornamental application, pigment extraction (carthamidin, an orange-yellow pigment), birdseed, livestock feed (yet, with low nutritional value, except for birds) and medicinal purposes, due to the tonic, laxative, antibacterial, hypotensive properties of leaf/flower decoctions and oil (OGTR 2019; OECD 2022).

Water is one of the main factors determining safflower productivity. The plant itself possesses adaptation mechanisms to hydric deficit, mainly by expanding its root system. However, actual seed and oil yield are affected. Studies show that a normal irrigation scheme, consisting of three water applications of 350-400 m^3/ha may lead to a 70-100% increase in seed biomass and 80-120% increase in oil yield/ha (although oil content in individual seeds seem to be constant; Parameshnaik et al. 2023)

The objective of this paper was to determine a wider array of bioactive compounds contents in CW 4440 cultivar of safflower grown locally and the way irrigation regime influences these contents.

MATERIAL AND METHODS

Biological material consisted of *Carthamus tinctorius* L. achenes, cultivar CW 4440, originating from the experimental farm of the „Ovidius" University, Constanța, in 2023.

Cultivation was initiated by ploughing the experimental field (September 25th, 2022), preparing seedbed (March 29th, 2023), and seeding on March 30th, 2023, with 12 kg seeds/ha and 200,000 viable seeds/m^2.

Two different experimental variants were taken into consideration: zero irrigation (control) and normal dose of 1,200 m^3/ha (3 applications of 400 m^3/ha each).

Achenes were collected and taken for laboratory analyses. For most analyses, they were ground and extracted in 70% ethanol at 10% final concentration.

Total carotenoids were determined by 10-fold dilution in 80% acetone and spectrophotometric absorption reading (ONDA UV-21 spectrophotometer) at 470 nm (Popoviciu et al. 2023). Among individual carotenoids, β-carotene, lycopene, lutein (and lutein esters), cryptoxanthin and zeaxanthin were determined by 10-fold dilution in acetone:petroleum ether, petroleum ether, ether, acetone, respectively ethanol and determining absorbance at 445, 446, 453, 505, 645, 663 nm according to Braniša et al. 2014, Sujith et al. 2010, Biehler et al. 2009, Butnariu et al. 2014.

The total amounts of phenolic compounds were determined by 10-fold dilution in ethanol and Folin-Ciocâlteu reaction. Absorbance was read at 765 nm against a gallic acid calibration curve (Popoviciu et al. 2023). Flavonoids and phenolic acids (hydroxybenzoic and hydroxycinnamic) were analyzed by 10-fold dilution in ethanol-hydrochloric acid mixture and spectrophotometry at 220, 275 325, 345 and 380 nm (Paula et al. 2017). Flavanols (catechins), by dilution in 70% ethanol and reading at 280 nm (Yaneva et al. 2020). Anthocyanins, by dilution in 70% ethanol and reading at 520 and 700 nm (Braniša et al. 2014).

Tannins were determined after thermal hydrolysis in water-hydrochloric acid mixture and reading at 550 nm (Moutari et al. 2018). Stilbenes, by dilution in 70% ethanol and reading at 304 nm (Bancuta et al. 2015). Coumarins, by dilution in 80% methanol and reading at 275 nm (Soares e Silva et al., 2012).

Total ascorbic and dehydroascorbic acid were determined by 10-fold ethanol dilution, reaction with ammonium molybdate and sulfuric acid, and spectrophotometric reading at 494 nm (Riscahyani et al. 2019). Total soluble carbohydrates were determined by reacting extracts with sulfuric acid and phenol (5%) and reading at 490 nm (Agrawal et al. 2015).

Lipids were determined by grinding achenes in petroleum ether, filtration, solvent evaporation at 35°C and gravimetry (Orphanides et al. 2011).

RESULTS AND DISCUSSIONS

The concentrations of various classes of compounds with bioactive potential are shown in Figures 1-2 and Table 1.

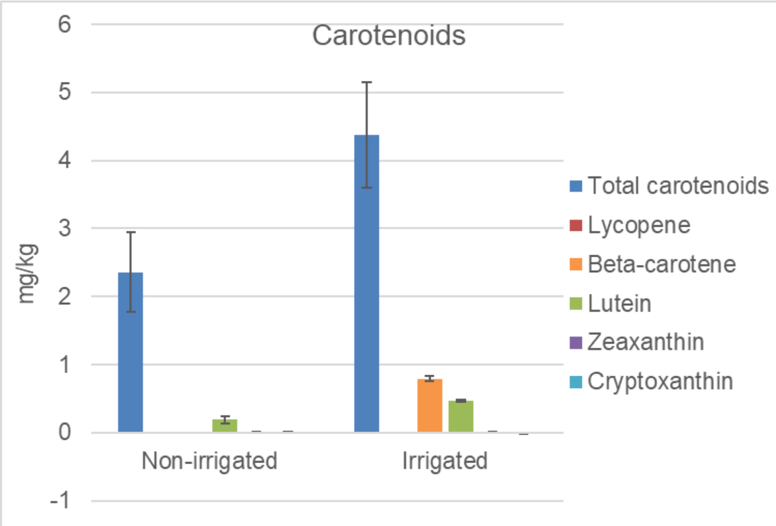

Figure 1. Concentrations of total and some individual carotenoids in safflower achenes (mg/kg FW).

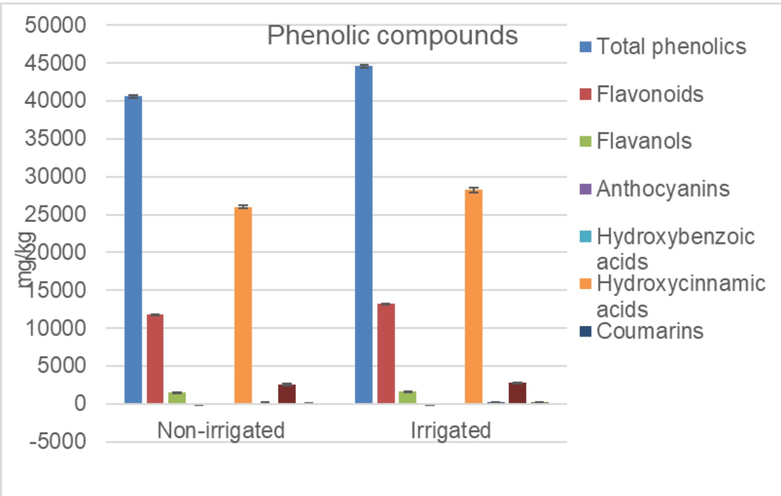

Figure 2. Concentrations of total phenolic compounds and some subclasses in safflower achenes (mg/kg FW).

Most types of compounds were positively correlated with irrigation dosage. Average total carotenoid content, for instance was 2.36 mg/kg in non-irrigated plants and 4.38 mg/kg in irrigated ones (Fig. 1). Among carotenoids, lycopene was found in amounts below detection limit for all samples, while sizeable β-carotene amounts were only found in irrigated plants (0.79 mg/kg). Lutein ranged from 0.19 mg/kg in non-irrigated to 0.47 mg/kg in irrigated safflower. Both xanthins were found in amounts below 0.01 mg/kg, with cryptoxanthin being the only compound inversely proportional to water dosage.

Table 1.

Other compound classes in safflower achenes (mg/kg FW).

	Non-irrigated	Irrigated
Ascorbic acid	349±19	756±4
Soluble Sugars	1,731±1	2,508±1
Oil	60,933±306	116,800±200

Carotenoid contents in safflower seeds seems to be highly variable among cultivars, since a study on 20 varieties found 3.75-18.97 mg/kg, usually dominated by xantophyll fraction (lutein, xanthins and related compounds; Tonguç et al., 2023). Another study, on oil fraction only, found total carotenoid values of 1.14-1.32 mg/g, but with a dominance of carotenes, of which β-carotene reached 0.30-0.35 mg/kg (Ben Moumen et al. 2014).

Average total phenolic content was less dependent upon irrigation, with 40,627-44,574 mg/kg (Fig. 2). Among them, hydroxycinnamic acids were the main fraction, with 26,023-28,233 mg/kg (63-64%). Hydroxybenzoic acids, on the other hand, were below detection limits. Flavonoids were the second major components, with 11,854-13,201 mg/kg (29-30%). Among flavonoids, flavanols were a significant fraction (1,469-1,595 mg/kg), while anthocyanins were found in minute amounts (3 mg/kg) and only in non-irrigated plants. Small fractions of coumarins were found (166-193 mg/kg), while stilbenes were major constituents (2,516-2,750 or over 6% of the phenolic inventory). A previous study on 20 safflower cultivars found total phenolic contents of just 5,100-14,000 mg/kg, but with a higher ratio of flavonoids (4,600-7,150 mg/kg; Tonguç et al., 2023).

Ascorbic acid amounts were relatively low (Table 1), while oil – the most valuable fraction of safflower seeds – ranged from 6.1 to 11.7% ot the achene fresh weight, with a 92% increase in irrigated plants. Oil is the most valuable component of safflower seeds and it may range up to 24-33% of the seed biomass (Tonguç et al., 2023).

Scientific studies on safflower reaction to different water regimes often focus only on biomass and a single biochemical fraction, namely oil. Results are highly variable, probably depending on cultivar and local conditions. Some experiments have shown irrigation leads to a major increase in oil yield (up to 100% and over), due to higher number of seeds and higher achene and seed biomass, while oil percentage remains constant (28-30% of seed weight according to Parameshnaik et al. 2023). Similar consequences were observed by Seify et al. (2023) on 24 safflower genotypes.

However, not all cultivars show such a stability in oil content. Some may show variations of 30% and over (Nabipour at al. 2007). In our case, the 92% variation was extreme, however reported to entire achene weight.

CONCLUSIONS

Safflower seeds from cultivar CW 4440 were found to contain low amounts of carotenoids, 2.38-4.38 mg/kg, with notable amounts of lutein and minor concentrations of xanthins. All carotenoids, except for cryptoxanthin, reached their highest values in irrigated plants, while β-carotene was found in detectable amounts only in irrigated plants.

Phenolic inventory, on the other hands, was high compared to known data in literature: 40,627-44,574 mg/kg. Of these, hydroxycinnamic acids were dominant, with 26,023-28,233 mg/kg (63-64%). Flavonoids reached 11,854-13,201 mg/kg (29-30%), including a major fraction of flavanols (1,469-1,595 mg/kg). Stilbenes were another major component, with 2,516-2,750 mg/kg. All the highest values were found in irrigated plants.

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