



Enhanced Biochemical and Morphological Parameters in *Eruca vesicaria* by Applications of date-palm seed Extract

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Abstract

The current global context stimulating agroecology and green agriculture need to explore for the novel sustainable and eco-conscious methods for safeguarding plants. The aim of this study is to exploit the rich contents of date palm seeds (DPS) as an appropriate organic elicitor to boost growth and plant secondary metabolism in *Eruca vesicaria*. The seeds of DPS were utilized in the extraction process using distilled water to create an aqueous extract, which underwent phytochemical characterization. In a pot experiment, *Eruca vesicaria* seeds were soaked in serial doses (0, 20, 40, 80, and 120 g l⁻¹) of DPS aqueous extract, and the 21-days old seedlings were collected. DPS extract analysis indicated adequate P and N contents, antioxidant compounds, and exhibited antioxidant activity. The primary components identified in the analysis of DPS were 2-Dodecenal, fatty acids and cyclopentane butyl-acid. Priming with DPS extract significantly improved its bio-stimulating capacity by enhancing fresh and dry biomasses, photosynthetic pigments, and primary metabolites in response to the optimal DSP concentration of 80 g l⁻¹. Stress biomarkers (H₂O₂ and malondialdehyde (MDA)) were found in the ranges of water-primed control except for the highest dose of DPS extract (120 g l⁻¹). Further, priming with DPS extract increased the secondary metabolites (total phenolics and flavonoids) besides the activity of polyphenol oxidase (PPO), suggesting an enhanced cell redox system. The expression patterns of a series of specific key genes included in secondary metabolism modulation as, *phenylalanine ammonia lyase* (PAL), *chalcone synthase* (CHS), *chalcone isomerase* (CHI), *flavonol synthase* (FLS), and *deoxyxylulose phosphate reductoisomerase* (DXR), were activated following treatments with DPS extract. Overall, the present study underscores that the application of DPS extract can stimulate the growth and bioactive constituents of *Eruca vesicaria*, thus elevating its potential as a nutraceutical and medicinal value.

Keywords Date palm seeds · Elicitation · *Eruca vesicaria* · Primary metabolites · Secondary metabolism

1 Introduction

Eruca vesicaria is an essential vegetable crop and a member of Brassicaceae family. It has high commercial value and serves as a vital source of significant metabolites (Barazani and Ziffer-Berger 2014; Veremeichik et al. 2023). With high

nutritional value, its leaves are commonly consumed as salad, containing a diverse range of bioactive constituents with health promoting traits (Bell et al., 2015; Veremeichik et al. 2023). The *Eruca vesicaria* leaves have been reported to possess six types of phytochemicals, including terpenoids (31.91%), phenolics (23.60%), aromatic and aliphatic esters (16.97%), heterocyclics (14.83%), sulfur containing organics (11.25%), and silyl compounds (1.44%) (Al bratty et al. 2022). The phytochemicals of *Eruca vesicaria* was reported to have many therapeutic benefits, including antimicrobial, anti-inflammatory, anticancer, antioxidant, and antigenotoxic (Awadelkareem et al. 2022). Relying on these significant properties, *Eruca* potential as vegetable crop can be harnessed to produce those valuable compounds and widen its cultivation. Thus, proposing new eliciting techniques could enhance the potent agricultural and pharmaceutical applications of plants.

The plant secondary metabolism is the synthetic route for these bioactive compounds, which operates indirectly

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during plant growth and development, and essentially for plant environment interactions (Carreón-Delgado et al. 2023; Biała and Jasiński 2018). These natural phytochemical compounds are naturally consumed in plant-based foods due to their several health benefits. Consequently, it is crucial to regulate the synthesis of these natural bioactive compounds and enhance their abundance in cultivated crops. Polyphenols, a major group of secondary metabolites, are synthesized via the phenylpropanoid pathway, initiated by Phenylalanine ammonia lyase (PAL) (Singh et al. 2021; Ferreira and Antunes 2021). Enzymes like chalcone synthase (CHS), chalcone isomerase (CHI), and flavonol synthase (FLS) play key roles in flavonoid production (Biała and Jasiński 2018; Lei et al. 2023). Flavonoids enhance stress tolerance, nutrient uptake, and interactions with nitrogen-fixing bacteria and mycorrhizal fungi, improving nutrient cycling (Del Valle et al., 2020; Singh et al. 2021).

Terpenoids, the largest group of organic compounds in plants, include primary and secondary metabolites like photosynthetic pigments, phytohormones, antioxidants, and essential oils (Pichersky and Raguso 2018; Gao et al. 2018). *Eruca vesicaria* leaves contain 31.91% terpenoids, such as cilonasterol and phytol, which offer health benefits (Al bratty et al. 2022). Terpenoid biosynthesis requires isopentenyl pyrophosphate (IPP) and dimethylallyl diphosphate (DMAPP), produced via the mevalonate (MVA) and methylerythritol phosphate (MEP) pathways in plastids (Xu et al. 2019; Wölwer-Rieck et al. 2014; Gao et al. 2018). The enzyme deoxyxylulose phosphate reductoisomerase (DXR) is crucial in the chloroplast pathway, converting deoxyxylulose phosphate (DXP) into MEP and subsequently into IPP, regulating terpenoid biosynthesis (Gao et al. 2018; Zhang et al. 2013). Therefore, the manipulation of specific enzymes expression related to key metabolic pathways is a major research target that could lead to improved health benefits and plant performance.

Towards the enhancement of organic plant productivity, natural bioproducts are encouraged to be effectively used. Therefore, the need to validate the availability of sustainable applications that enhance plant health and growth is essential. The seeds of the date palm (*Phoenix dactylifera*) are often considered waste products and disposed of in large quantities in Middle Eastern countries. However, these cost-effective date palm seeds have the potential to serve as a valuable source of various components that offer significant nutritional benefits. These components include minerals, proteins, oil, fibres, fatty acids, and phenolic, which have high nutritional value (Al-Najar et al. 2019). Previous studies have established that these seeds exhibit remarkable antioxidant activity, surpassing that of standard antioxidants. This can be attributed to the occurrence of several bioactive phenolic compounds, like naringenin and rutin (Bentrad and Gaceb-Terrak 2020). The date seed components were

recorded to possess several biological properties that might be used in food production, cosmetics applications, water toxins removal, bio-fuel production, and medicinal supplements (Al-Najar et al. 2019; Alkhoori et al. 2022). Despite that fact, this study represents the first exploration into the accommodation of utilizing DPS extract within agricultural application. Consequently, the aim of this investigation is to underscore the efficacy of employing date palm seeds extract as an environmentally friendly application to boost growth and trigger the production of valuable secondary metabolites in *Eruca vesicaria* seedlings.

2 Materials and Methods

2.1 Preparation of Date Palm Seed (DPS) Extract

The dry fruits of date palm (*Phoenix dactylifera*), whose local name is Aswani, were procured from market to obtain the seeds. The whole seeds were simply used in the extraction method without crushing and soaked in distilled water. About 20 g of date seeds were soaked in 500 ml of distilled water for 4 days at 4 °C, then crushed by an electric blender for 2 min before filtration, which was finally adjusted to 1 L. The same steps were repeated to prepare the aqueous date palm seeds at 40, 80, and 120 g l⁻¹. The obtained DPS extracts were freshly utilized for seed priming treatments.

2.2 Phytochemical Analysis of DPS Extract

For phytochemical analysis, DPS aqueous extract was evaporated in the vacuum chamber of a rotary evaporator. The resulting crystalline residues were dissolved in 90% ethanol and then analyzed using gas chromatography-mass spectrometry (GC-MS) and phytochemical analysis. The ethanolic fraction (1 µl) was injected into the GC-MS (Perkin Elmer Clarus 580/560S). The chemical composition of the crude extract was determined based on the GC retention time (RT) on the capillary column (Elite-5MS, 30 m, 0.25mmID 0.25 µm df). The resulting mass spectra were compared to a database of standard compounds (50 to 620Da).

As for the phytochemical analysis, 20 ml DPS extract (20 g l⁻¹) were digested using HNO₃: H₂O₂ mixture (5:3, v/v) to detect N, P, K, Ca, and Mg levels. The colorimetric technique using Rochelle reagent was employed to quantify the nitrogen content, while the molybdenum blue method was utilized to determine the phosphorus levels, both referenced against standard calibration curves as per Allen et al. (1989). Inductively coupled plasma optical spectroscopy (Polyscan 61E, Thermo Jarrell-Ash Corp., Franklin, MA, USA) was used to estimate the levels of potassium, calcium, and magnesium.

2.3 Antioxidant Activity Assays

The antioxidant scavenging capacity was assayed based on three techniques: DPPH (2, 2-Diphenyl-1-picrylhydrazyl), phosphomolybdate (PMA), and ferric reducing power (FRP).

DPPH radical scavenging activity (Bondet et al. 1997) was estimated by mixing 0.1 ml extract with 3.9 ml of a DPPH solution (0.03 g l⁻¹ in methanol). After 1 h of dark incubation, the decreased absorbance at 517 nm was monitored against a blank sample. The percentage of scavenged DPPH was measured from the following equation, where A_o is blank absorbance and A_s is sample absorbance: DPPH scavenging % = $\frac{A_o - A_s}{A_o} \times 100$.

The PMA method (Ahmed et al. 2012) was carried out by adding 0.1 ml extract to 3 ml phosphomolybdate reagent, (28 mM sodium phosphate, 4 mM ammonium molybdate, and 0.6 M sulfuric acid). After incubation in a boiling water bath for 30 min, the mixture was cooled, and the absorbance was determined at 695 nm. The PMA antioxidant capacity was measured as mg g⁻¹ ascorbic acid (ASA) equivalent.

The FRP assay (Oyaizu 1986) was applied by incubating a mixture of 1 ml extract, 1 ml 0.2 M sodium phosphate buffer (pH 6.6), and 1 ml of K₃Fe(CN)₆ (1%, w/v) at 50 °C for 20 min. To the mixture, about 1 ml trichloroacetic acid (20%, w/v) was added and centrifuged at 5000 rpm for 15 min. Thereafter, the obtained upper layer was mixed with 0.2 ml FeCl₃ (0.1%, w/v) while photochemically measured at 700 nm against a blank without K₃Fe(CN)₆. The FRP was expressed as mg g⁻¹ ASA equivalent.

2.4 Experimental Design

The seeds of *Eruca vesicaria* Mill. were surface sterilized using 5% hypochlorite solution for 5 min and washed several times with distilled water. The seeds were further divided into two groups, the first was considered as control and soaked in distilled water for 10 h. The second group was separated into four sets, which were primed by immersing in different levels of DPS extract (20, 40, 80, and 120 g l⁻¹) for 10 h. The five priming treatments were kept at room temperature under continuous stirring. Each group of seeds was sown in plastic pots (20 cm diameter and 15 cm depth) containing 5 kg clay-sandy soil mixture (2:1 w/w). The treatments were represented by three pots per treatment and subjected to normal greenhouse conditions with 11 h photoperiod at 25/15 °C day/night and 60% relative humidity. The pots received 400 ml (50% field capacity) tap water once weekly till 21 days of growth. The seedlings were collected and washed to evaluate their growth and phytochemical parameters.

2.5 Evaluation of Biomass and Photosynthetic Pigments

The collected seedlings (21 days old) were washed thoroughly and fresh weights were measured to estimate fresh biomass (FM). For dry biomass (DM) determination, fresh seedlings were dried at 50 °C for 3 days. As for chlorophylls (Chl a, Chl b) and carotenoids estimation, about 0.1 g of fresh leaves were extracted in acetone according to Arnon (1949) and Horvath et al. (1972), respectively. The spectroscopic absorbance was recorded at 663, 644, and 480 nm to express their contents by mg g⁻¹ DM.

2.6 Evaluation of Plant Primary and Secondary Metabolites

The total carbohydrates (TCs) and proteins (TPs) were estimated using borate extract of dry leaves powder. As described by Masuko et al. (2005) with slight modification, 0.1 ml of extracted samples was incubated at 30 °C for 30 min with a 1: 5 mixture of 5% (w/v) phenol and concentrated sulfuric acid. The TCs content was determined as mg g⁻¹ DM by reading the absorbance at 490 nm with the aid of glucose calibration curve. The method ascribed by Bradford (1976) was utilized to measure TPs by mixing 3 ml Commassie Brilliant Blue reagent with 0.1 ml borate buffer extract. The absorbance of produced blue color was measured against a standard protein (bovine serum albumin) calibration curve to determine TPs (mg g⁻¹ DM).

Total phenolics and flavonoids levels were estimated by using ethanolic extract of shoot, according to Jindal and Singh (1975) and Chang et al. (2002), respectively with modification. To determine the phenolic content, the ethanolic extract of the sample was mixed with Folin-Ciocalteu's reagent and 20% Na₂CO₃ and incubated for one hour. The absorbance of the mixture was then measured at 650 nm against a gallic acid standard curve (0–1 mg l⁻¹, R² = 0.9942). For the quantification of total flavonoids, a mixture consisting of the sample extract, 10% AlCl₃, 1 M K-acetate, and 95% ethanol was prepared. The absorbance was determined at 417 nm and compared to a standard of quercetin curve (0–1 mg l⁻¹, R² = 0.9848).

2.7 Evaluation of Stress Biomarkers

The content of H₂O₂ and malondialdehyde (MDA) were assessed according to Velikova et al. (2000) and Heath and Packer (1968), respectively. Fresh leaves (0.1 g) were extracted in 0.1% trichloroacetic acid (TCA) to estimate H₂O₂. The plant extract was mixed with phosphate buffer (pH 7.0) and KI₂ (1 M), where the absorbance was read at 390 nm. To measure MDA, a product of lipid peroxidation, leaf was extracted in a 5% (w/v) TCA solution. The

reaction mixture consisted of the sample extract and 0.67% (w/v) TBA, which was then boiled for 20 min then cooled. The samples absorbance was measured at two wavelengths, specifically 532 and 600 nm.

2.7.1 Estimation of Polyphenol Oxidase and Phenylalanine ammonia Lyase Activity

The fresh leaves of *E. vesicaria* were extracted using phosphate buffer (pH 7) to detect the activity of polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL). PAL activity was assayed according (Şirin et al. 2016) in a reaction mixture contained enzyme extract, 100 mM Tris-HCl (pH 8.8), 40 mM L-phenylalanine, detecting the trans-cinnamic acid formation at 290 nm. After incubating at room temperature for half an hour, the reaction was stopped by adding 4 M HCl and PAL specific activity was expressed as $\mu\text{M g}^{-1} \text{ FM}$. PPO activity was measured by screening purpurogallin formation, according to (Kumar and Khan 1982). The reaction was started by adding enzyme extract to a mixture of 2 mM pyrogallol (dissolved in 0.1 M phosphate buffer (pH 6) and incubated for 10 min at 25 °C. The reaction was stopped by H_2SO_4 (2.5 N) to record the absorbance at 420 nm. The PPO activity was expressed as $\mu\text{M g}^{-1} \text{ FM min}$ using the extinction coefficient of $26.40 \text{ M}^{-1} \text{ cm}^{-1}$.

2.7.2 Quantitation of Relative Expression Levels of Studied Genes

To extract total RNA from fresh *Eruca vesicaria* leaf tissues, a pulverization method using liquid nitrogen was employed. The RNA extraction process was carried out using the RNeasy Mini Kit from Qiagen. Complementary DNA (cDNA) was then obtained through a reverse transcription reaction using the Superscript III Reverse Transcriptase kit from

Invitrogen. This reaction was performed in a thermocycler. The expression patterns of key genes were analyzed using quantitative real-time polymerase chain reaction (qRT-PCR) on a Rotor-Gene 6000 instrument (QIAGEN, ABI System, USA). The qRT-PCR reaction mixture included SYBR Green PCR Master Mix from Fermentas and specific primer pairs, as outlined in Table 1. The expression of the target genes was normalized to the reference gene β -actin. All samples were tested in triplicate to ensure accuracy and consistency. The relative expression levels of the studied genes were calculated using the $2^{-\Delta\Delta\text{CT}}$ method. (Livak and Schmittgen 2001).

2.7.2.1 Statistical Analysis The results were described as the average of three replicates, and the standard error (SE) was calculated. Statistical analysis was performed using one-way ANOVA, by using Duncan's test to determine significant differences between treatments. The XLSTAT software (version 2014.5.03) was used for these analyses, with a significance level set at $p < 0.05$. Furthermore, the XLSTAT software was also utilized to calculate the Pearson correlation coefficient and conduct a Principal Component Analysis (PCA) to explore the relationships between different parameters and varying concentrations of DPS extract.

3 Results

3.1 Quantitative Characterization of DPS Extract

The phytochemical characterization of DPS extract is represented in Table 2. The mineral analysis of the DPS extract revealed that it had the highest level of P at $8.2 \pm 0.4 \text{ mg g}^{-1}$ of DM. The N content was $4.1 \pm 0.4 \text{ mg g}^{-1}$ DM, while K and Ca were both around 1.3 mg g^{-1} DM, 1.2 mg g^{-1} DM,

Table 1 Sequences of specific primers used in qRT-PCR

Gene	Abbreviation	Direction	Sequences (5'–3')
Reference gene	β -Actin	F	GTGGGCCGCTCTAGGCACCAA
		R	CTCTTTGATGTCACGCACGATTTC
Chalcone synthase	CHS	F	AGGCTAACAGAGGAGGGTA
		R	CCAATTTACCGGCTTTCT
Chalcone isomerase	CHI	F	TGGTGGCCTAGACAACGATGAGTT
		R	TCACACTCCCAACTTGGTTTCCT
Flavonol synthase	FLS	F	TTAAAGGAAGGTCTCGGTGGCGAA
		R	TCATTGGTGACGATGAGTGCGAGT
Deoxy-D-xylulose 5-phosphate reductoisomerase	DXR	F	TTGGTTGCTGAGCTAAAAGAAG
		R	CTTGTTGAAAAGTGTGGCAGAG
Phenylalanine ammonia lyase	PAL	F	AGAACGGTGTCTGCTCTTCAG
		R	TGTGGCGGAGTGTGTAATG
Polyphenol oxidase	PPO	F	CATGCTCTTGATGAGGCGTA
		R	CCATCTATGGAACGGGAAGA

respectively. The Mg level was the lowest at 0.2 ± 0.1 mg g⁻¹ DM. The DPS extract also demonstrated significant antioxidant activity, indicating the presence of beneficial antioxidant compounds. The antioxidant activity of DPS extract was confined on the basis of DPPH scavenging, PMA, and FRP assays. In this context, the phenolic contents were greatly observed in the DPS extract by 60.1 ± 9.8 mg g⁻¹ DM and total flavonoids contents were found by 4.4 ± 0.9 mg g⁻¹ DM.

3.2 GC-MS Analysis of DPS Extract

The primary phytochemical components identified through GC-MS analysis were detailed in Table 3 based on the chromatogram depicted in the Fig. 1. The most abundant compound detected at a retention time (RT) of 15.318 to 15.143 min was 2-Dodecenal and 2-Dodecenal, (E)-. Following this, palmitic acid was the second-highest component

observed at an RT of 36.997 min. Additionally, various fatty acids like myristic acid, Oleic Acid, and linoleic acid were identified at RTs of 16.274, 28.909, and 38.923, respectively. Other fatty alcohols, such as Hexadecanol and 9-Tetradecen-1-ol were present at RTs of 23.412 and 13.733 min. Furthermore, carboxylic acid, including Cyclopentane, butyl-acid, was detected at an RT of 15.528. Lastly, 4-Hydroxyproline was observed in specific quantities at an RT of 15.668 min.

3.3 Growth and Photosynthetic Pigments

The results shown in Table 4 indicated that seed priming with DPS extract had an enhanced impact on physical growth parameters. The different doses of DPS extract led to a steady increase in the fresh and dry biomass. The highest dose of 80 g l⁻¹ resulted in the most significant improvement in both fresh and dry biomass by 250% and 177%, respectively, compared to the water primed control. The positive response of priming, particularly, with moderate DPS doses (40 and 80 g l⁻¹), was significantly demonstrated by the marked increase in the contents of Chl a and Chl b, as shown in Table 4. Relative to the control, 80 g l⁻¹ DPS treatment resulted in the maximum elevation of Chl a and Chl b levels by 38% and 61%, respectively. The carotenoids levels did not exhibit significant changes after DPS priming, as revealed in Table 4.

3.4 Plant Primary and Secondary Metabolites

The content of TCs and TPs, as metabolic markers, were identified as illustrated in Fig. 2a and b. Their improvement was evident by all sets of DPS priming treatments compared to the control. The priming with 80 g l⁻¹ caused the most significant elevation in TCs and TPs by 186% and 125%, respectively. Moreover, the over-accumulation of total phenolics and flavonoids was recorded with DPS priming showing the highest enhancement at the moderate 80 g l⁻¹ DPS dose by 132% and 38%, respectively, relative to the control (Fig. 2c, d).

Table 2 Quantitative characterization of date palm seeds (DPS) extract

Parameters	Results	Unit
Total nitrogen	4.1 ± 0.4	mg g ⁻¹ DM
Total phosphorus	8.2 ± 0.4	mg g ⁻¹ DM
Total potassium	1.3 ± 0.3	mg g ⁻¹ DM
Total calcium	1.2 ± 0.1	mg g ⁻¹ DM
Total magnesium	0.2 ± 0.1	mg g ⁻¹ DM
Total phenolic contents	60.1 ± 9.8	mg g ⁻¹ DM
Total flavonoid contents	4.4 ± 0.9	mg g ⁻¹ DM
DPPH scavenging	63.1 ± 1.7	%
PMA assay	5.4 ± 0.3	mg ASA equivalent g ⁻¹ DM
FRP assay	96.3 ± 0.2	mg ASA equivalent g ⁻¹ DM

DPPH 2, 2 Diphenyl – 1-picrylhydrazyl, *PMA* Phosphomolybdate, *FRP* ferric reducing power, *DM* Dry biomass, *ASA* Ascorbic acid. The data represents means $\pm$ standard error ($n=3$). Different lower-case letters are used to indicate statistically significant differences at Duncan's test ($P < 0.05$)

Table 3 Phytochemical components detected in date palm seeds (DPS) extract by GC-MS

No	Area %	Compounds	RT	Height	Molecular formula
1	12.545	2-Dodecenal	15.318	3,808,730,368	C ₁₂ H ₂₂ O
2	5.718	2-Dodecenal, (E)-	15.143	2,027,950,720	C ₁₂ H ₂₂ O
3	4.860	Palmitic acid	36.997	134,514,080	C ₁₆ H ₃₂ O ₂
4	1.829	Myristic acid	16.274	1,163,078,528	C ₁₄ H ₂₈ O
5	1.513	Hexadecanol	23.412	321,032,128	C ₁₆ H ₃₀ O
6	1.374	9-Tetradecen-1-ol	13.733	622,556,352	C ₁₄ H ₂₈ O
7	0.779	Cyclopentane, butyl-acid	15.528	356,900,192	C ₉ H ₁₆ O ₂
8	0.726	Oleic Acid	28.909	46,386,428	C ₁₈ H ₃₄ O ₂
9	0.649	4-Hydroxyproline	15.668	303,657,184	C ₅ H ₉ O ₃ N
10	0.534	linoleic acid	38.923	49,586,272	C ₁₈ H ₃₂ O

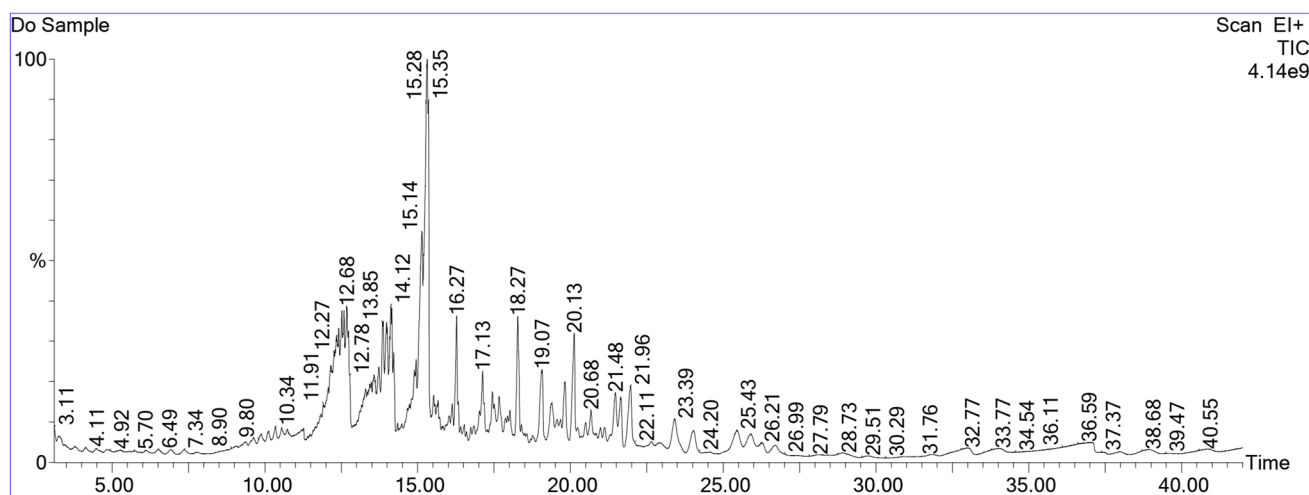


Fig. 1 GC-MS chromatogram of the phytochemical components of date palm seeds (DPS) extract

Table 4 The biomasses and photosynthetic pigments levels of *Eruca vesicaria* seedlings after seed priming with date palm seeds (DPS) extract (0, 20, 40, 80, 120 g l⁻¹)

Treatments	FM(g/plant)	DM(g/plant)	Chl a (mg g ⁻¹ DM)	Chl b (mg g ⁻¹ DM)	Carotenoids (mg g ⁻¹ DM)	Total pigments (mg g ⁻¹ DM)
Control	2.11 ± 0.36 ^c	0.22 ± 0.01 ^c	1.18 ± 0.11 ^c	0.62 ± 0.11 ^b	0.21 ± 0.06 ^a	2.02 ± 0.1 ^c
20 g/l	4.8 ± 0.33 ^b	0.41 ± 0.04 ^b	1.327 ± 0.10 ^{bc}	0.68 ± .09 ^b	0.22 ± 0.04 ^a	2.23 ± .017 ^{bc}
40 g/l	6.6 ± 0.2 ^a	0.55 ± 0.02 ^a	1.56 ± 0.02 ^{ab}	0.92 ± 0.06 ^{ab}	0.22 ± 0.02 ^a	2.7 ± 0.07 ^a
80 g/l	7.4 ± 0.2 ^a	0.61 ± 0.005 ^a	1.63 ± 0.01 ^a	1 ± 0.06 ^a	0.27 ± 0.04 ^a	2.9 ± .04 ^a
120 g/l	4.8 ± 0.46 ^b	0.44 ± 0.02 ^b	1.37 ± 0.12 ^{abc}	0.8 ± 0.1 ^{ab}	0.22 ± 0.06 ^a	2.39 ± 0.17 ^b

FM fresh biomass, DM Dry biomass, Chl a Chlorophyll a, Chl b Chlorophyll b. The data represents means ± standard error ($n=3$). Different lowercase letters are used to indicate statistically significant differences at Duncan's test ($P<0.05$)

3.5 Stress Markers and Activities of PAL and PPO

To evaluate the intimal status of cells after DPS extract treatment, H₂O₂ and MDA were assessed as stress markers in *E. vesicaria* leaves (Fig. 3a, b). Relative to the control, the seed priming with the lowest and moderate doses (20, 40, and 80 g l⁻¹) of DPS extract had no significant impact among H₂O₂ and MDA. However, the highest dose (120 g l⁻¹) exhibited a marked increase in their levels than the control, indicating oxidative stress. Concerning PAL and PPO activities (Fig. 3c, d), they were significantly stimulated after DPS priming treatments, and 80 g l⁻¹ DPS extract caused a remarkable improvement by 47% and 144%, respectively, comparable to the control and other DPS levels.

3.6 RT-PCR of the Specific key Genes

The study investigated the relative gene expression of several key enzymes, including *PAL*, *CHS*, *CHI*, *FLS*, *DXR*, and *PPO* (Fig. 4). The results showed that priming the plants with the DPS extract significantly elevated the expression of

these genes, indicating higher transcript levels compared to the control. Specifically, the moderate dose of DPS extract (80 g l⁻¹) was found to be the most effective in increasing the expression of *PAL*, *CHS*, *CHI*, *FLS*, and *PPO* by 86%, 240%, 151%, 440%, and 278%, respectively, than the water-primed control. As observed from current results, *DXR* expression level caused a non-significant increase at 120 g l⁻¹ DPS treatments, while priming with 80 g l⁻¹ caused the most favorable increase by 347% relative to the control. At 40 g l⁻¹ extract treatment; the relative expression of all genes was lower than their maximum overexpression levels but still higher than control levels.

3.7 Correlation among Different Parameters

Pearson correlation coefficient (r) was used to analyze the relationships between physiological and molecular characteristics observed in various treatments with DPS extract application (as shown in Fig. 5). The findings revealed strong positive correlations between phenolic and flavonoid contents, growth, and photosynthetic pigments. The

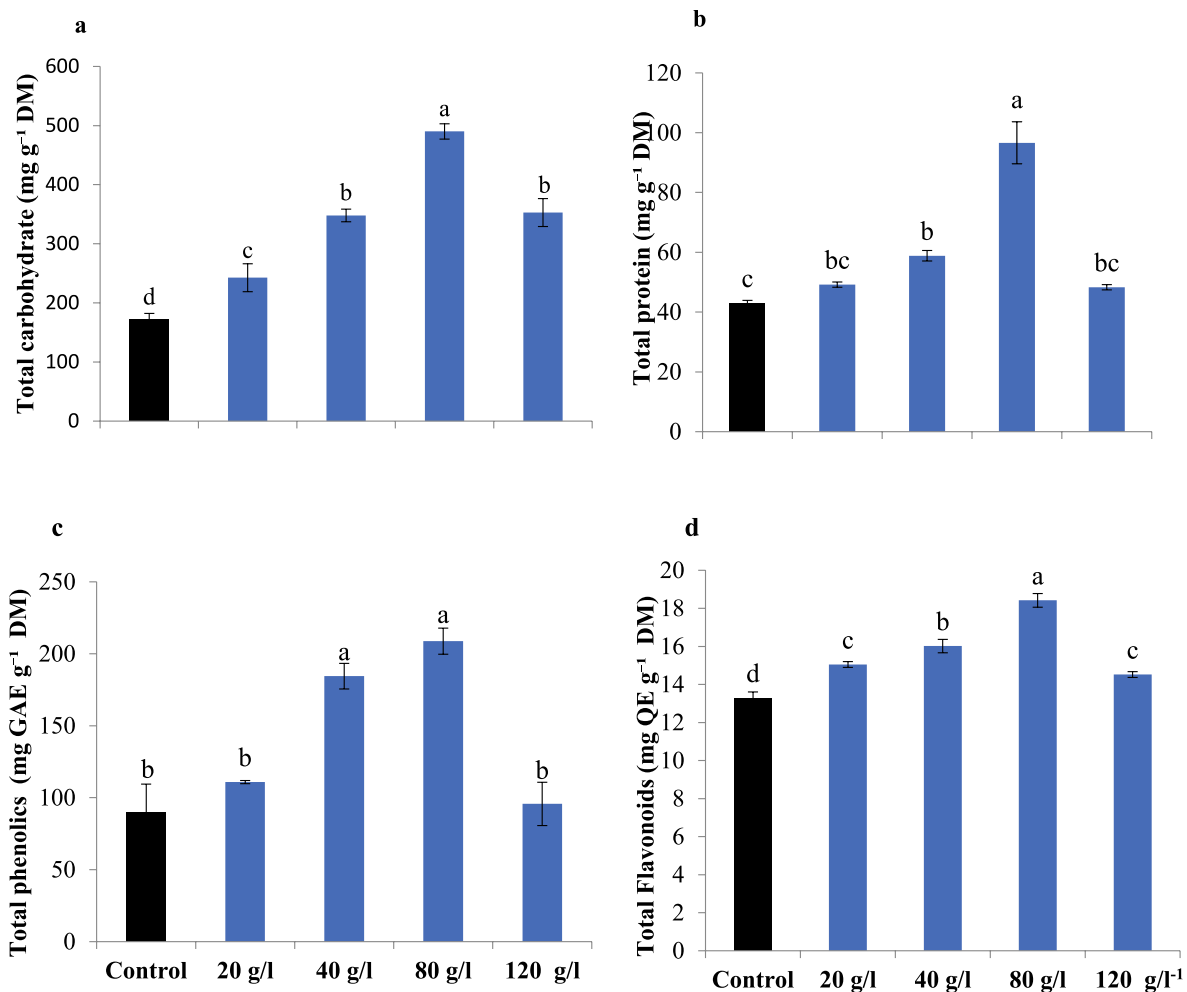


Fig. 2 The levels of total carbohydrate (**b**), total protein (**a**), phenolics (**c**) and flavonoids (**d**) in *Eruca vesicaria* seedlings after seed priming with date palm seeds (DPS) extract (0, 20, 40, 80, 120 g l⁻¹). The data

represents means $\pm$ standard error ($n=3$). Different lowercase letters are used to indicate statistically significant differences at Duncan's test ($P < 0.05$)

phenolic and flavonoid contents were also found to be positively related with augmented expression levels of *PAL*, *CHS*, *CHI*, *FLS*, and *DXR* genes. Furthermore, the growth, and photosynthetic pigments showed positive correlations with the expression levels of *CHS*, *CHI*, and *FLS* genes. The *DXR* and *PPO* genes were significantly correlated with carotenoids, as well as primary and secondary metabolites in plants. Conversely, the MDA and H₂O₂ contents showed negative correlations with fresh and dry biomasses, as well as primary and secondary metabolites in plants.

3.8 Principle Component Analysis

The results of the study employed principal component analysis (PCA) to observe the relationship between DPS extract treatments, physiological parameters, and expressions of the studied genes (Fig. 6). The analysis revealed that the first four principal components (PCs) encompassed the entire

variation observed in the data. The top two PCs, with eigenvalues greater than or equal to 1, explained 93.74% of the total variation. The PC1 represented a significant portion, accounting for 79.76% of the overall variation and showed significant correlations with various attributes in the following order: *FLS*, flavonoids, *PPO*, *PAL* activity, Chl a, Chl b, *CHI*, TCs, *CHS*, DM, FM, TPs, *DXR*, *PAL*, phenolics, carotenoids, and *PPO* activity, in descending order. On the other hand, PC2 explained 13.98% of the overall variation and was primarily influenced by MDA and H₂O₂.

4 Discussion

Plants are known to produce huge levels of various primary and secondary metabolites. Primary metabolites are normally directed to photosynthesis, respiration, and other vital activities of normal growth, including the metabolism

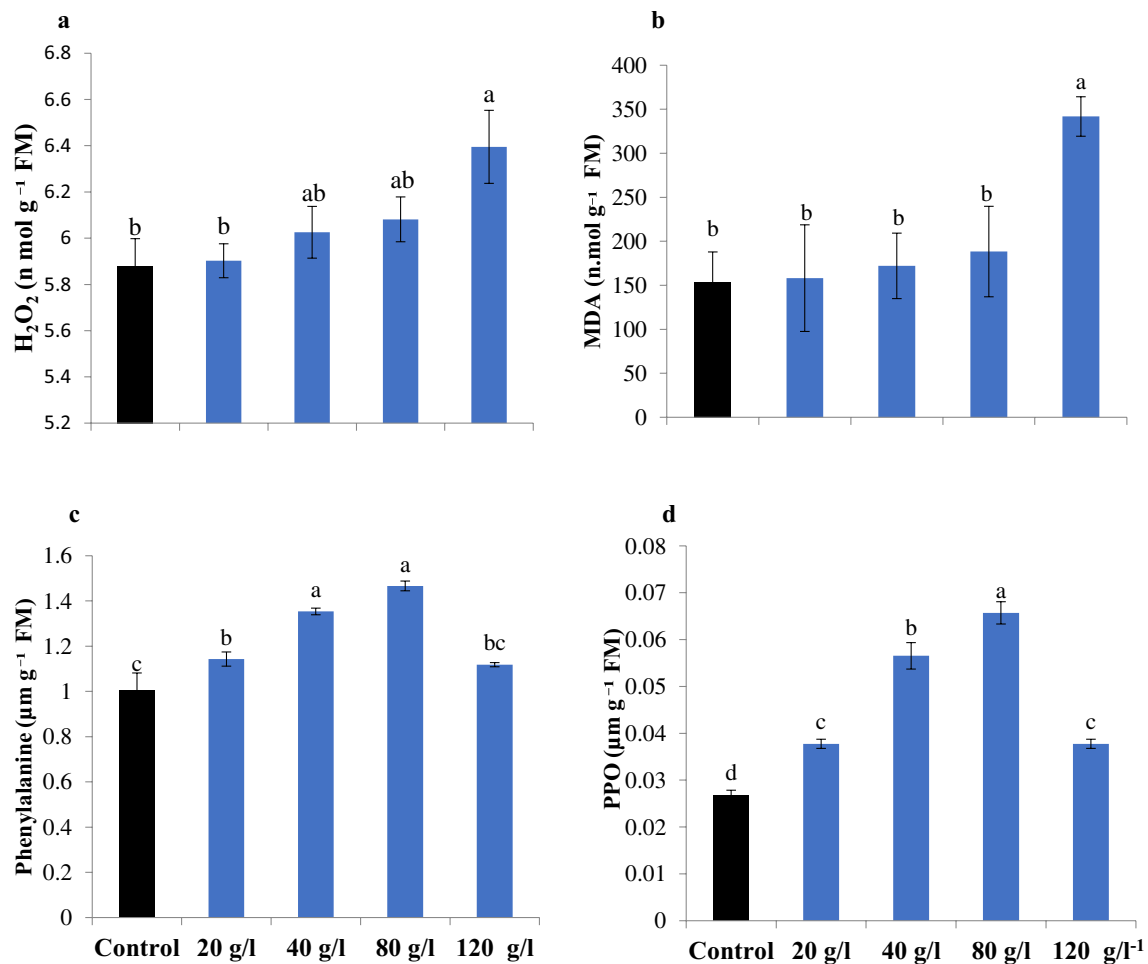


Fig. 3 The levels of Stress markers ((a) H_2O_2 and (b) malondialdehyde (MDA)) and activities of (c) Phenylalanine and (d) polyphenol oxidase (PPO) in *Eruca vesicaria* seedlings after seed priming with date palm seeds (DPS) extract (0, 20, 40, 80, 120 g l⁻¹). The data rep-

resents means $\pm$ standard error ($n=3$). Different lowercase letters are used to indicate statistically significant differences at Duncan's test ($P<0.05$)

of fat, protein, and carbohydrate. Alternatively, secondary metabolites are produced in diverse plant tissues to perform several activities and enhance plant adaption either in situ or ex-situ (Singh et al. 2021). Their production are modulated by various chemical or biological elicitors that could boost plant defense mechanisms or via stresses including light, ultraviolet radiation, drought, and salinity (Galicia-Campos et al. 2022; Nabavi et al. 2020).

The DPS extract was analyzed to identify its components and understand its growth-promoting effects. The promising fertilizing potential of the DPS extract may be due to its great levels of macronutrients and antioxidants. The P and N contents were represented the highest, where K and Ca ions were detected with low quantities (Table 2). Further, the plentiful levels of antioxidants compounds, including total phenolics and flavonoids, could indicate the high antioxidant activity of DPS extract on the basis of DPPH scavenging, PMA, and FRP results, suggesting its eliciting potential.

Plant extracts could act as bioactive stimulants and exhibit high antioxidant activity, which depends on several complex antioxidant compounds such as phenolics and flavonoids (ElSayed et al. 2022; Nessem et al. 2023). This obtaining might propose a DPS extract as a promotor for plant growth and its defense responses.

The GC analysis of DPS extract reveals a variety of compounds contributing to its strong antioxidant abilities. The most prevalent is 2-Dodecenal, known for its antioxidant properties, helping to combat oxidative damage, and role in plant cell biochemistry, especially in chloroplasts (Sharopov et al. 2015; Hatanaka et al. 1989). The second major constituent was palmitic acid, which is a saturated fatty acid present in various plant sources, plays a significant role in plant health, enhances seedling development, and interacts with pathogens (Liu et al. 2017; Ma et al. 2021). The presence of fatty acids and their derivatives was distinct in DPS extract, such as myristic acid, oleic acid, and linoleic acid.

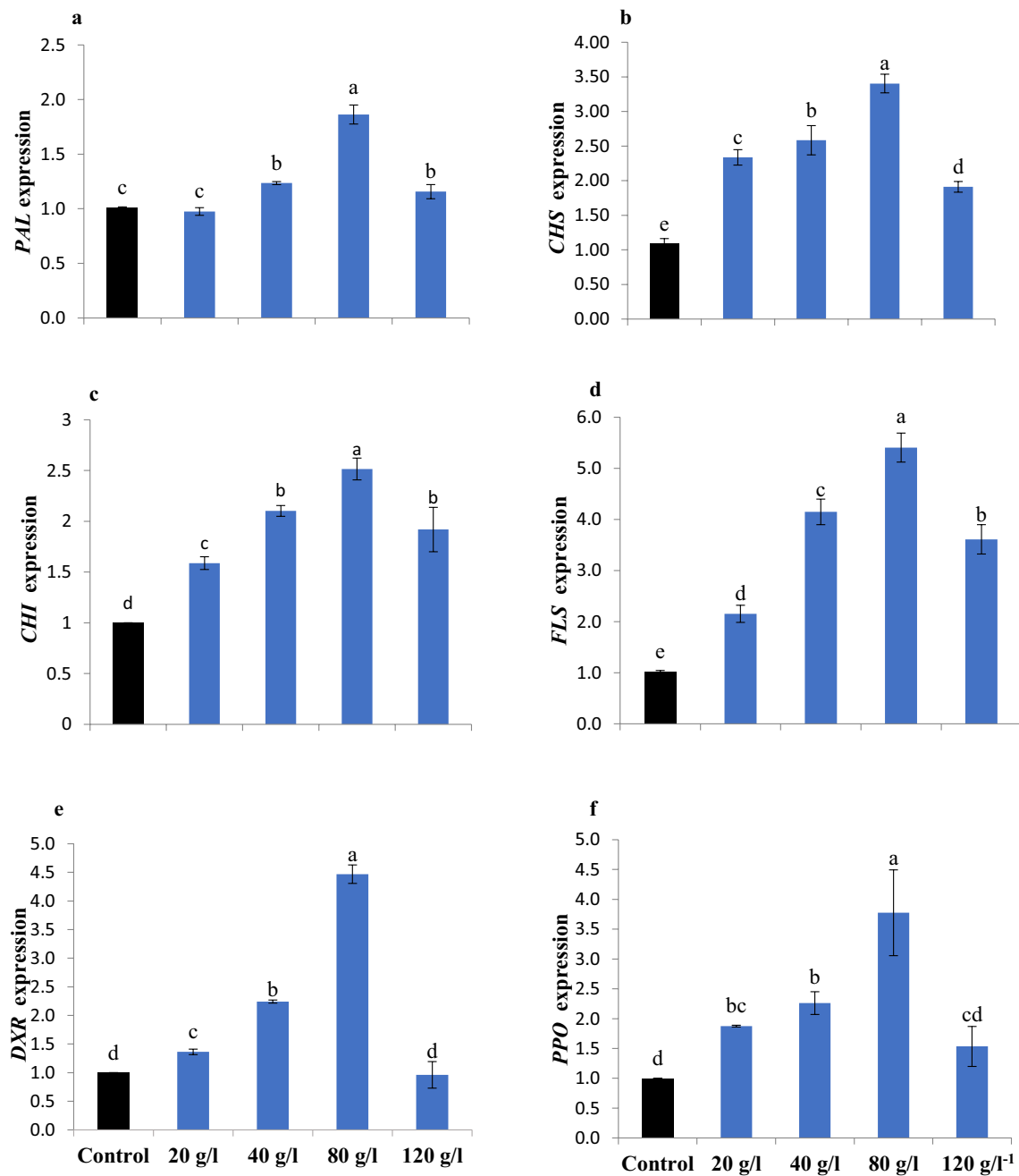


Fig. 4 The effect of different date palm seeds (DPS) extract concentrations (0, 20, 40, 80, 120 g l⁻¹) on (a) Phenylalanine ammonia lyase (PAL), (b) Chalcone synthase (CHS), (c) Chalcone isomerase (CHI), (d) Flavonol synthase (FLS), (e) Deoxy-D-xylulose 5-phos-

phate reductoisomerase (DXR), and (f) Polyphenol oxidase (PPO) relative expressions of *Eruca vesicaria* seedlings. The data represents means $\pm$ standard error ($n=3$). Different lowercase letters are used to indicate statistically significant differences at Duncan's test ($P < 0.05$)

Further, the fatty alcohols hexadecanol and 9-tetradecen-1-ol were detected in definite amounts. In a similar report, the characterization of date palm seeds revealed their richness of many bioactive components, including unsaturated fatty acids and phenols such as myristic and oleic acids (Harkat et al. 2022). Interestingly, the date seeds contain important antioxidants and phenolic compounds, which make up 65

to 78% of their composition (Fikry et al. 2019). Further, the presence of cyclopentane butyl-acid, which is an analogue of jasmonic acid, has been found to enhance cotyledon toughness and stimulate the biosynthesis of specific compounds in plants (Bodnaryk and Yoshihara 1995; Stintzi et al. 2001). Also, in DPS extract, 4-hydroxyproline is a major component of hydroxyproline-rich glycoproteins (HRGPs) found in

	FM	DM	Chl a	Chl b	Carotenoids	TCs	TPs	Phenolics	Flavonoids	H2O2	MDA	PAL	PPO	PAL	CHS	CHI	FLS	DXR
DM	1.00																	
Chl a	0.98	0.98																
Chl b	0.94	0.95	0.98															
Carotenoids	0.69	0.68	0.71	0.72														
TCs	0.90	0.92	0.91	0.95	0.81													
TPs	0.79	0.78	0.83	0.84	0.97	0.87												
Phenolics	0.88	0.87	0.94	0.91	0.75	0.80	0.87											
Flavonoids	0.92	0.91	0.93	0.90	0.90	0.90	0.96	0.93										
H2O2	0.30	0.35	0.27	0.39	0.07	0.52	0.09	-0.03	0.12									
MDA	0.07	0.13	0.03	0.15	-0.11	0.31	-0.12	-0.27	-0.11	0.97								
PAL	0.95	0.94	0.98	0.95	0.78	0.88	0.89	0.98	0.97	0.11	-0.13							
PPO	0.71	0.74	0.68	0.76	0.76	0.91	0.75	0.51	0.74	0.69	0.55	0.63						
PAL	0.75	0.76	0.82	0.87	0.95	0.90	0.98	0.83	0.91	0.23	0.02	0.86	0.80					
CHS	0.96	0.95	0.93	0.87	0.82	0.87	0.87	0.88	0.97	0.14	-0.08	0.95	0.71	0.81				
CHI	0.97	0.98	0.96	0.96	0.73	0.97	0.82	0.83	0.91	0.47	0.25	0.92	0.84	0.82	0.92			
FLS	0.94	0.93	0.96	0.95	0.87	0.93	0.95	0.95	0.99	0.19	-0.04	0.98	0.75	0.93	0.95	0.94		
DXR	0.79	0.78	0.85	0.84	0.94	0.83	0.99	0.92	0.96	-0.02	-0.24	0.92	0.65	0.96	0.88	0.79	0.95	
PPO	0.89	0.87	0.90	0.87	0.94	0.89	0.98	0.91	1.00	0.08	-0.13	0.95	0.74	0.93	0.96	0.88	0.98	0.97

Pearson correlation coefficient (r)

Fig. 5 Pearson correlation coefficient for primary and secondary metabolite pathways determined at different concentrations of date palm seeds (DPS) extract in *Eruca vesicaria* seedlings (0, 20, 40, 80, 120 g l⁻¹) (Values in bold are a significant ($p \leq 0.05$)). The parameters analyzed were fresh biomass (FM), dry biomass (DM), chlorophyll a (Chl a), chlorophyll b (Chl b), total carbohydrates (TCs), total pro-

teins (TPs), the activity of enzymes phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO), the stress marker H₂O₂, malondialdehyde (MDA), and gene expression of *Phenylalanine ammonia lyase* (PAL), *Chalcone synthase* (CHS), *Chalcone isomerase* (CHI), *Flavonol synthase* (FLS), *Deoxy-D-xylulose 5-phosphate reductoisomerase* (DXR), and *Polyphenol oxidase* (PPO)

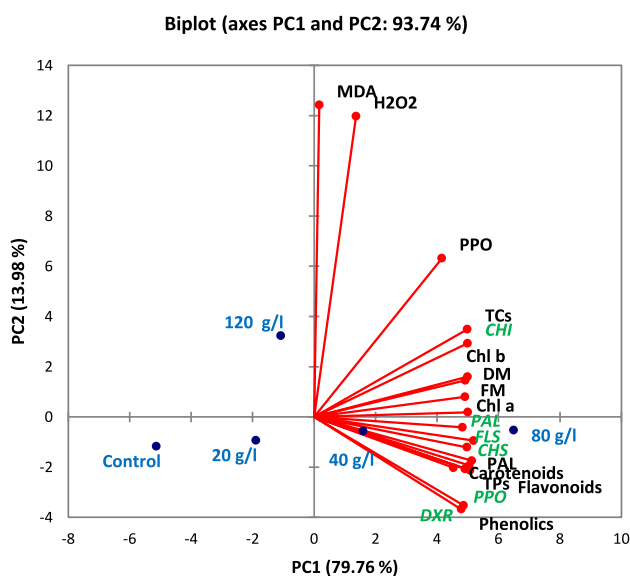


Fig. 6 Principal component analysis (PCA) for primary and secondary metabolites pathway determined at different concentrations of date palm seeds (DPS) extract at *Eruca vesicaria* seedlings (0, 20, 40, 80, and 120 g l⁻¹). The parameters analyzed were fresh biomass (FM), dry biomass (DM), chlorophyll a (Chl a), chlorophyll b (Chl b), total carbohydrates (TCs), total proteins (TPs), the activity of enzymes phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO), the stress marker H₂O₂, malondialdehyde (MDA), and gene expression of *Phenylalanine ammonia lyase* (PAL), *Chalcone synthase* (CHS), *Chalcone isomerase* (CHI), *Flavonol synthase* (FLS), *Deoxy-D-xylulose 5-phosphate reductoisomerase* (DXR), and *Polyphenol oxidase* (PPO)

plant cell walls. These HRGPs are essential for plant growth, development, and morphogenesis, influencing cell shape, differentiation, and polarity (Kavi Kishor et al. 2015; Xu et al. 2011). Thus, their biological characteristics and chemical composition need to be fully investigated to be further exploited in valuable applications.

Currently, seed priming with different sets of DPS extract resulted into significant increments in fresh and dry biomasses and the levels of photosynthetic pigments (chl a, chl b and total pigments) in *Eruca vesicaria* seedlings. The most favorable enhancing impact was recorded with the moderate doses 40 and 80 g l⁻¹ DPS relative to the non-treated control. The mineral components of the DPS extract, including key macronutrients like N, P, K, Ca and Mg, appear to be directly linked to enhancing metabolic processes and plant biomass. In this context, the present report is the first to investigate DPS extract ability as a bio-stimulant in a seed priming approach. Delivering vital nutrients that operates during seed establishment is the major advantage of seed priming (Ragab and Saad-Allah 2020). The stimulation of chlorophylls levels and thus their biosynthesis would be linked to enhanced photosynthetic process and growth level. DPS extract minerals may be directly incorporated in or indirectly stimulated the biosynthetic pathway of chlorophylls (El-Shenody et al. 2023). Similarly, seed nutrimpriming with bio-stimulants was identified to improve seed germination and seedling growth of *Ceratothera trilobal* (Masondo et al. 2018). Although the DPS stimulating impact represented

by enhanced biomasses, the carotenoids content showed non-significant changes by DPS priming as compared to the control. It is known that the elevated carotenoids levels usually related to combating oxidative stress constraints as antioxidant (Nessem et al. 2023) which might point to the normal cell machinery.

To explore the effect of DPS extract application on *Eruca vesicaria* seedlings, primary metabolic markers was assessed. The obtaining results indicated that seed priming with variable levels of DPS extract significantly intensified TCs and TPs where 80 g l⁻¹ offered the pronounced levels compared to the control. The increased TCs and TPs are mainly corresponded to the promoted metabolic processes and high rates of photosynthesis which manifested with increased biomasses and pigments. Similarly, seed priming with natural extracts was reported to supplement plants with essential nutrients and increase essential metabolites including carbohydrates and proteins enhancing growth rate (El-Shenody et al. 2023; Kasim et al. 2017). This mean that the DPS extract can enhance seed germination and seedling development due to the presence of growth promoting substance like polyphenol which support better root and shoot development, making plants stronger and healthier from the early stage.

In this regard, DPS priming hastened the over-production of total phenolics and flavonoids where was 80 g l⁻¹ DPS dose revealed their highest levels. The stimulating capacity of DPS exposure could be proved by the potent elevation of such secondary metabolites. Phenolics and flavonoids are non-enzymatic antioxidant with scavenging potential that could perform several imperative functions in developmental processes (Saad-Allah and Ragab 2020; Elsherif et al. 2024). Phenolics have vital importance as cell wall constituents and contributes in plant growth and development (Cheynier et al. 2013; Sen and Puthur 2020). As a result of DPS application, primary and secondary metabolites of *Eruca vesicaria* have intensified, indicating the effective shift from primary metabolism to the up-regulation of phenylpropanoid pathway without harming plant growth.

To evaluate the metabolic status after DPS application and induced secondary metabolites, stress markers (MDA and H₂O₂) were detected. The DPS application (20, 40, and 80 g l⁻¹) caused non-significant change in MDA and H₂O₂ contents, matching control levels to prove non-stress condition of plant tissues. The obtained results provided strong evidence through a negative Pearson correlation between the levels of malondialdehyde, a marker of oxidative stress, and various plant parameters including fresh and dry biomasses, as well as primary and secondary metabolites in plants. This finding indicates that the application of the DPS extract plays a dual role in neutralizing oxidative stress and eliciting beneficial responses in plants. With respect to DPS concentrations, 120 g l⁻¹ DPS greatly induced MDA and

H₂O₂ levels compared to the control indicating oxidative stress. The high dosage of DPS provides increased access to its active ingredients, which may create stressful conditions and result in the production of ROS. Nonetheless, the heightened levels of stress markers could be associated with the improved antioxidant activity of PPO to slightly relieve ROS and manage their toxicity. This obtaining associated with 120 g l⁻¹ DPS treatment is manifested by the maintained growth rate and vital physiological processes when compared to the control (Boeckx et al. 2015).

Owing to DPS extract moderate level (80 g l⁻¹), the activity of PPO (Fig. 3d) was remarkably stimulated due to the increased expression of *PPO* gene (Fig. 4f) compared to the water-primed control. PPO is widely associated in normal cell metabolism such as adjustment of oxygen levels in plastids, incorporation in the aromatic biopolymers such as hesperidin, auron and lignin. Further, PPO could comprise in the biosynthesis and degradation of metabolites depending on the developmental stages, environmental circumstances and the availability and specificity of phenolic compounds (Zhang 2023). In the present results, priming with DPS extract upregulated *PPO* gene expression and activity which possibly offers an enhancement of the cell redox system and provoking defense responses. In this context, the transcript levels of *PPO* genes in plant cells can be upregulated due to many factors, thereby benefiting from its principal roles under normal and stressed conditions (Zhang 2023).

Seed priming with DPS resulted into PAL activation (Fig. 3c), which can be considered as an indication of the promotion of secondary metabolism, representing a direct shift from primary metabolism. (Barros and Dixon 2020). The significant induction in PAL activity is mainly connected to the overexpression of *PAL* gene (Fig. 4a). Thus, the increment in phenolics and flavonoids levels by DPS extract application indicating the activation of phenylpropanoid pathways, as evidenced by the increased activity and expression of *PAL*. The strong Pearson correlation depicted in Fig. 6 provided clear evidence of the observed findings. The activity of the PAL enzyme has been connected to the levels of anthocyanins and other phenolic compounds found in strawberry fruit. Additionally, the *StlA* gene from the bacterium *Photorhabdus luminescens*, which encodes a PAL enzyme, has been shown to play a role in the production of a stilbene-based antibiotic (Liu et al. 2021).

In order to validate the enhancement of flavonoid biosynthesis following DPS treatment, the relative expression of various genes encoding enzymes involved in flavonoid synthesis was assessed. The present results declare the eliciting potential of priming application by DPS extract, which enhances total phenolics and flavonoids in *Eruca vesicaria* leaves. Further, 80 g l⁻¹ DPS extract resulted in a significant improvement in *CHS*, *CHI*, and *FLS* expression genes relative to the control, which was prospectively related to their

actions and enzyme activities. The repression of CHS in *Solanum lycopersicum* was found to be a critical limiting agent in total flavonoid levels (Bovy et al. 2007). As similar with our results, increasing transcript levels of *CHI* were positively associated with flavonoid content in *A. thaliana* (Jiang et al. 2015), and tobacco plants (Liu et al. 2021). Another gene coded for the principal enzyme in flavonoid metabolites synthesis is *FLS*, which is essentially integrated as a rate-limiting enzyme in the synthesis of flavonols (Liu et al. 2021). *FLS* causes the desaturation of dihydroflavonols to produce flavonols such as kaempferol, quercetin, and myricetin (Lei et al. 2023). In accordance with our results, the increased expression of *FLS* accounted for the overproduction of kaempferol and quercetin in tobacco (Park et al. 2020). Therefore, the obtained results demonstrated the DPS's interesting ability to promote secondary metabolism pathways and thus the overproduction of bioactive compounds.

The results of the study revealed a noteworthy increase in the expression levels of the DXR gene when seeds were primed with an 80 g l⁻¹ DPS extract. This rise in DXR transcript levels likely resulted in an elevation in the functional protein and enzyme activity within the leaves of *Eruca vesicaria* plants. The findings were stayed by a significant Pearson correlation presented in Fig. 6, demonstrating a strong relationship between DXR expression level and the production of primary and secondary metabolites in plants. Previous studies have associated DXR overexpression with increased terpenoid production, highlighting DXR as a key regulator of terpenoid biosynthesis (Pan et al. 2015). In this context, overexpression of DXR has been shown to enhance the accumulation of *Mentha piperita* monoterpenes and essential oils by 50% (Gao et al. 2018).

5 Conclusion

This work introduces a pioneering use of date palm seeds (DPS) extract as an ecologically sustainable plant elicitor. Understanding the underlying mechanisms and optimizing elicitation conditions of date palm seeds extract was related to its phytocomponents. Its evoking impacts were manifested with *Eruca vesicaria* stimulated growth including its biomass, photosynthetic pigments, primary and secondary metabolites, thereby establishing it as a successful bio-elicitor. Notably, *Eruca* defense responses were induced by date palm seeds extract translated by overexpression of several secondary metabolism genes involved in the synthesis of phenylpropanoids, flavonoids, and terpenoids. Therefore, the stimulation and elicitation capacity of date palm seeds extract was demonstrated in *Eruca vesicaria* plants. However, additional research is required to comprehensively explore the inducing and fertilizing capacity of DPS.

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Data Availability The authors declare that the data presented in this manuscript is their own work. All the data collected in this experiment were given in the manuscript and supplementary data. The data would be made available on request to the corresponding author.

Declarations

Competing Interests The authors declare no competing interests.

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