Industrial Crops & Products 235 (2025) 121830

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

Ocium basilicum essential oil biostimulant activity and protective effects on cadmium-induced DNA damage and oxidative stress in *Raphanus sativus* L

Martina Dentato [a], Flavio Polito [b,1], Adriana Basile [a], Vincenzo De Feo [b], Elena De Marino [a], Alessia Postiglione [a,*], Viviana Maresca [c]

[a] *Department of Biology, University of Naples Federico II, Naples 80126, Italy*
[b] *Department of Pharmacy, University of Salerno, Fisciano 84084, Italy*
[c] *Department of Life Science, Health, and Health Professions, Link Campus University, Rome 00165, Italy*

ARTICLE INFO

Keywords:
Ocimum basilicum L.
Raphanus sativus L.
Essential oil
Antioxidant activity
DNA damage
Seed germination

ABSTRACT

The ability of the essential oil (EO) of the aerial parts of *Ocimum basilicum* L. cv 'Prospera' and its main constituents to reduce cadmium (Cd) toxicity in *Raphanus sativus* L. was tested. To study the tolerance induced by EO to Cd toxicity, the percentage of seed germination and hypocotyl-root length, DNA damage and antioxidant response were examined. The exogenous application of EO produces a recovery in the percentage of seed germination and hypocotyl-root length; it also reduces Cd-induced oxidative stress, as demonstrated by the reduction in the content of reactive oxygen species (ROS) and the increased activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione-S-transferase (GST). Furthermore, the application of EO produces a reduction in DNA damage. To investigate these promising results, both enzymatic activity and DNA damage were individually evaluated by testing the most common constituents in EO: endo-fenchol (21.5 %), eugenol (20.4 %) and carvacrol (10.2 %). These compounds replicated the antioxidant and genoprotective effects observed with the whole oil, with endo-fenchol and eugenol showing the strongest activity. Our findings clearly demonstrate that *O. basilicum* EO possesses a potent bioprotective effect against Cd-induced oxidative stress and genotoxicity, acting both through enzymatic activation and the inherent antioxidant properties of its major constituents. This study supports the use of basil EO as a natural, sustainable strategy to enhance plant tolerance to heavy metal stress, with promising applications in environmental and agricultural biotechnology.

1. Introduction

Over the last decades, heavy metal pollution has become a threat to the environment and human health (Mishra et al., 2019). In plant and animal cells, heavy metals have been reported to damage cellular organelles and components such as cell membrane, mitochondria, endoplasmic reticulum, and nuclei (Banfalvi, 2011). Metal ions have been found to interact with DNA and nuclear proteins, causing genotoxicity and negative conformational changes (Beyersmann and Hartwig, 2008). Furthermore, heavy metals can generate oxidative pressure in the plant cell, with the consequent toxicity. In this context, several antioxidant mechanisms have been observed in plants, such as changes in antioxidant activities for scavenging the Reactive Oxygen Species (ROS) in different compartments inside plant cells.

Cadmium (Cd) is an ecologically dangerous toxic metal that damages the health of all living organisms: plants, animals and humans. Cd targets several essential cellular components, including the cell membrane, mitochondria, endoplasmic reticulum, and nuclei. The first event that occurs when Cd enters the plant cell cytoplasm is a high accumulation of ROS, which can cause lipid peroxidation, protein oxidation, enzyme activity alteration, DNA damage and interact with other plant cell constituents (Genchi et al., 2020a). Consequently, plants growing in the presence of Cd show biochemical and physiological disorders such as growth inhibition, damage to membrane functions, alteration of ion homeostasis, decrease of water and nutrient transport, inhibition of photosynthesis, alteration of metabolism, altered activities of several

---

[*] Corresponding author.

E-mail addresses: martina.dentato@unina.it (M. Dentato), fpolito@unisa.it (F. Polito), adbasile@unina.it (A. Basile), defeo@unisa.it (V. De Feo), elena.demarino@unina.it (E. De Marino), alessia.postiglione@unina.it (A. Postiglione), v.maresca@unilink.it (V. Maresca).

[1] This author contributed equally to this work.

https://doi.org/10.1016/j.indcrop.2025.121830
Received 27 June 2025; Received in revised form 1 August 2025; Accepted 28 August 2025

key enzymes and even cell death (Li et al., 2023), therefore, with evident effects both at a morphological and physiological level (Shanying et al., 2017). Since Cd enters the food chain through plants, it can damage the health of all living organisms including humans and is therefore considered one of the most ecologically dangerous elements. Furthermore, it is interesting to determine how plants respond to Cd, but also, and perhaps above all, how it is possible to reduce the toxic effects induced by the metal on the health of plant organisms, especially those important for human nutrition. The use of bioprotective products on plants grown in soils polluted by metals is an important approach to mitigate the adverse effects of metal contamination and safeguard plant health. Bioprotective products or biostimulants, are substances derived from natural sources, such as microorganisms, plant extracts, which can help plants tolerate stress conditions, including metal toxicity. Certain plant extracts contain phytochemicals with metal-chelating, antioxidant, and detoxifying properties. These extracts can be used as bioprotective products to enhance plant tolerance to metal stress and mitigate the adverse effects of metal contamination on plant growth and development. Many plant essential oils (EOs) have already been tested for their potential antioxidant activity. *Ocimum basilicum* L., commonly called sweet basil, is an herb belonging to the Lamiaceae family, widely used as a culinary and medicinal plant. Native to India and other parts of Asia, it is now cultivated globally due to its versatility and rich phytochemical profile (Spence, 2024). In addition to its culinary use, ethnopharmacological evidence supports its traditional application in treating convulsions, diarrhea, epilepsy, gout, nausea, sore throat, toothache, bronchitis, and even cancer (Hamid et al., 2024). Experimental studies have shown that the EOs derived from *O. basilicum* contain biologically active constituents with notable antioxidant and antimicrobial properties (Mahendran and Vimolmangkang, 2023). However, the chemical composition of basil EO is not uniform and has been extensively investigated for its variation across cultivars and geographical regions. Basil oils are generally classified into four primary chemotypes, each associated with distinct regions and dominant constituents. The European type, grown in Europe, the United States, and parts of Africa, is rich in linalool and methyl chavicol. The Reunion type, commonly found in the Comoros and Seychelles Islands and Reunion Island, is characterized by a high concentration of methyl chavicol. The tropical type, native to India, Pakistan, Guatemala, Haiti, and some African regions, is dominated by methyl cinnamate. Lastly, a chemotype with eugenol as the main component is prevalent in North Africa, Eastern Europe, Russia, and parts of Asia (Chalchat et al., 1999). In addition to these major chemotypes, several basil EOs have been reported to contain varying proportions of camphor, linalool, eugenol, methyl chavicol, and methyl cinnamate, depending on environmental factors such as climate, soil composition, harvest time, and phenological stage (Ahmed et al., 2019). These chemical differences significantly influence the biological activities of each oil, including their antioxidant potential. For example, oils rich in eugenol or carvacrol may exhibit stronger radical scavenging and cytoprotective properties compared to those dominated by methyl chavicol or linalool. Given this variability, it is essential to characterize the chemical profile of basil EO from specific cultivars and origins to fully understand and optimize their biological application. Within the framework of our ongoing research on endemic Mediterranean plants (De Feo et al., 2003) and the bioactivity of essential oils (Maresca et al., 2023), we focused on the antioxidant properties of the EO extracted from the aerial parts of *O. basilicum* cv. ‘Prospera’, cultivated in Southern Italy. This cultivar is notable for its high content of endo-fenchol, eugenol, and carvacrol, compounds known for their potent antioxidant and genoprotective effects. In this study, we aimed to evaluate the ability of *O. basilicum* EO and its main constituents to mitigate cadmium (Cd)-induced toxicity in Raphanus sativus L. Specifically, we assessed their impact on seed germination, hypocotyl-root axis length, reactive oxygen species (ROS) levels, antioxidant enzyme activity, and DNA integrity under Cd stress, to determine their protective potential in enhancing plant resilience.

2. Materials and methods

2.1. Plant material

Ocimum basilicum cv ‘Prospera’ was grown at the Caselle farm located in Pontecagnano (Salerno, Italy), 40°38′40″ N, 14°52′34″ E, 34 m asl, and harvested in June 2022. A voucher specimen of the plant is stored in the herbarium of the Medical Botany Chair at the University of Salerno, labelled as DF/2022/278.

2.2. Extraction of the essential Oil

Two kg of fresh aerial parts were subjected to hydrostillation for 2 h, following the method reported in the European Pharmacopoeia (Council of Europe, 2020) with a 0.01 % yield. The EO was dried over anhydrous Na_2SO_4 and stored in sealed vials under nitrogen headspace in the dark, at 4 °C.

2.3. Cadmium and essential oils treatments on R. sativus L.

R. sativus L. seeds were surface sterilized using 70 % ethanol (2 min) and 2 % NaClO with the addition of a few drops of Triton X-100 (Sigma-Aldrich Co., St Louis, MO, US) (5 min) and, subsequently, washed (10 min) with sterile distilled water. Seeds were exposed to treatments including Cd alone, EO alone, Cd+EO, and individual EO components, ensuring controlled comparisons. Incubation occurred in germination chambers, and parameters were measured after 3–7 days. Then, i) 20 seeds were soaked with solutions containing only water (control), ii) 20 seeds were soaked with solutions containing only the essential oils at the two concentrations (0.16 and 0.4 % (v/v)), iii) 20 seeds soaked with solutions only cadmium (1.5 mM $CdCl_2$), iv) 20 seeds soaked with the combination of Cd and essential oil, v) 20 seeds soaked with endo-fenchol, eugenol and carvacrol, vi) 20 seeds soaked with the combination of Cd and eugenol, carvacrol, fenchol for 24 h. Successively closed inside Petri dishes equipped with a Whatman filter (11 cm in diameter) saturated with 5 mL of distilled water. All Petri dishes were then incubated for seven days in the dark in a germination chamber at 20 ± 1 ∘C and hydrated with spray every two days with the corresponding solutions of imbibition treatment prepared by dissolving 5 % dimethyl sulfoxide (DMSO) (PanReac AppliChem, Barcelona, Spain) in water containing a surfactant Tween 20. At the end of the experiments, the treated and untreated samples (control) were used for the analysis.

2.4. Seed germination and hypocotyl-root length

Observations were made using a binocular (M3Z binocular from WILD, Heerbrugg, Switzerland). Seedlings were measured using a calibrated, square graticule eyepiece. *R. sativus* seed germination percentages were evaluated by examining 20 seeds per replicate at 3 days, and hypocotyl-root length was measured on 15 roots per replicate at 7 days after inoculation (Basile et al., 2011).

2.5. Detection of ROS and antioxidant activity enzyme

The samples of *R. sativus* for each treatment were homogenized, to obtain a total extract, with PBS buffer solution (Sigma-Aldrich Co., St Louis, MO, US) (0.1 mL of 50 mM, pH 7.4) using a sterile pestle and mortar. The extract was used to evaluate the levels of ROS and the activity of the antioxidant enzymes.

To ROS, homogenates were transferred to a 96-well plate, incubated with 5 μM H_2DCFDA for 30 min at 37 ± 1 °C and analyzed using a with an automatic plate reader. ROS quantity was monitored by fluorescence (excitation wavelength of 350 nm and an emission wavelength of 600 nm).

CAT (Sigma-Aldrich Co., St Louis, MO, US), SOD (19160, Sigma-Aldrich Co., St Louis, MO, US), and GST (CS0410, Sigma-Aldrich Co., St Louis, MO, US) were evaluated following the kit protocols. The level of ROS and the antioxidant activity enzyme was detected using a microplate reader (Bio-Rad Laboratories Inc., Hercules, CA, USA). For each sample, 3 replicates were performed.

2.6. Comet assay

The hypocotyl roots of *R. sativus* for each treatment were sliced using a fresh razor blade. The plate was kept tilted on ice so that the isolated nuclei would collect in a cold Tris buffer. The protocol was performed as reported by Maresca et al. (2015). After treatment, approximately 150 mg of plant tissue was placed in a 60-mm Petri dish on ice with 1.5 mL of cold 400 mM Tris buffer (pH 7.5), gently sliced with a fresh razor blade, and the dish was kept tilted to allow nuclei to collect in the buffer. The suspension was filtered through a 20 µm nylon mesh to remove debris. Equal volumes (500 µL) of the nuclear suspension and 1 % low melting point (LMP) agarose (in PBS, 37°C) were mixed, and 80 µL was pipetted onto microscope slides pre-coated with 1 % normal melting point (NMP) agarose. Slides were covered with coverslips and placed on ice for 5 min. Coverslips were then removed, and slides were transferred to a horizontal electrophoresis tank containing cold alkaline buffer (1 mM Na_2EDTA, 300 mM NaOH, pH >13). The nuclei were incubated for 15 min to allow the DNA to unwind prior to electrophoresis at 0.72 V/cm (26 V, 300 mA) for 5 min at 4°C.

A fluorescence microscope was used to examine the slides, analysing a minimum of 50 randomly selected nuclei from each slide and avoiding overlapping figures. A computerized image-analysis system (Comet-Score) was employed. Twenty-five nuclei were scored per slide, three slides were evaluated per treatment and each treatment was repeated at least twice. From the repeated experiments, DNA damages, Tail moment (tail length times percentage of DNA in the tail), and the Olive moment (distance between the centers of the head and tail times the rate of DNA in the tail) from each slide were calculated.

2.7. Statistical analysis

ROS production, SOD, CAT, GST enzyme activities, seed germination and hypocotyl-root length were examined by one-way analysis of variance (ANOVA) and Tukey's test. In all the figures, values are presented as mean ± st. err; numbers not accompanied by the same letter are significantly different at $P < 0.05$. Statistical software was used to analyze all data (StatSoft 7.0, Tulsa, OK, USA)

3. Results

3.1. Composition of the essential oil

The results of the characterization of the essential oil were published by Landi et al. (2025). The most abundant components were endo-fenchol (21.5 %) and eugenol (20.4 %). Many literature studies reported the chemical composition of the EO from different basil varieties and cultivars, but only few studies reported the composition of the EO of the cv 'Prospera'. Our results largely agree with the terpene amounts found in the 'Prospera' grown in Israel, in which eugenol was the main constituent (Birenboim et al., 2022). However, the chemical composition of the basil EO depends on both exogenous and endogenous factors, first the harvesting period and the phenological stage of the plant.

3.2. Seed germination and hypocotyl-root length

The percentage germination and hypocotyl-root axis length on *R. sativus* were evaluated in the control samples (Ctrl), in samples exposed to treatment with $CdCl_2$ (Cd), in samples treated with EO at two concentrations 0.16 % (EO1) and 0.4 % (EO2) (v/v) and exposed to $CdCl_2$ and EO treatment (CdEO1 and CdEO2) (Table 1). Percentage germination and hypocotyl-root axis length decreased dramatically in Cd samples compared to Ctrl. In samples EO1 and EO2 percentage germination and hypocotyl-root axis length are comparable to Ctrl. Instead, a decrease is observed in the CdEO1 and CdEO2 samples compared to the Cd samples.

Table 1

Percentage germination (at 3 days) and hypocotyl-root axis length (cm) (at 7 days) in *R. sativus* in the control samples (Ctrl), in samples without treatment with EO and exposed to treatment with $CdCl_2$ (Cd), in samples treated with EO at two concentration 0.16 % and 0.4 % (v/v) and without treatment with $CdCl_2$ (EO1 and EO2), in samples treated with EO at two concentration 0.16 % and 0.4 % (v/v) and exposed to $CdCl_2$ treatment (CdEO1 and CdEO1). Bars not accompanied by the same letter (a–d) were significantly different at $p < 0.05$. Data are mean of three independent experiments ± SE ($n = 5$).

Treatment	Seed germination (%)	hypocotyl-root axis length (cm)
Ctrl	55.0 ± 1.7^{a}	7.0 ± 1.1^{a}
Cd	13.2 ± 0.8^{b}	2.5 ± 0.9^{b}
EO1	58 ± 1.5^{a}	6.8 ± 1.7^{a}
EO2	56.2 ± 2.0^{a}	7.2 ± 1.2^{a}
CdEO1	36.4 ± 1.3^{c}	3.2 ± 1.4^{c}
CdEO2	43.2 ± 1.9^{d}	5.8 ± 1.0^{d}

3.3. Detection of ROS and antioxidant activity enzyme

The antioxidant response in *R. sativus* was assessed by quantifying Reactive Oxygen Species (ROS) levels and the activity of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione S-transferase (GST). The treatments included: (i) control samples (Ctrl); (ii) samples exposed to $CdCl_2$ without essential oil (EO) treatment (Cd); (iii) samples treated with EO at two concentrations, 0.16 % and 0.4 % (v/v), without $CdCl_2$ exposure (EO1 and EO2); and (iv) samples treated with EO at the same concentrations and exposed to $CdCl_2$ (CdEO1 and CdEO2) (Fig. 1).

Exposure to Cd significantly increased ROS levels in *R. sativus* compared to the control ($p < 0.05$), confirming the pro-oxidant effect of Cd stress. Treatment with basil essential oil at two concentrations (EO1 and EO2) did not elevate ROS and maintained values statistically comparable to the control group ($p > 0.05$). Notably, co-treatment with Cd and EO (CdEO1 and CdEO2) significantly reduced ROS levels compared to Cd alone ($p < 0.05$), although levels remained higher than in the control. A dose-dependent trend was observed, with CdEO2 producing the most pronounced ROS reduction.

The activity of antioxidant enzymes also responded to Cd and EO treatments. CAT activity was significantly induced by Cd ($p < 0.05$) and further increased under EO treatments, particularly in CdEO2, suggesting enhanced enzymatic defense. SOD activity followed a similar trend: Cd triggered a sharp increase, and EO co-treatments (CdEO1, CdEO2) maintained elevated but significantly lower levels than Cd alone, implying partial alleviation of oxidative stress. GST activity also peaked in Cd-treated plants, while EO and Cd+EO treatments significantly attenuated this response, especially at the higher EO concentration.

Together, these results indicate that basil EO contributes to redox homeostasis by lowering ROS and modulating antioxidant defenses, with CdEO2 demonstrating superior efficacy.

To dissect the contribution of individual EO constituents, plants were treated with endo-fenchol (EF), eugenol (EU), and carvacrol (CA), with or without Cd co-exposure (Fig. 2).

Cd treatment alone significantly elevated ROS levels compared to the control ($p < 0.05$), whereas EF, EU, and CA alone had no such effect, maintaining ROS at control-like levels. Co-treatments (Cd-EF, Cd-EU, Cd-CA) significantly lowered ROS compared to Cd alone ($p < 0.05$), with Cd-EF and Cd-EU being the most effective.

For CAT, SOD, and GST activity, Cd exposure consistently induced a marked increase. Treatment with EO constituents alone led to slight to moderate increases in enzyme activity, likely due to mild stress

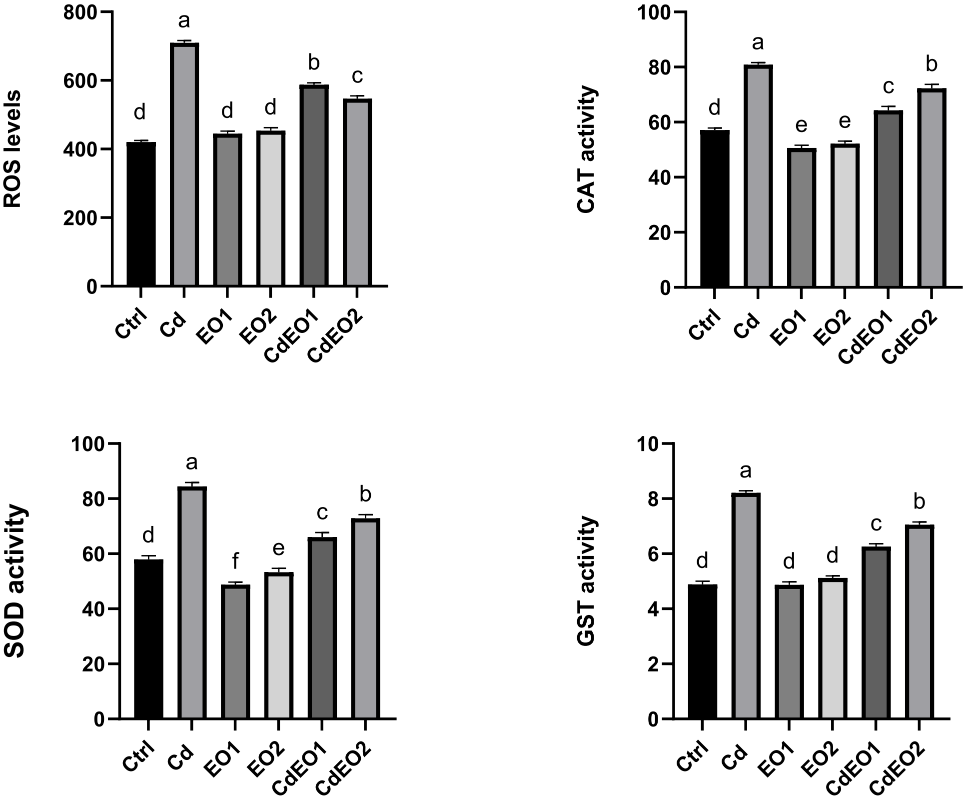

Fig. 1. ROS production (fluorescence intensity) and antioxidant/detoxifying enzyme activities SOD (activity %), CAT (U/mg of protein), and GST (μmol/mL/min) in *R. sativus* in the control samples (Ctrl), in samples without treatment with EO and exposed to treatment with $CdCl_2$ (Cd), in samples treated with EO at two concentration 0.16 % and 0.4 % *(v/v)* and without treatment with $CdCl_2$ (EO1 and EO2), in samples treated with EO at two concentration 0.16 % and 0.4 % *(v/v)* and exposed to $CdCl_2$ treatment (CdEO1 and CdEO1). Bars not accompanied by the same letter (a–d) were significantly different at $p < 0.05$. Data are mean of three independent experiments ± SE (n = 5).

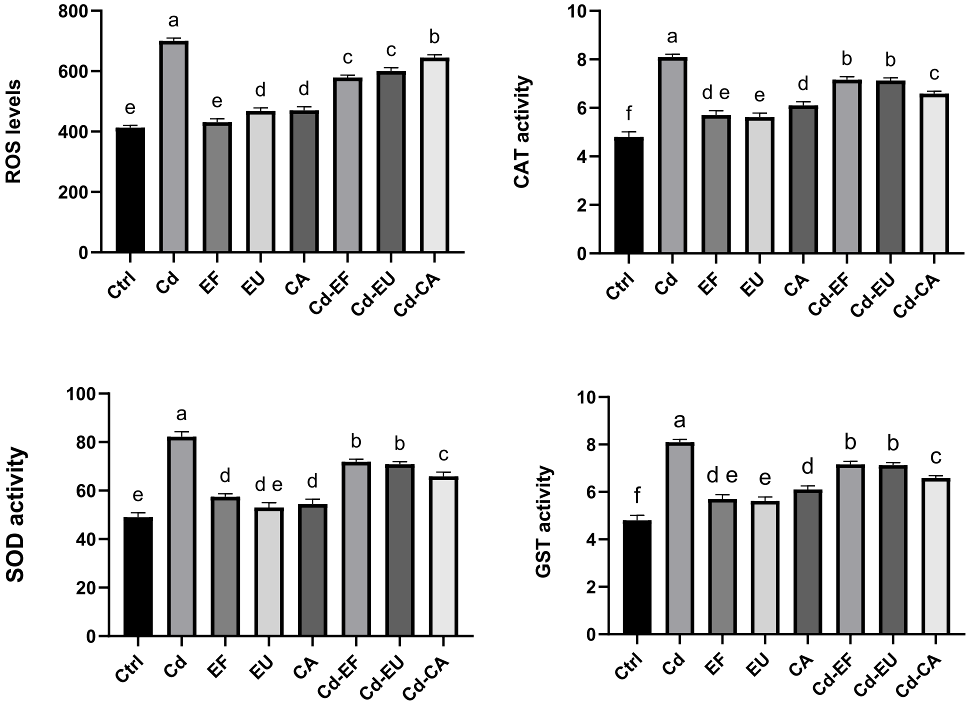

Fig. 2. ROS production (fluorescence intensity) and antioxidant/detoxifying enzyme activities SOD (activity %), CAT (U/mg of protein), and GST (μmol/mL/min) in *R. sativus* in the control samples (Ctrl), in samples without treatment with endo-fencol (EF), eugenol (EU) and carvacrol (CA) and exposed to $CdCl_2$ (Cd), in samples treated with endo-fencol, eugenol and carvacrol and without $CdCl_2$, in samples treated with endo-fencol, eugenol and carvacrol and exposed to $CdCl_2$. Bars not accompanied by the same letter (a–d) were significantly different at $p < 0.05$. Data are mean of three independent experiments ± SE (n = 5).

signaling. However, co-treatments with Cd and EO components significantly reduced enzyme activity compared to Cd alone, particularly with Cd-EF and Cd-EU, supporting their role in mitigating oxidative stress.

Overall, these findings reveal that all three EO components possess antioxidant-modulating properties, with endo-fenchol and eugenol offering the most effective protection. Their ability to reduce ROS levels and moderate antioxidant enzyme activity under Cd stress underscores their role as key contributors to the bioprotective action of basil EO.

3.4. Comet assay

To assess the genoprotective effects of *O. basilicum* EO and its main bioactive constituents, a comet assay was performed to evaluate DNA integrity in *R. sativus* subjected to Cd exposure.

The effect of whole EO (Fig. 3), Cd exposure caused a significant increase ($p < 0.05$) in all comet assay parameters (% DNA in tail, Olive moment, and Tail moment) compared to the control, confirming the genotoxic effects of Cd. In contrast, plants treated with EO alone (EO1 and EO2) showed values comparable to the control, indicating that EO itself did not induce DNA damage.

Co-treatment with Cd and EO (CdEO1 and CdEO2) resulted in a significant reduction in DNA damage across all parameters compared to Cd-only samples ($p < 0.05$), though values remained slightly above control levels. No significant differences were observed between CdEO1 and CdEO2 treatments, suggesting that both EO concentrations exert a protective effect against Cd-induced genotoxicity.

To identify which EO components contributed most to the observed protective effect, the comet assay was repeated using the individual compounds EU, CA, and FE (Fig. 4).

Cd treatment again caused a significant elevation in DNA damage markers. Treatments with EU, CA, or EF alone produced values similar to the control group, confirming that none of the individual compounds had genotoxic effects.

Notably, co-treatment with Cd and EO constituents (Cd-EU, Cd-CA, Cd-EF) significantly reduced DNA damage compared to Cd alone ($p < 0.05$). Among the treatments, Cd-EF and Cd-CA were the most effective in reducing % DNA in tail, Olive moment, and Tail moment values, bringing them closer to control levels. These results suggest a strong genoprotective role for endo-fenchol and carvacrol, likely due to their antioxidant properties and relative abundance in the EO composition.

4. Discussion

The findings of this study highlight the bioprotective potential of *O. basilicum* L. cv. 'Prospera' essential oil (EO) and its major constituents (endo-fenchol, eugenol, and carvacrol) in mitigating cadmium (Cd)-induced toxicity in *R. sativus* L. Notably, EO application significantly improved seed germination and hypocotyl root development, traits typically suppressed under Cd stress. This improvement is likely due to EO's ability to modulate oxidative stress responses and enhance cellular resilience.

One of the key protective mechanisms is the observed reduction in reactive oxygen species (ROS) levels. Cd stress is known to cause excessive ROS production, leading to oxidative damage and cellular dysfunction (Genchi et al., 2020b; Demidchik, 2015). While co-treatments with EO (CdEO1 and CdEO2) did activate antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione S-transferase (GST), their activation levels were lower than those in Cd-only treatments. This suggests that the reduction in ROS is only partially due to enzymatic activity and likely also involves the intrinsic antioxidant properties of basil EO (Grondona et al., 2014; Romano et al., 2022).

Cd exposure is also associated with DNA damage via oxidative stress, resulting in mutations and impaired cellular function (Demidchik, 2015; Saha et al., 2017). In this study, EO treatment reduced DNA damage, indicating a role in maintaining genomic integrity and further supporting its genoprotective effects. The comet assay confirmed that CdEO2, representing the highest EO concentration, was most effective in reducing genotoxic damage.

This protective effect was validated by analyzing the EO's major bioactive components. Endo-fenchol, eugenol, and carvacrol each significantly reduced oxidative and genotoxic stress compared to Cd alone. Endo-fenchol and eugenol were particularly effective, possibly due to their higher relative abundance in the EO (Naghdi Badi et al., 2017; Diniz do Nascimento et al., 2020).

Within the field of agricultural sciences, interest in natural compounds with bioprotective, antioxidant, and biostimulant properties is growing. Heavy metals such as Cd continue to pose a significant threat to crop productivity due to their capacity to induce oxidative damage, reduce biomass, and impair yield (Maresca et al., 2018). Cd induces ROS via several mechanisms: it displaces iron in proteins, leading to Fenton-type reactions (Jomova and Valko, 2011); it reduces glutathione (GSH) levels by stimulating phytochelatins (PC) synthesis (Goncharuk and Zagoskina, 2023) and it inhibits antioxidant enzymes while activating ROS-generating enzymes like NADPH oxidase (Jomova and Valko, 2011; Demidchik, 2015). ROS-related DNA damage, including double-strand breaks, further exacerbates Cd toxicity.

Cd contamination from anthropogenic sources can affect plant development throughout the life cycle, impacting germination and root biomass production (Saha et al., 2017; Carvalho et al., 2023; Asgharipour et al., 2011). In this context, the use of natural compounds,

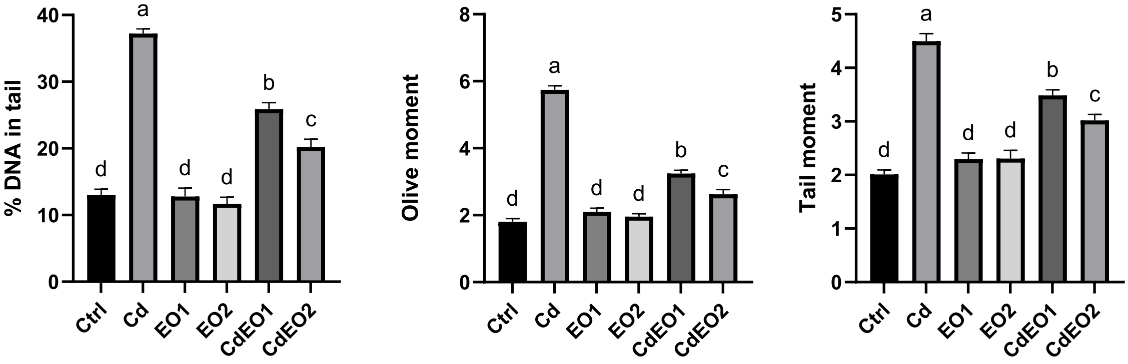

Fig. 3. Comet assay results, DNA damage, Olive moment, and Tail moment in *R. sativus* in the control samples (Ctrl), in samples without treatment with EO and exposed to $CdCl_2$ (Cd), in samples treated with EO at two concentration 0.16 and 0.4 % (v/v) and without $CdCl_2$ (EO1 and EO2), in samples treated with EO at two concentration 0.16 and 0.4 % (v/v) and exposed to $CdCl_2$ (CdEO1 and CdEO2). Bars not accompanied by the same letter (a–d) were significantly different at $p < 0.05$. Data are mean values of three independent experiments ± SE ($n = 5$).

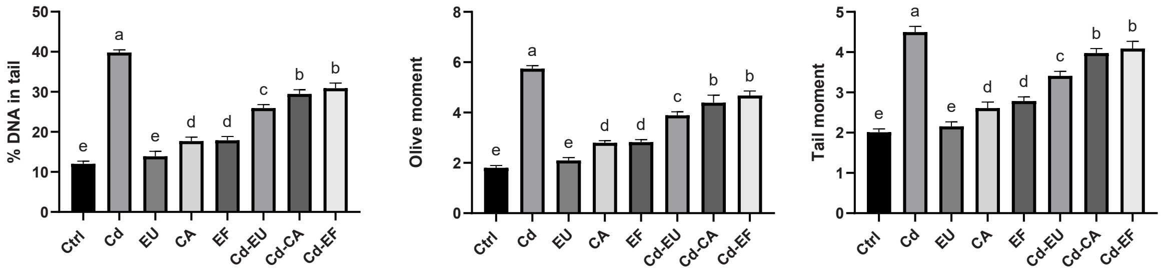

Fig. 4. Comet assay results, DNA damage, Olive moment, and Tail moment in *R. sativus* in the control samples (Ctrl), in samples without treatment with endo-fencol (EF), eugenol (EU) and carvacrol (CA) and exposed to $CdCl_2$ (Cd), in samples treated with endo-fencol, eugenol and carvacrol and without $CdCl_2$, in samples treated with eugenol endo-fencol, eugenol and carvacrol to $CdCl_2$.

especially plant-derived secondary metabolites, is being explored as a strategy to enhance plant growth and mitigate stress from abiotic and biotic factors (Mrid et al., 2021a). Basil EO, along with other Lamiaceae essential oils, is gaining attention for its potential to alleviate heavy metal toxicity in plants (Grondona et al., 2014). Our results confirm that basil EO acts as a biostimulant and bioprotector, promoting root development and reducing Cd-induced toxicity in *R. sativus*.

Unlike many studies that focus on aqueous extracts or microbial metabolites (Szparaga, 2023; Kisiriko et al., 2021; Mrid et al., 2021b), this work demonstrates that essential oils, particularly from basil, are a promising but underexplored resource. Basil EO contains bioactive compounds with known antioxidant capacity capable of modulating the activity of endogenous antioxidant enzymes (Romano et al., 2022). Compounds such as fenchone, eugenol, and carvacrol have been shown to reduce lipid peroxidation and protect DNA from oxidative damage (Naghdi Badi et al., 2017; Diniz do Nascimento et al., 2020; Kousar et al., 2023; Xu et al., 2024; Rezaie et al., 2020).

Furthermore, this study supports the applicability of the comet assay in plant systems. Although traditionally used in animal cells, it effectively revealed DNA damage and its reduction following EO treatments, highlighting its utility in plant genotoxicity research. The significant reduction in DNA strand breaks in EO-treated *R. sativus* confirms EO's genoprotective role.

In conclusion, basil EO demonstrates promising antioxidant and genoprotective properties against Cd-induced stress. Its bioactive components effectively reduce ROS and DNA damage, restore redox balance, and promote plant growth. Future research should focus on elucidating the molecular mechanisms underlying these effects and evaluating EO efficacy in vivo, particularly in phytoremediation applications. The integration of such bioprotective strategies into agricultural practices represents a sustainable and eco-friendly approach to improving plant resilience and productivity in metal-contaminated environments. Careful selection and assessment of these products based on plant specificity and environmental impact will be essential for their successful application.

5. Conclusion

This study demonstrated that the essential oil of *Ocimum basilicum* cv. 'Prospera' and its main constituents, endo-fenchol, eugenol, and carvacrol, can effectively mitigate cadmium-induced oxidative and genotoxic stress in *Raphanus sativus*. The treatments significantly improved seed germination, promoted root development, reduced ROS levels, enhanced antioxidant enzyme activity, and decreased DNA damage. Among the tested constituents, endo-fenchol and eugenol showed the strongest protective effects. These findings support the potential application of basil essential oil as a natural, eco-friendly biostimulant and bioprotective agent in agriculture, particularly in environments contaminated by heavy metals. Natural products, particularly plant extracts, therefore represent the main source of bioactive compounds. In addition to these, the usefulness of animal-derived compounds has also been established in recent decades (Amin et al., 2022). Further research is encouraged to explore the molecular mechanisms involved and to evaluate the field-level applicability of these natural compounds.

CRediT authorship contribution statement

Alessia Postiglione: Writing – review & editing, Visualization, Validation, Supervision, Investigation, Conceptualization. **Elena De Marino:** Investigation. **Viviana Maresca:** Writing – review & editing, Validation, Supervision, Project administration, Investigation, Formal analysis, Conceptualization. **Martina Dentato:** Writing – original draft, Software, Methodology, Data curation. **Flavio Polito:** Writing – original draft, Software, Methodology, Data curation. **Vincenzo De Feo:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Formal analysis. **Adriana Basile:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Funding

This research was funded by MISE project titled: LIFECYCLE INDUSTRIAL MANAGER BIOSENSOR BASED CUP: B69J23000720005.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

References

Ahmed, A.F., Attia, F.A., Liu, Z., Li, C., Wei, J., Kang, W., 2019. Antioxidant activity and total phenolic content of essential oils and extracts of sweet basil (Ocimum basilicum L.) plants. Food Sci. Hum. Well 8 (3), 299–305.

Amin, B.H., Amer, A., Azzam, M., Abd El-Sattar, N.E., Mahmoud, D., Al-Ashaal, S., Hozzein, W.N., 2022. Antimicrobial and anticancer activities of periplaneta americana tissue lysate: an in vitro study. J. King Saud. Univ. Sci. 34 (5), 102095.

Asgharipour, M.R., Khatamipour, M., Razavi-Omrani, M., 2011. Phytotoxicity of cadmium on seed germination, early growth, proline and carbohydrate content in two wheat varieties. Adv. Environ. Biol. 5 (4), 559–565.

Banfalvi, G., 2011. Cellular Effects of Heavy Metals. Springer, Dordrecht, The Netherlands, pp. 3–28.

Basile, A., Cobianchi, R.C., Rigano, D., Senatore, F., Bruno, M., Rosselli, S., Sorbo, S., 2011. Potential allelopathic activity of sideritis italica (Miller) greuter et burdet essential oil. Plant Biosyst. 145 (1), 241–247.

Beyersmann, D., Hartwig, A., 2008. Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms. Arch. Toxicol. 82 (8), 493–512. https://doi.org/10.1007/s00204-008-0313-y.

Birenboim, M., Chalupowicz, D., Maurer, D., Barel, S., Chen, Y., Falik, E., Shimshoni, J. A., 2022. Optimization of sweet basil harvest time and cultivar characterization using near-infrared spectroscopy, liquid and gas chromatography, and chemometric statistical methods. J. Sci. Food Agric. 102 (8), 3325–3335.

Carvalho, M.E., Agathokleous, E., Nogueira, M.L., Brunetto, G., Brown, P.H., Azevedo, R. A., 2023. Neutral-to-positive cadmium effects on germination and seedling vigor, with and without seed priming. J. Hazard. Mater. 448, 130813.

Chalchat, J.C., Garry, R.P., Sidibé, L., Harama, M., 1999. Aromatic plants of Mali (I): chemical composition of essential oils of ocimum basilicum L. J. essent. Oil Res. 11 (3), 375–380.

Council of Europe, 2020. European Pharmacopoeia, tenth ed. Council of Europe, Strasbourg, France.

De Feo, V., Bruno, M., Tahiri, B., Napolitano, F., Senatore, F., 2003. Chemical composition and antibacterial activity of the essential oils of thymus spinulosus ten. (Lamiaceae). J. Agric. Food Chem. 51, 3849–3853.

Demidchik, V., 2015. Mechanisms of oxidative stress in plants: from classical chemistry to cell biology. Environ. Exp. Bot. 109, 212–228.

Diniz do Nascimento, Lidiane, et al., 2020. Bioactive natural compounds and antioxidant activity of essential oils from spice plants: new findings and potential applications. Biomolecules 10 (7), 988.

Genchi, G., Sinicropi, M.S., Lauria, G., Carocci, A., Catalano, A., 2020a. The effects of cadmium toxicity. Int. J. Environ. Res. Public Health 17 (11), 3782.

Genchi, G., Sinicropi, M.S., Lauria, G., Carocci, A., Catalano, A., 2020b. The effects of cadmium toxicity. Int. J. Environ. Res. Public Health 17 (11), 3782.

Goncharuk, Evgenia A., Zagoskina, Natalia V., 2023. "Heavy metals, their phytotoxicity, and the role of phenolic antioxidants in plant stress responses with focus on cadmium. Molecules 28 (9), 3921.

Grondona, E., Gatti, G., López, A.G., Sánchez, L.R., Rivero, V., Pessah, O., Ponce, A.A., 2014. Bio-efficacy of the essential oil of oregano (Lamiaceae. Ssp. Hirtum). Plant Foods Hum. Nutr. 69, 351–357.

Hamid, S., Oukil, N.F., Moussa, H., Djihad, N., Mróz, M., Kusznierewicz, B., Chebrouk, F., 2024. Chemical and biological characterization of ocimum basilicum L. Phenolic extract and essential oil derived through ultrasound and microwave-assisted extraction techniques. Food Biosci. 60, 104359.

Jomova, K., Valko, M., 2011. Advances in metal-induced oxidative stress and human disease. Toxicology 283 (2-3), 65–87.

Kisiriko, M., Anastasiadi, M., Terry, L.A., Yasri, A., Beale, M.H., Ward, J.L., 2021. Phenolics from medicinal and aromatic plants: characterisation and potential as biostimulants and bioprotectants. Molecules 26 (21), 6343.

Kousar, M.U., Jabeen, A., Fatima, T., Hussain, S.Z., Zargar, I.A., Amin, T., Yaseen, M., 2023. Optimization of ultrasonic-assisted extraction of eugenol-rich fraction from basil leaves: characterization of extract for phenols, flavonoids and antioxidant activity. Food Chem. Adv. 3, 100374.

Landi, S., Polito, F., Esposito, S., Sorbo, S., Cianciullo, P., Postiglione, A., Maresca, V., 2025. Protective effect of ocimum basilicum L. Essential oil on lactuca sativa L. Treated with cadmium. Plant Physiol. Biochem. 219, 109437.

Li, Y., Rahman, S.U., Qiu, Z., Shahzad, S.M., Nawaz, M.F., Huang, J., Cheng, H., 2023. Toxic effects of cadmium on the physiological and biochemical attributes of plants, and phytoremediation strategies: a review. Environ. Pollut. 325, 121433.

Mahendran, G., Vimolmangkang, S., 2023. Chemical compositions, antioxidant, antimicrobial, and mosquito larvicidal activity of ocimum americanum L. And ocimum basilicum L. Leaf essential oils. Complement. Med. Ther. 23 (1), 390.

Maresca, V., Badalamenti, N., Ilardi, V., Bruno, M., Basile, A., 2023. The antioxidant properties and protective capacity of prangos trifida and cachrys cristata essential oils against cd stress in lunularia cruciata and brassica napus. Antioxidants 12 (4), 793.

a) Maresca, V., Fusaro, L., Sorbo, S., Siciliano, A., Loppi, S., Paoli, L., Basile, A., 2018. Functional and structural biomarkers to monitor heavy metal pollution of one of the most contaminated freshwater sites in Southern Europe. Ecotoxicol. Environ. Saf. 163, 665–673.b) Shahid, M., Khalid, S., Abbas, G., Shahid, N., Nadeem, M., Sabir, M., Dumat, C., 2015. Heavy metal stress and crop productivity. CPGEI 1–25.

Maresca, V., Fusaro, L., Sorbo, S., Siciliano, A., Loppi, S., Paoli, L., Basile, A., 2018. Functional and structural biomarkers to monitor heavy metal pollution of one of the most contaminated freshwater sites in Southern Europe. Ecotoxicol. Environ. Saf. 163, 665–673.

Mishra, S., Bharagava, R.N., More, N., Yadav, A., Zainith, S., Mani, S., Chowdhary, P., 2019. Heavy metal contamination: an alarming threat to environment and human health. Environ. Biotechnol. 103–125.

Mrid, R.B., Benmrid, B., Hafsa, J., Boukcim, H., Sobeh, M., Yasri, A., 2021a. Secondary metabolites as biostimulant and bioprotectant agents: a review. Sci. Total Environ. 777, 146204.

Mrid, R.B., Benmrid, B., Hafsa, J., Boukcim, H., Sobeh, M., Yasri, A., 2021b. Secondary metabolites as biostimulant and bioprotectant agents: a review. Sci. Total Environ. 777, 146204.

Naghdi Badi, H.A., et al., 2017. An overview on two valuable natural and bioactive compounds, thymol and carvacrol, in medicinal plants. J. Med. Plants 16 (63), 1–32.

Rezaie, R., Abdollahi Mandoulakani, B., Fattahi, M., 2020. Cold stress changes antioxidant defense system, phenylpropanoid contents and expression of genes involved in their biosynthesis in Ocimum basilicum L. Sci. Rep. 10 (1), 5290.

Romano, R., De Luca, L., Aiello, A., Pagano, R., Di Pierro, P., Pizzolongo, F., Masi, P., 2022. Basil (Ocimum basilicum L.) leaves as a source of bioactive compounds. Foods 11 (20), 3212.

Saha, Jayanta K., et al., 2017. Soil Pollution-an Emerging Threat to Agriculture. Springer, Singapore.

Shanying, H.E., Xiaoe, Y.A.N.G., Zhenli, H.E., Baligar, V.C., 2017. Morphological and physiological responses of plants to cadmium toxicity: a review. Pedosphere 27 (3), 421–438. https://doi.org/10.1016/S1002-0160(17)60339-4.

Spence, C., 2024. Sweet basil: an increasingly popular culinary herb. J. Gastron Food Sci., 100927

Szparaga, A., 2023. From biostimulant to possible plant bioprotectant agents. Agric. Eng. 27 (1), 87–98.

Xu, J., Wang, T., Sun, C., Liu, P., Chen, J., Hou, X., Zhang, L., 2024. Eugenol improves salt tolerance via enhancing antioxidant capacity and regulating ionic balance in tobacco seedlings. Front. Plant. Sci. 14, 1284480.