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Exploring microalgae and endophyte as biostimulants: Antioxidant and anti-inflammatory properties of *Cannabis sativa* L. sprouts under standard and enrichment conditions

Andrea Cavallero [1], Elisa Chelucci [1], Carolina Chiellini, Morena Gabriele [*]

Institute of Agricultural Biology and Biotechnology, Italian National Research Council, Via Moruzzi 1, 56124, Pisa, Italy

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ABSTRACT

Sprouts from germinated seeds of edible herbaceous plants are richer in bioactive compounds and micronutrients than mature plants. In recent years, the consumption of sprouted seeds in diets and the interest in exploring vegetable sprout's potential beneficial effects on human health has increased. In this work, the five-day-old sprouts of *Cannabis sativa* L. cultivar "Futura 75" were investigated and characterized. Hemp sprouts were obtained by germination tests under standard and enrichment conditions using the microalgal strain *Chlorella* sp. C2 and the bacterium *Sphingomonas* sp. Can_S11. The biochemical properties of hemp sprouts were analyzed for total polyphenol and flavonoid content and antioxidant activities (DPPH, ABTS, FRAP, ORAC, and Fe^{2+}-chelating activity) by *in vitro* assays. A significant increase in phenolic compounds and greater antioxidant and anti-radical activities were observed for hemp sprouts obtained under enrichment conditions (dry microalgal biomass C2 and the bacterium Can_S11). Additionally, these sprouts and *C. sativa* seeds demonstrated enhanced protective effects on inflamed A549 cells, effectively mitigating the TNF-α induced changes and reducing the expression of IL-8 and COX-2. Overall, PGP (Plant Growth Promoting) microorganisms and microalgae represent a good strategy to promote the development of hemp sprouts and increase their phytochemical content, and antioxidant and anti-inflammatory activity.

1. Introduction

Sprouts derived from the germination of edible herbaceous plant seeds contain higher concentrations of bioactive compounds and micronutrients than their mature counterparts. In recent years, there has been a growing trend towards incorporating sprouted seeds into the human diet, driven by increasing interest in their potential health benefits (Benincasa et al., 2019). Indeed, sprouts are known for their high nutritional value (e.g., protein content), health-promoting bioactive compounds (e.g., polyphenols), micronutrients (e.g., carotenoids, vitamins C, B_1, B_2, B_6, E), and low anti-nutrients content than edible adult plants, making them promising functional foods with beneficial effects on human health (Aborus et al., 2017; Chiriac et al., 2020; Donkor et al., 2012; Gan et al., 2017; Jeong et al., 2018; Le et al., 2020; Mir et al., 2021). Additionally, it is known that germination is recognized to be a good alternative source of phytonutrients and bioactive compounds, promoting their bioavailability due to the well-documented composition changes related to the biochemical reactions involved in this process (Benincasa et al., 2019; Idowu et al., 2020). The latter can enhance nutritional and nutraceutical properties, increasing the consumption of sprouts in the human diet.

Among edible plants, hemp (*Cannabis sativa* L., belonging to the Cannabaceae family) is an annual herbaceous plant native to Central Asia. It has been primarily utilized for medical treatments, food, and fiber production (Frassinetti et al., 2018). Industrial hemp (*C. sativa* subsp. sativa) contains bioactive molecules, such as phenolic compounds and terpenes, while possessing a low level of the psychoactive compound D9-tetrahydrocannabinol (THC) compared to *C. sativa* subsp. Indica (less than 20% THC) (Andre et al., 2016). The nutritional potential of hemp can be further enhanced through the germination process, which has been shown to induce the production of anti-inflammatory compounds such as prenylflavonoids and cannflavins A and B (Werz et al.).

Techniques collectively called "elicitation" are employed to boost the

* Corresponding author.
E-mail address: morena.gabriele@cnr.it (M. Gabriele).
[1] Andrea Cavallero and Elisa Chelucci contributed equally to this work.

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levels of phytochemical compounds in sprouts. Elicitation refers to a specific response of plants to a stressor, known as the elicitor, which can be pathogens or other environmental factors. This response triggers certain biochemical pathways, leading to the synthesis of secondary metabolites (Szulc et al., 2024). These strategies are useful to induce physiological changes in plant species and to improve the phytochemical profile and biological activities of sprouts (Liu et al., 2019). Among these novel biostimulants, bacterial endophytes and microalgae, have shown promise as potential strategies for enhancing the sustainability and profitability of agricultural production (Liu et al., 2019).

The bacterial endophytes isolated from plants, known as "Plant Growth Promotion Bacteria" (PGPB), have been demonstrated to be a potential strategy to make agricultural production more sustainable and profitable, due to their ability to promote plant growth and increase the content of bioactive molecules (Liu et al., 2019). Indeed, bacterial endophytes can modulate plant phytohormone releases, such as cytokinins and gibberellins, necessary for plant survival and development. However, the most studied auxin produced by PGPB is the indole-3-acetic acid (IAA), which stimulates seed germination and growth processes and modulates metabolite production (Glick, 2012).

Moreover, microalgae are a good source of various nutrients, including carbohydrates, proteins, lipids, amino acids, and vitamins, as well as natural phytohormones like auxins and cytokinins. Microalgal biostimulants are administered to plants and soil via fertigation, spray application on leaves, or seed treatment to promote germination (Colla & Rouphael, 2020) and chlorophylls and carotenoids' release, increase the root system development and the nutrient uptake from the soil, and release organic compounds necessary to ameliorate the activity of other microorganisms that can benefit plants (Chiaiese et al., 2018; Colla & Rouphael, 2020).

Given the anti-inflammatory properties of hemp sprouts, as highlighted by its ability to produce compounds like prenylflavonoids and cannflavins, there is potential for its use in addressing chronic inflammatory diseases. One such disease is chronic obstructive pulmonary disease (COPD), a progressive respiratory disease characterized by an irreversible obstruction of the airways, caused by both genetic and environmental factors, primarily cigarette smoking. COPD is associated with oxidative stress and chronic inflammation of the lung tissue. To date, COPD represents one of the main causes of morbidity and mortality (Rajković et al., 2018) and drugs currently used can reduce the symptoms but do not allow a complete recovery. Among the possible candidates, there are natural extracts that, due to their anti-inflammatory potential, are already used e.g. for the treatment of acute or chronic bronchitis such as *Pelargonium sidoides* (Kocic et al., 2019) and *Echinacea purpurea* (Agbabiaka et al., 2008) extract. Instead, Li et al. (2021) demonstrated the efficacy of Chinese herbaceous extracts in significantly improving COPD in rats.

The nutritional properties of hemp have paved the way for its plant derivatives to be integrated into human nutrition as promising functional foods (Rehman et al., 2021). As observed by Anil et al. (2021) the extract derived from the inflorescences of *Cannabis sativa* reduced IL-6 and IL-8 levels in TNF-α inflamed A549 lung cells, which are associated with inflammation linked to COVID-19. Among its plant derivatives, the seeds of *Cannabis sativa* L. cv. Futura 75, known for being a significant source of biologically active compounds, has the potential to positively impact our health (Frassinetti et al., 2018). This suggests they may hold promise for treating the inflammatory component associated with COPD.

Therefore, this study aimed to evaluate the germination rate and the nutraceutical properties of *C. sativa* sprouted seeds, under standard and enrichment conditions. For the latter, the microalgal *Chlorella* sp. strain C2 was chosen to stimulate *Cannabis sativa* L. seeds germination, and the endophytic bacterium Can_S11 was selected for its remarkable ability to produce indoleacetic acid (Chiellini et al., 2022; Gabriele et al., 2022). Besides, the anti-inflammatory effect of seed and sprout extracts was evaluated on tumor necrosis factor-alpha (TNFα)-inflamed A549 human pulmonary cell line.

Table 1
Experimental groups and germination conditions of *Cannabis sativa* L. sprouts.

Group	Germination conditions
CTRL	Standard culture medium
BI	Can_S11 bacterial inoculum
BS	Can_S11 bacterial spread on the culture medium surface
C2 D	Dried microalgal C2 biomass added to the culture medium
C2 F	Seed imbibition in fresh microalgal C2 biomass before seeding
BI + C2 D	BI and C2 D combination
BI + C2 F	BI and C2 F combination
BS + C2 D	BS and C2 D combination
BS + C2 F	BS and C2 F combination

2. Materials and methods

2.1. Chemicals and reagents

Reagents and standards employed were of analytical grade. Agar, Folin-Ciocalteu reagent, saline solution (NaCl 0.9% *w/v*), sodium carbonate, ethylenediaminetetraacetic acid (EDTA), sodium acetate, potassium chloride, gallic acid, sodium hydroxide, quercetin dihydrate, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ), ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), ferrous sulfate heptahydrate ($FeSO_4{\cdot}7H_2O$), ferrozine, sodium nitrite ($NaNO_2$), aluminum chloride ($AlCl_3$), Tryptic Soy Broth (TSB), cell culture reagents, media, and medium supplements were purchased from Sigma-Aldrich (Saint Louis, MO, USA). Ethanol and methanol were supplied from VWR (Radnor, PA, USA).

2.2. Plant material and biostimulants

Cannabis sativa L. cultivar Futura 75 seeds were kindly offered by ASSOCANAPA, Carmagnola, Turin, Italy. The nutraceutical features of hemp seed were previously characterized (Frassinetti et al., 2018).

After three washes (5 min each) with sterile double-distilled water (bdH_2O), the hemp seeds were plated with sterile forceps in 100 mm Petri dishes (10 seeds/Petri dish). Agar (0.7%) culture medium was employed for the germination tests. The latter was carried out by sprouting seeds on the only standard culture medium, and by adding the dry and fresh biomass microalgal strain C2 cultured as described by Chiellini et al. (2022), and/or the *Sphingomonas* sp. strain Can_S11, isolated from the *Cannabis sativa* L. seeds and selected as a biostimulant for germination for the high IAA production and resistance to streptomycin, oxidative stress (H_2O_2), and water potential stress (PEG 6000) (Gabriele et al., 2022).

2.3. Hemp sprouts' germination

The germination tests were performed by placing with sterile forceps 10 *Cannabis sativa* L. cultivar Futura 75 seeds for each 100 mm Petri dish, in which the standard culture medium, prepared with distilled water supplemented with 0.7% agar, was previously added (CTRL). Nevertheless, the germination tests were conducted also upon enrichment conditions, consisting of the addition of the microalgae C2 and/or the bacterial endophyte Can_S11. To obtain the dried microalgal biomass C2, the fresh one was centrifugated at 2314×*g* (Jouan CR 31 centrifuge, Newport Pagnell, UK) for 10 min and maintained at 45 °C to dry; after that, it was pulverized with a sterile mortar and added (0.25 mg mL^{-1}) to the culture medium until its complete homogenization before pouring it into the Petri dishes. For treatment with fresh microalgal biomass, the seeds were soaked in the microalgal culture and gently shaken overnight at 25 °C before seeding. For the Can_S11 treatments, the bacterium was administered by adding 25 μL of bacterial

inoculum (corresponding to about 3×10^9 CFU mL^{-1}) around each seed (BI) or by bacterial spread on the culture medium surface (BS). The germination conditions selected are listed in Table 1. Germination tests were assessed to ensure maximum sterility, under the biological flow, avoiding any contamination. During germination time, seeds were exposed to a 16/08 h day-night cycle with PPFD of 70 μmol photons m^{-1} s^{-1} and maintained at a controlled temperature of 22 ± 2 °C until sprouts' collection on the fifth day according to Gabriele et al. (2022).

2.4. Morphological characterization and extraction of hemp sprouts

The hemp sprouts obtained from each germination condition were collected with sterile forceps after five days (T5). For the morphological characterization of hemp sprouts, the following parameters were evaluated: sprouted seeds' number, fresh weight (g), total sprout length (cm), and root and aerial part length (cm). To determine the nutraceutical content and the potential antioxidant capacity of five-day-old hemp sprouts, these were extracted as described by Frassinetti et al. (2018) with some modifications. Briefly, 10 mL of 80% ethanol were added to 2 g of freshly harvested sprouts; subsequently, these were homogenized by using Ultraturrax (Kinematica Polytron PT MR 2100, Eschbach, Germany), and kept in agitation in the dark overnight. The following day, the mixtures were centrifuged at 6620×*g* (Jouan CR 31 centrifuge, Newport Pagnell, UK) for 10 min, and the extracts were stored at - 20 °C in the dark until use. Hemp seeds were extracted using the same protocol.

2.5. Phytochemical profile of hemp sprouts' extracts

The phytochemical profile of five-day-old sprout extracts was evaluated by quantifying the total polyphenol (TPC) and flavonoid (TFC) content. TPC and TFC were carried out according to Gabriele et al. (2015). TPC valued as Folin–Ciocalteu (FC) reducing capacity was expressed as mg of gallic acid equivalents (GAE) g^{-1} fresh weight (FW). Briefly, 100 μL of each hemp sprouts extract or gallic acid (15.62–1000 μM) were mixed with 500 μL of FC reagent (0.2 N) and kept for 5 min in the dark. Subsequently, 400 μL of Na_2CO_3 (0.7 M) were added and, after 2 h of incubation in the dark at room temperature, the absorbance was measured at 760 nm (PerkinElmer UV/VIS Lambda 365, Waltham, MA, USA).

TFC was determined employing the aluminum chloride colorimetric method and reported as mg quercetin equivalents (QE) g^{-1} fresh weight (FW). Briefly, 200 μL of hemp sprout extracts or standard were added to 800 μL of distilled water and 60 μL of 5% $NaNO_2$. After 5 min of incubation at room temperature, 60 μL of 10% $AlCl_3$ were added. The reactions were neutralized with 400 μL of NaOH (1 M) and 480 μL of distilled water after 6 min of incubation. Following 30 min, the absorbance was read at 430 nm (PerkinElmer UV/VIS Lambda 365, Waltham, MA, USA).

2.6. In vitro antioxidant activities of hemp sprouts

2.6.1. $ABTS^{+}$ radical scavenging activity

The 2-2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) radical cation ($ABTS^{\cdot+}$) scavenging activity of hemp sprouts' extracts was assessed as described by Chelucci et al. (2023). Briefly, 190 μL of each extract were added to 1 mL of $ABTS^{\cdot+}$ solution, previously diluted in methanol to obtain an absorbance of 0.7 ± 0.02 at 734 nm (PerkinElmer UV/VIS Lambda 365, Waltham, MA, USA). After 10 min of incubation, the samples' absorbance was measured at 734 nm. The percentage $ABTS^{\cdot+}$ reduction was measured using the following formula: % $ABTS^{\cdot+}$ reduction = $[(A_i - A_f)/A_i] \times 100$, where A_i is the initial absorbance and A_f is the final absorbance. The $ABTS^{\cdot+}$ scavenging activity was reported as Trolox equivalent antioxidant capacity (TEAC) using a Trolox standard curve (5–1000 μg mL^{-1}).

2.6.2. $DPPH^{\bullet}$ antiradical activity

The $DPPH^{\bullet}$ antiradical activity of each hemp sprout extract was performed as reported by Chiellini et al. (2022). The results were expressed as the percentage of $DPPH^{\bullet}$ inhibition corresponding to the antiradical activity (ARA) and calculated as follows: ARA % = $[1 - (A_S/A_C)] \times 100$, where the A_S is the absorbance of the sample and the A_C is the absorbance of the DPPH solution.

2.6.3. Oxygen radical absorbance capacity (ORAC) assay

The Oxygen Radical Absorbance Capacity (ORAC) of hemp sprouts' extracts was carried out as described by Gabriele et al. (2015). The results were reported as ORAC units corresponding to micromoles of Trolox equivalents (TE)/g FW. Briefly, 10 mM fluorescein in Tris-HCl (pH 8) was employed as a probe, and 500 mM AAPH in 75 mM phosphate buffer pH 7.4 (PB) as the peroxyl radical generator. For each sample (100 μL), a solution containing 800 μL of fluorescein (40 nM) and 100 μL of AAPH (40 mM) was prepared, whose 200 μL were plated and the fluorescence decay was read at 485 nm excitation and 514 nm emission (VictorTM X3 Multilabel Plate Reader, Waltham, MA).

2.6.4. Ferric reducing antioxidant power (FRAP) assay

The FRAP assay describes the capacity of reducing ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) and it was assessed as previously described by Chelucci et al. (2023). Briefly, 2500 μL of FRAP buffer (300 mM acetate buffer pH 3.6, 10 mM TPTZ in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ at a ratio of 10:1:1) were added to 85 μL of hemp sprouts' extracts, and the absorbance was read at 593 nm (PerkinElmer UV/VIS Lambda 365, Waltham, MA, USA) after 30 min of incubation at room temperature. The relative data were reported as Fe^{2+} equivalents (μM), employing a calibration curve of $FeSO_4 \cdot 7H_2O$ (range: 15.62–2000 μM).

2.6.5. Fe^{2+} chelation ability

The ability of aqueous hemp sprout extracts (200 mg mL^{-1}) to chelate Fe^{2+} was evaluated by a colorimetric assay as previously reported (Chelucci et al., 2023). Briefly, 800 μL ultrapure water and 100 μL of $FeSO_4$ (0.3 mM) were mixed with 250 μL of each aqueous extract and kept 5 min at room temperature. Subsequently, 150 μL of ferrozine (0.8 mM) were added to the solution, after an incubation of 5min at room temperature. After 15 min, the absorbance was measured at 562 nm (PerkinElmer UV/VIS Lambda 365, Waltham, MA, USA) and the results were reported as mg EDTA equivalent (EDTAE)/g FW using a calibration curve of EDTA (3.125–75 μg mL^{-1}).

2.7. A549 cells treatments and viability

The human A549 (ATCC® CCL-185™; lung tissue, alveolar epithelium) was grown in Dulbecco's Modified Eagle's Medium high glucose (Sigma Aldrich Chemie GmbH, Steiheim, Germany) supplemented with 10% fetal bovine serum and antibiotics at 37 °C with 95% humidity and 5% CO_2. All treatments were carried out using DMEM medium, without phenol red and FBS, containing antibiotics. A549 were pre-treated for 1 h with or without seeds/sprouts extracts, then exposed for 4 h with or without 50 ng mL^{-1} TNF-α. A549 cell viability was evaluated by the MTT assay as previously described (Gabriele et al., 2018).

2.8. RNA extraction, reverse transcriptase-PCR, and quantitative real-time PCR

Total RNA was isolated from A549 cells using the E.Z.N.A.® Total RNA Kit I (OMEGA Bio-Tek, Norcross, GA, USA) and reverse-transcribed using the iScript™ cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA). Quantitative Real-Time PCR was performed using the SsoFast™ EvaGreen® Supermix (Bio-Rad, Hercules, CA, USA) in a CFX Connect Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). IL-8, COX-2, and β-actin gene primers were described previously (Gabriele et al., 2018). The gene expression was calculated by the $2^{-\Delta\Delta CT}$ relative

quantification method.

2.9. Immunoblot analysis

For western blotting, total protein from A549 cells was extracted with RIPA buffer containing cOmplete™ protease inhibitor cocktail (Sigma-Aldrich) and incubated for 30 min on ice. Next, proteins were centrifuged at 12000×*g* (Jouan CR 31 centrifuge, Newport Pagnell, UK) for 30 min at 4 °C. Protein concentrations were quantified with Bradford assay (Sigma-Aldrich Saint Louis, MO, USA). Protein (45 μg) was separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto nitrocellulose membranes. After blocking with 5% non-fat milk for 2 h, membranes were incubated with specific primary antibodies COX2 and GADPH (1:1000 SAB5700721 and 1:1000 SAB2108266 Sigma-Aldrich) at 4 °C overnight. Subsequently, membranes were incubated with peroxidase-conjugated secondary antibodies (1:2000 A0545 Sigma-Aldrich) at room temperature for 1 h. After three washes with TBST, immunoreactive proteins were visualized using a chemiluminescence reaction kit (Clarity Western ECL substrates; Bio-Rad Laboratories) and signals were acquired using a ChemiDoc Imaging System (Bio-Rad Laboratories, Hercules, CA, USA). Protein band intensities were analyzed using the Image Lab Software (Bio-Rad Laboratories, Hercules, CA, USA).

2.10. Statistical analysis

Statistical analysis was performed using GraphPad Prism version 9.00 for macOS (GraphPad Software, San Diego, CA, USA). Assays were carried out in triplicate, and results were expressed as mean values ± standard deviation (SD). Differences between samples were analyzed by one-way analysis of variance (ANOVA) with Dunnett or Bonferroni multiple comparison tests. A p-value lower than 0.05 was considered statistically significant.

3. Results and discussion

3.1. Hemp sprouts' germination and morphological characterization

Hemp sprouts were obtained through germination tests under standard conditions (CTRL) using a sterile bdH_2O and agar (0.7%) culture medium. Under enriched conditions, the medium was supplemented with selected biostimulants, including the microalgal Chlorella-like strain C2 and the bacterial *Sphingomonas* sp. strain Can_S11. According to our previous works (Frassinetti et al., 2018; Gabriele et al., 2022), medium-length (4–5 cm) hemp sprouts with two to four leaves could be harvested after five-day (T5) exposure of *C. sativa* L. seeds to 16 h to light and 8 h to darkness (day-night cycle) with PPFD of 70 μmol photons m^{-1} s^{-1} upon a controlled temperature (22 ± 2 °C).

Appropriate light exposure is crucial for regulating and promoting germination processes (Chanyenga et al., 2012; Motsa et al., 2015; Zhang et al., 2020), with its effectiveness influenced by light intensity, type, and duration. Zhang et al. (2020) reviewed the impact of LED lighting on enhancing the nutritional value, phytochemical content, antioxidant properties, and growth of sprouts and microgreens. Similarly, Xiang et al. (2017) have observed a higher phenolic content in sweet corn (*Zea mays* L.) sprouts, germinated under light, than those exposed to dark. Moreover, for some seeds, it has been observed that exposure to light results in sprouts with more developed hypocotyls and higher fresh weight (Zhang et al., 2020). Conversely, for other seeds (e.g., *Glycine* max L. seeds), germination under the dark is preferred (Y. Chen & Chang, 2015), while for other ones (e.g., *Dipogon lignosus* L. and *Vigna radiata* mung beans) no differences were observed between dark or light exposure in terms of germination yield (Chanyenga et al., 2012; Islam, 2017). However, the effects of light must be considered in combination with germination temperature (Chanyenga et al., 2012), which differs depending on the crop seeds (Motsa et al., 2015).

Table 2
Morphological characterization of *Cannabis sativa* L. sprouts under different germination conditions collected after five days (T5). Data were reported as mean ± SD of three replicates. The germination conditions selected were the following: I) standard culture medium (CTRL); II) with Can_S11 bacterial inoculum (BI); III) with Can_S11 bacterial spread on the culture medium surface (BS); IV) addition of dried microalgal C2 biomass (C2 D) to the culture medium; V) seed imbibition in fresh microalgal C2 biomass before seeding (C2 F); VI) BI and C2 D combination; VII) BI and C2 F combination; VIII) BS and C2 D combination; IX) BS and C2 F combination. For each selected morphological parameter, a statistical analysis was performed by one-way ANOVA with Tukey's post-hoc test: *p < 0.05 vs CTRL (culture medium alone).

Germination Conditions	Sprouted Seeds (T5)	Fresh Weight (g)	Sprouts Length (cm)	Roots Length (cm)	Aerial part Length (cm)
CTRL	6–7	0.067 ± 0.016	5.081 ± 1.658	2.319 ± 1.194	2.761 ± 0.856
BI	3–5	0.059 ± 0.018	3.690 ± 1.411	1.470 ± 0.590	2.220 ± 0.950
BS	3–4	0.061 ± 0.014	5.143 ± 1.457	2.314 ± 0.659	2.829 ± 0.846
C2 D	6–7	0.068 ± 0.016	5.215 ± 1.835	2.765 ± 1.462	2.473 ± 0.650
C2 F	1–3*	0.057 ± 0.011	4.125 ± 1.919	1.350 ± 1.136	2.775 ± 0.789
BI + C2 D	6–8	0.065 ± 0.016	5.657 ± 1.392	2.763 ± 1.027	2.894 ± 0.602*
BI + C2 F	2–3*	0.053 ± 0.018	3.540 ± 1.630	1.100 ± 0.587	2.440 ± 1.092
BS + C2 D	4–6	0.071 ± 0.018	5.340 ± 1.558	1.870 ± 1.032	3.470 ± 0.863
BS + C2 F	1–3*	0.063 ± 0.022	4.633 ± 1.916	1.633 ± 1.363	3.000 ± 0.687

According to the literature, fresh and dry microalgae biomass are employed as biostimulants and/or biofertilizers to increase agricultural sustainability to cope with the increase in world population and the resulting environmental impact of intensified agricultural production (Alvarez et al., 2021; Gonçalves, 2021; Ronga et al., 2019). Furthermore, microalgal biomass is known to promote development, growth, and crop yield by improving their resistance to stressogenic conditions (Colla & Rouphael, 2020; Dineshkumar et al., 2018; Gonçalves, 2021) and also by promoting seed germination (Dineshkumar et al., 2018).

According to Chiellini et al. (2022), the microalgal *Chlorella* sp. strain C2 was chosen to stimulate *Cannabis sativa* L. seeds germination and promote the sprout's development in a short time. On the other hand, bacterial endophytes known as PGPB are also considered a good strategy to improve seed germination. Hence, we used the endophytic bacterium Can_S11, previously isolated from sterilized hemp seeds and selected for its remarkable ability to produce indoleacetic acid (Gabriele et al., 2022), a hormone capable of promoting plant growth (Glick, 2003). Therefore, the use of natural soil conditioners such as microalgae and bacteria can increase crop production without high costs, reducing chemical fertilizers or pesticides' practice, which damage environmental and human health, obtaining sustainable and nutrient-rich products (Chiaiese et al., 2018; Delshadi et al., 2017; Dineshkumar et al., 2018; Ribeiro et al., 2019).

The *Cannabis sativa* L. sprouts under the selected germination conditions (listed in Table 1) were collected after five days (T5) and evaluated morphologically. As reported in Table 2, the measured morphological parameters are the following: I) number of sprouted seeds; II) fresh weight (g); III) sprouts length (cm); IV) roots length (cm); V) aerial part length (cm).

Table 2 includes seed counts per plate, from which sprouts with developed roots and two or four leaves were obtained after five days. Optimal enrichment conditions for germination involved using dry microalgal biomass C2 D [0.25 mg mL^{-1}], combined with bacterium Can_S11 (BI and BS). This combination enhanced seed germination and

Table 3
The total polyphenol content (TPC) and total flavonoid content (TFC) of hemp sprout extracts (80% ethanol) were determined by spectrophotometric assays. Results were reported as mean ± SD of three replicates. The germination conditions selected were the following: I) standard culture medium (CTRL); II) Can_S11 bacterial inoculum (BI); III) Can_S11 bacterial spread on the culture medium surface (BS); IV) dried microalgal C2 biomass (C2 D) added to the culture medium; V) seed imbibition in fresh microalgal C2 biomass before seeding (C2 F); VI) BI and C2 D combination; vii) BI and C2 F combination; VIII) BS and C2 D combination; IX) BS and C2 F combination. The letters ^{a,b,c,d,e,f,g,h} indicate the significant difference between the treatments. Statistical analyses were performed by one-way ANOVA, followed by Tukey's post-hoc test; *p < 0.01 and **p < 0.001 vs CTRL (culture medium alone).

Germination Conditions	TPC (mg GAE/g FW)	TFC (mg QE/g FW)
CTRL	$2.20^{a} \pm 0.02$	$1.63^{a} \pm 0.01$
BI	$2.25^{ab} \pm 0.01$	$1.46^{b} \pm 0.03$*
BS	$2.06^{c} \pm 0.002$*	$1.21^{c} \pm 0.03$*
C2 D	$2.40^{d} \pm 0.01$*	$2.21^{d} \pm 0.02$*
C2 F	$2.29^{ab} \pm 0.01$	$1.6^{a} \pm 0.03$
BI + C2 D	$2.95^{e} \pm 0.01$**	$2.68^{e} \pm 0.01$**
BI + C2 F	$1.79^{f} \pm 0.01$*	$1.79^{f} \pm 0.06$*
BS + C2 D	$2.26^{ab} \pm 0.03$	$1.99^{g} \pm 0.03$*
BS + C2 F	$1.56^{g} \pm 0.03$*	$0.76^{h} \pm 0.02$*

seedling growth, likely due to microalgae providing essential nutrients and growth-promoting compounds (Garcia-Gonzalez & Sommerfeld, 2016; Navarro et al., 2021). Microalgae appeared to act synergistically with the bacterium Can_S11 likely contributing to the production of indoleacetic acid, a growth-promoting hormone. Microbial treatments have increased phytohormone levels, enhancing root and sprout growth (Mazepa et al., 2021). Overall, microorganisms like bacteria and microalgae serve as effective biofertilizers and biostimulants, promoting plant development by producing growth-promoting metabolites. The combined activity of microalgal biomass C2 and bacterium Can_S11 shows promising results in enhancing hemp seed germination and sprout development.

3.2. Phytochemical profile of hemp sprouts' extracts

The total content of polyphenols and flavonoids determined in hemp sprout extracts are summarized in Table 3.

Overall, hemp sprouts exhibited a fair content of total polyphenols (TPC) and flavonoids (TFC). CTRL sprouts showed higher TPC and TFC content than those obtained by Frassinetti et al. (2018) using similar protocols (TPC = 1,2 ± 0,02 mg GAE/g FW and TFC = 1,03 ± 0,15 mg QE/g FW).

As shown in Table 3, BI + C2 D hemp sprouts' extracts reported the highest TPC and TFC levels. Notably, BI + C2 D and C2 D conditions differed significantly ($p < 0.001$) from CTRL and all treatments in the total polyphenol and flavonoid content. Therefore, microalgal C2 biomass seems to increase the phytochemical content of hemp sprouts, according to previous nutraceutical characterization assessed by Chiellini et al. (2022). Nevertheless, hemp sprouts obtained under C2 D conditions (C2 D, BI + C2 D, and BS + C2 D) presented significantly ($p < 0.001$) higher polyphenol and flavonoid content than ones treated with C2 F (C2 F, BI + C2 F, and BS + C2 F). Concerning this, given that the seeds were soaked with fresh microalgal biomass overnight, while the dry one was added directly to the growth medium, it may be assumed that the exposure time to the treatment may have influenced the polyphenol and flavonoid content. In addition, dried microalgae are commonly employed in food supplements' formulation, functional foods production, and crop treatment (Chiellini et al., 2022; Gallego et al., 2018), increasing their phytochemical content and nutraceutical properties. On the other hand, hemp sprouts treated with BI (BI, BI + C2 D, and BI + C2 F) reported TPC and TFC significantly higher than ones grown under BS conditions (BS, BS + C2 D, and BS + C2 F). These conditions differ in the bacterium administration.

Table 4
Anti-radical and antioxidant *in vitro* activity of *Cannabis sativa* L. sprouts obtained from each treatment used for germination tests. Results were reported as mean ± SD of three replicates. The letters ^{a,b,c,d,e,f} in the columns indicate the significant differences between the treatments. Statistical analyses were performed by one-way ANOVA, followed by Tukey's post-hoc test; *p < 0.01 and **p < 0.001 vs CTRL (culture medium alone). n.d.: not determined.

Germination Conditions	ABTS (μM TEAC)	DPPH (% ARA)	ORAC (μM TE/g FW)	FRAP (Fe^{2+} μM)	Fe^{2+}-chelating activity (mg EDTA eq/g FW)
CTRL	$0.89^{a} \pm 0.03$	$22.6^{a} \pm 0.68$	$41.29^{a} \pm 1.94$	$787.17^{a} \pm 2.91$	$0.31^{a} \pm 0.01$
BI	$0.87^{ab} \pm 0.01$	$23.96^{a} \pm 1.18$	$38.81^{ab} \pm 1.82$	$685.54^{b} \pm 41.39$	$0.33^{a} \pm 0.02$
BS	$0.62^{c} \pm 0.02$	$17.07^{b} \pm 0.91$	$30.53^{b} \pm 0.18$	$530.44^{c} \pm 35.67$	$0.14^{b} \pm 0.02$
C2 D	$1.13^{d} \pm 0.01$***	$11.68^{c} \pm 0.22$	$55.19^{c} \pm 3.44$	$746.74^{ab} \pm 3.68$	$0.23^{a} \pm 0.01$
C2 F	$0.77^{b} \pm 0.02$	n.d.	$47.60^{ac} \pm 2.24$	$488.84^{cd} \pm 3.97$	$1.37^{c} \pm 0.01$
BI + C2 D	$1.48^{e} \pm 0.01$***	$27.02^{d} \pm 0.35$*	$81.83^{d} \pm 14.55$***	$909.58^{e} \pm 7.95$*	$0.26^{a} \pm 0.01$
BI + C2 F	$1.05^{d} \pm 0.07$	$1.45^{e} \pm 0.84$***	$30.41^{b} \pm 1.43$	$512.01^{cf} \pm 0.87$	$1.38^{c} \pm 0.002$
BS + C2 D	$0.99^{ae} \pm 0.02$	$14.27^{bc} \pm 0.77$	$37.47^{ab} \pm 2.02$	$717.75^{ab} \pm 5.14$	$0.14^{b} \pm 0.02$
BS + C2 F	$0.87^{b} \pm 0.02$	$3.83^{e} \pm 0.12$	$29.07^{b} \pm 0.05$	$423.6^{d} \pm 6.49$	$0.32^{a} \pm 0.02$

3.3. In vitro antioxidant activities of hemp sprouts

In vitro antioxidant and anti-radical capacities of *Cannabis sativa* L. sprout extracts were evaluated by ABTS, DPPH, ORAC, FRAP, and Fe^{2+}-chelating activity assays (Table 4).

The ABTS and DPPH assays were performed to determine the free radicals scavenging capacity exerted by *Cannabis sativa* L. sprouts. The results of the ABTS assay, obtained for each treatment tested, were reported as Trolox equivalent antioxidant capacity (TEAC, μM) in Table 4. Notably, C2 D and BI + C2 D conditions showed significantly higher anti-radical activity to the CTRL, confirming the findings obtained from morphological characterization and TPC and TFC content. On the other hand, DPPH values were expressed as the percentage of the samples' ability to inhibit DPPH· radicals. Specifically, BI + C2 D treatment exhibited significantly higher anti-radical activity than the control ($p < 0.01$). Furthermore, BI + C2 D exerted the greatest anti-radical activity compared to the other treatments evaluated, presenting the highest percentage of DPPH· radical inhibition. In contrast, spouts obtained from BI + C2 F presented the lowest DPPH· radical scavenging capacity which was significantly lower ($p < 0.001$) than all other conditions except BS + C2 F. In addition, as demonstrated in analyses previously described, BI treatment was significantly more effective ($p < 0.05$) than BS one, despite the endophytic bacteria used being the same (Can_S11). This significant difference was also observed for treatments that include C2 dry microalgal biomass, i.e. BI + C2 D and BS + C2 S. Consistently, the use of fresh C2 F biomass (C2 F, BI + C2 F, and BT + C2 F) led to significantly lower values ($p < 0.05$) than conditions involving the use of dry one.

Similarly, the ORAC assay was performed to evaluate the antioxidant activity of the sprouts. BI + C2 D reported a significantly higher value ($p < 0.001$) compared to all treatments, especially to the control showing halved activity. Consistent with previous analysis, significant differences were observed between the C2 D and BS + C2 D treatments compared to the control and BI + C2 D ($p < 0.001$). Notably, findings obtained for the control were comparable to those of hemp sprouts harvested on day 5 (48 ± 1 μmol TE/g FW) and analyzed by Frassinetti

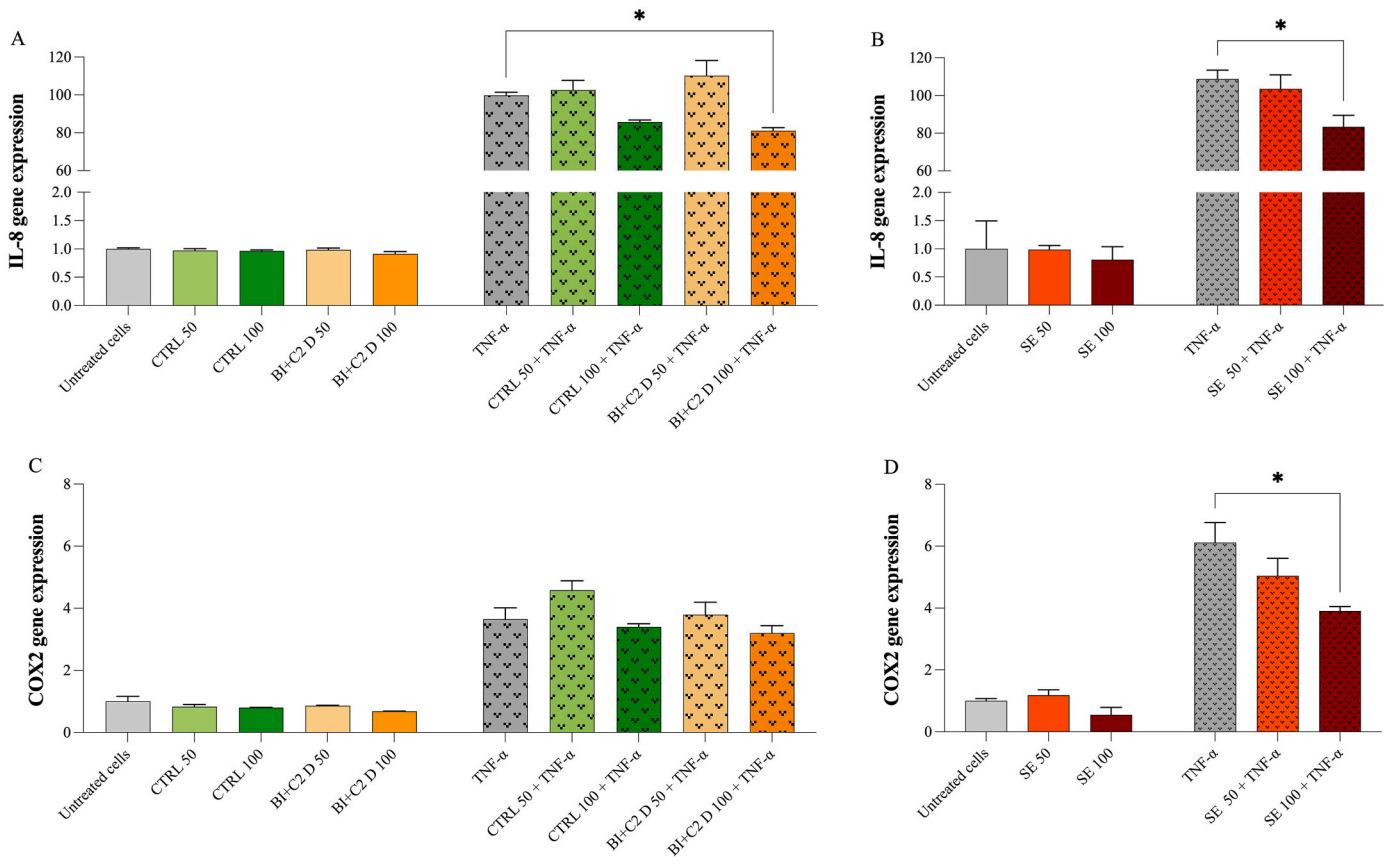

Fig. 1. IL-8 (A and B) and COX2 (C and D) gene expression in A549 cells 1 h pre-treated with 50 μg mL^{-1} or 100 μg mL^{-1} of sprouts (CTRL, control sprouts; BI + C2 D, BI + C2 D treated sprouts) and seeds extracts (SE), then exposed 4 h with (right side) or without (left side) to 50 ng mL^{-1} TNF-α. One-way ANOVA and Dunnett's post-hoc test: * different from TNF-α 50 ng mL^{-1}: *$p < 0.05$.

et al. (Frassinetti et al., 2018).

Regarding the FRAP assay, BI + C2 D treatment resulted in sprouts with the highest antioxidant activity, which differed significantly from the control and all treatments ($p < 0.001$). Nevertheless, no significant difference was reported between the C2 D condition and control. In contrast, all treatments using C2 F showed significantly lower values than the control.

Finally, treatments with the highest Fe^{2+}- chelating activity were C2 F and BI + C2 F (Table 3). Thus, unlike the other assays, treatments with C2 F promoted the presence of antioxidant compounds capable of chelating Fe^{2+} in hemp sprouts. Instead, BS and BS + C2 D presented the lowest value. Therefore, conditions with Can_S11 and C2 D showed the lowest Fe^{2+}- chelating activity.

Except for the latter assay, the findings obtained confirm that C2 dry microalgal biomass and Can_S11 bacteria could be used as biostimulants to improve the phenolic content and promote the antioxidant activity of *Cannabis sativa* L. sprouts. Indeed, these microorganisms present and/or synthesize auxins and cytokinins, phytohormones known to enhance antioxidant properties (Mazepa et al., 2021). In addition, the highest TPC and TFC were observed in the BI + C2 D treatment, which also presented higher antioxidant and anti-radical activity (except for Fe^{2+}chelating activity).

3.4. Effect of hemp seeds and sprouts extract on TNF-α inflamed A549 pulmonary cell line

Inflammation serves as a vital physiological response of the body to disease, infection, and injury, involving the modulation of numerous inflammatory mediators through diverse pathways and mechanisms (L. Chen et al., 2017). Nevertheless, uncontrolled and inadequate activation of this response can result in chronic inflammation (L. Chen et al., 2017), carrying significant negative health implications. Today, owing to their minimal side effects and notable anti-inflammatory properties, natural products and plant-derived compounds stand out as promising assets for the management and treatment of chronic pulmonary inflammation, such as COPD (Li et al., 2021).

Given that sprouted *C. sativa* seeds under the BI + C2 D condition demonstrated elevated levels of polyphenols and flavonoids compared to the control ones, we subsequently investigated their potential anti-inflammatory impact on tumor necrosis factor-alpha (TNF-α)-inflamed A549 cells. Furthermore, considering their good nutraceutical profile (Frassinetti et al., 2018), we evaluated the anti-inflammatory effects of hemp seed extracts.

Extracts derived from *C. sativa* seeds and sprouts were evaluated for their potential cytotoxic effects on A549 viability and the expression of key inflammatory genes under basal and inflammatory conditions. Preliminary findings indicate that neither the seed nor the sprout extracts influenced the viability of A549 cells (data not shown). Furthermore, these extracts did not induce significant changes in the basal gene expression levels of IL-8 and COX-2 if compared to untreated cells (Fig. 1).

Upon exposure to a concentration of 50 ng mL^{-1} TNF-α, A549 cells exhibited a pronounced upregulation in both IL-8 and COX-2 gene expression levels relative to untreated cells ($p < 0.001$), underscoring the pro-inflammatory nature of TNF-α stimulation. Interestingly, under the inflammatory condition, pre-treatment with *C. sativa* seeds extract at a concentration of 100 μg mL^{-1} demonstrated a notable attenuation in the IL-8 (Fig. 1B) and COX-2 (Fig. 1D) (20% and 30%, respectively) upregulated expression. Hemp seeds contain some bioactive compounds like lignanamides and other phenylpropionamides that may exert an

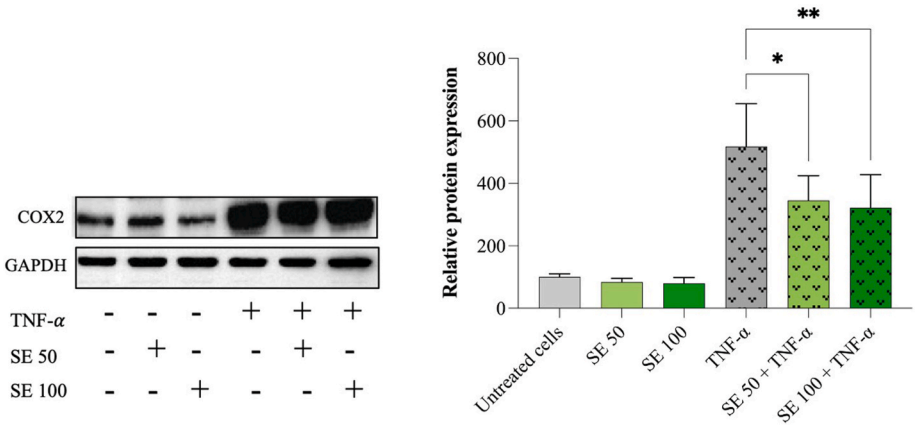

Fig. 2. Western blot results and semi-quantitative analysis of COX2 in A549 cells 1 h pre-treated with 50 μg mL^{-1} or 100 μg mL^{-1} of seeds extracts (SE), then exposed 4 h with or without 50 ng mL^{-1} TNF-α. GAPDH was used as a reference. One-way ANOVA and Dunnett's post-hoc test: * different from TNF-α 50 ng mL^{-1}: *$p < 0.05$, **$p < 0.01$.

anti-inflammatory effect as observed by Wang et al. (2019) in LPS-stimulated BV2 microglia cells. Following a similar approach, Martinez et al. (2020) identified N-trans-caffeoyltyramine as a significant compound in a fraction derived from an extract of defatted hemp seeds, which exhibited anti-inflammatory properties in LPS-treated human primary monocytes. Moreover, when A549 cells were pre-treated with the highest dose of BI + C2 D sprout extract (100 μg mL^{-1}) and exposed to TNF-α stimulus a 20% reduction in IL-8 expression was observed (Fig. 1A). Interestingly, seed germination involves the synthesis of new compounds with anti-inflammatory potential. For example, as observed by Werz et al., hemp seeds are normally free of cannflavins and sprouting induced the formation of cannflavin A and B. Furthermore, it is worth noting that the anti-inflammatory effects of sprouts are not unique to hemp. Observations of similar effects have been made with the sprouts of other plants, such as garlic and wheat (Gdula-Argasińska et al., 2017; Tomé-Sánchez et al., 2020).

Western blotting confirmed the impact of hemp seed extracts on A549 inflamed cells at the COX2 protein level (Fig. 2).

In line with gene expression data, the administration of TNF-α to A549 cells resulted in a significant elevation in COX2 protein levels compared to untreated cells ($p < 0.001$). Remarkably, pretreatment with the extract led to a 40% reduction in COX-2 levels induced by the pro-inflammatory stimulus.

4. Conclusion

This study on the seeds and five-day-old sprouts of *Cannabis sativa* L. cultivar "Futura 75" has revealed promising insights into their potential as a health-promoting dietary component. Utilizing Plant Growth Promoting (PGP) microorganisms and microalgae, specifically the Chlorella-like strain C2 and the bacterial *Sphingomonas* sp. strain Can_S11, we observed significant enhancements in the phytochemical profile, antioxidant capacity, and anti-inflammatory properties of the hemp sprouts. The enrichment conditions resulted in a notable increase in phenolic compounds and improved antioxidant and anti-radical activities. Moreover, treatments with treated sprout extract and seed extract demonstrated a comparable potential to mitigate TNF-α induced alterations and reduce IL-8 expression in inflamed A549 cells. However, a remarkable protective effect on COX2 expression was observed only with the seed extract. These findings highlight the potential of leveraging PGP microorganisms and microalgae to enhance the development of hemp sprouts, thereby augmenting their health-promoting benefits. Further research in this direction holds promise for expanding our understanding of the sprouted seed's nutritional and therapeutic properties and their implications for human health.

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CRediT authorship contribution statement

Andrea Cavallero: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation. **Elisa Chelucci:** Writing – original draft, Investigation, Formal analysis, Data curation. **Carolina Chiellini:** Validation, Supervision, Resources, Investigation, Funding acquisition, Conceptualization. **Morena Gabriele:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Investigation, Funding acquisition, Data curation, Conceptualization.

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

Aborus, N. E., Čanadanović-Brunet, J., Ćetković, G., Šaponjac, V. T., Vulić, J., & Ilić, N. (2017). Powdered barley sprouts: Composition, functionality and polyphenol digestibility. *International Journal of Food Science and Technology, 52*(1), 231–238. https://doi.org/10.1111/ijfs.13274

Agbabiaka, T. B., Guo, R., & Ernst, E. (2008). Pelargonium sidoides for acute bronchitis: A systematic review and meta-analysis. *Phytomedicine: International Journal of Phytotherapy and Phytopharmacology, 15*(5), 378–385. https://doi.org/10.1016/j.phymed.2007.11.023

Alvarez, A. L., Weyers, S. L., Goemann, H. M., Peyton, B. M., & Gardner, R. D. (2021). Microalgae, soil and plants: A critical review of microalgae as renewable resources for agriculture. *Algal Research, 54*, Article 102200. https://doi.org/10.1016/j.algal.2021.102200

Andre, C. M., Hausman, J.-F., & Guerriero, G. (2016). Cannabis sativa: The plant of the thousand and one molecules. *Frontiers in Plant Science, 7*. https://www.frontiersin.org/articles/10.3389/fpls.2016.00019.

Anil, S. M., Shalev, N., Vinayaka, A. C., Nadarajan, S., Namdar, D., Belausov, E., Shoval, I., Mani, K. A., Mechrez, G., & Koltai, H. (2021). Cannabis compounds exhibit anti-inflammatory activity in vitro in COVID-19-related inflammation in lung epithelial cells and pro-inflammatory activity in macrophages. *Scientific Reports, 11*. https://doi.org/10.1038/s41598-021-81049-2

Benincasa, P., Falcinelli, B., Lutts, S., Stagnari, F., & Galieni, A. (2019). Sprouted grains: A comprehensive review. *Nutrients, 11*(2). https://doi.org/10.3390/nu11020421. Article 2.

Chanyenga, T. F., Geldenhuys, C. J., & Sileshi, G. W. (2012). Germination response and viability of an endangered tropical conifer *Widdringtonia whytei* seeds to temperature and light. *South African Journal of Botany, 81*, 25–28. https://doi.org/10.1016/j.sajb.2012.04.002

Chelucci, E., Chiellini, C., Cavallero, A., & Gabriele, M. (2023). Bio-functional activities of tuscan bee pollen. *Antioxidants, 12*(1). https://doi.org/10.3390/antiox12010115. Article 1.

Chen, Y., & Chang, S. K. C. (2015). Macronutrients, phytochemicals, and antioxidant activity of soybean sprout germinated with or without light exposure. *Journal of Food Science, 80*(6), S1391–S1398. https://doi.org/10.1111/1750-3841.12868

Chen, L., Deng, H., Cui, H., Fang, J., Zuo, Z., Deng, J., Li, Y., Wang, X., & Zhao, L. (2017). Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget, 9*(6), 7204–7218. https://doi.org/10.18632/oncotarget.23208

Chiaiese, P., Corrado, G., Colla, G., Kyriacou, M. C., & Rouphael, Y. (2018). Renewable sources of plant biostimulation: Microalgae as a sustainable means to improve crop performance. *Frontiers in Plant Science, 9*. https://www.frontiersin.org/articles/10.3389/fpls.2018.01782.

Chiellini, C., Serra, V., Gammuto, L., Ciurli, A., Longo, V., & Gabriele, M. (2022). Evaluation of nutraceutical properties of eleven microalgal strains isolated from different freshwater aquatic environments: Perspectives for their application as nutraceuticals. *Foods, 11*(5). https://doi.org/10.3390/foods11050654. Article 5.

Chiriac, E. R., Chițescu, C. L., Sandru, C., Geană, E.-I., Lupoae, M., Dobre, M., Borda, D., Gird, C. E., & Boscencu, R. (2020). Comparative study of the bioactive properties and elemental composition of red clover (Trifolium pratense) and alfalfa (medicago sativa) sprouts during germination. *Applied Sciences, 10*(20). https://doi.org/10.3390/app10207249. Article 20.

Colla, G., & Rouphael, Y. (2020). Microalgae: New source of plant biostimulants. *Agronomy, 10*(9). https://doi.org/10.3390/agronomy10091240. Article 9.

Delshadi, S., Ebrahimi, M., & Shirmohammadi, E. (2017). Influence of plant-growth-promoting bacteria on germination, growth and nutrients' uptake of Onobrychis sativa L. under drought stress. *Journal of Plant Interactions, 12*(1), 200–208. https://doi.org/10.1080/17429145.2017.1316527

Dineshkumar, R., Kumaravel, R., Gopalsamy, J., Sikder, M. N. A., & Sampathkumar, P. (2018). Microalgae as bio-fertilizers for rice growth and seed yield productivity. *Waste and Biomass Valorization, 9*(5), 793–800. https://doi.org/10.1007/s12649-017-9873-5

Donkor, O. N., Stojanovska, L., Ginn, P., Ashton, J., & Vasiljevic, T. (2012). Germinated grains – sources of bioactive compounds. *Food Chemistry, 135*(3), 950–959. https://doi.org/10.1016/j.foodchem.2012.05.058

Frassinetti, S., Moccia, E., Caltavuturo, L., Gabriele, M., Longo, V., Bellani, L., Giorgi, G., & Giorgetti, L. (2018). Nutraceutical potential of hemp (Cannabis sativa L.) seeds and sprouts. *Food Chemistry, 262*, 56–66. https://doi.org/10.1016/j.foodchem.2018.04.078

Gabriele, M., Parri, E., Felicioli, A., Sagona, S., Pozzo, L., Biondi, C., Domenici, V., & Pucci, L. (2015). Phytochemical composition and antioxidant activity of Tuscan bee pollen of different botanic origins. *Italian Journal of Food Science, 27*(2), 248–259.

Gabriele, M., Pucci, L., Árvay, J., & Longo, V. (2018). Anti-inflammatory and antioxidant effect of fermented whole wheat on TNFα-stimulated HT-29 and NF-κB signaling pathway activation. *Journal of Functional Foods, 45*, 392–400. https://doi.org/10.1016/j.jff.2018.04.029

Gabriele, M., Vitali, F., Chelucci, E., & Chiellini, C. (2022). Characterization of the cultivable endophytic bacterial community of seeds and sprouts of Cannabis sativa L. And perspectives for the application as biostimulants. *Microorganisms, 10*(9). https://doi.org/10.3390/microorganisms10091742. Article 9.

Gallego, R., Montero, L., Cifuentes, A., Ibáñez, E., & Herrero, M. (2018). Green extraction of bioactive compounds from microalgae. *Journal of Analysis and Testing, 2*(2), 109–123. https://doi.org/10.1007/s41664-018-0061-9

Gan, R.-Y., Lui, W.-Y., Wu, K., Chan, C.-L., Dai, S.-H., Sui, Z.-Q., & Corke, H. (2017). Bioactive compounds and bioactivities of germinated edible seeds and sprouts: An updated review. *Trends in Food Science & Technology, 59*, 1–14. https://doi.org/10.1016/j.tifs.2016.11.010

Garcia-Gonzalez, J., & Sommerfeld, M. (2016). Biofertilizer and biostimulant properties of the microalga Acutodesmus dimorphus. *Journal of Applied Phycology, 28*(2), 1051–1061. https://doi.org/10.1007/s10811-015-0625-2

Gdula-Argasińska, J., Paśko, P., Sułkowska-Ziaja, K., Kała, K., & Muszyńska, B. (2017). Anti-inflammatory activities of garlic sprouts, a source of α-linolenic acid and 5-hydroxy-l-tryptophan, in RAW 264.7 cells. *Acta Biochimica Polonica, 64*(3), 551–559. https://doi.org/10.18388/abp.2017_1534

Glick, B. R. (2003). Phytoremediation: Synergistic use of plants and bacteria to clean up the environment. *Biotechnology Advances, 21*(5), 383–393. https://doi.org/10.1016/S0734-9750(03)00055-7

Glick, B. R. (2012). Plant growth-promoting bacteria: Mechanisms and applications. *Scientifica, 2012*, Article e963401. https://doi.org/10.6064/2012/963401

Gonçalves, A. L. (2021). The use of microalgae and cyanobacteria in the improvement of agricultural practices: A review on their biofertilising, biostimulating and biopesticide roles. *Applied Sciences, 11*(2). https://doi.org/10.3390/app11020871. Article 2.

Idowu, A. T., Olatunde, O. O., Adekoya, A. E., & Idowu, S. (2020). Germination: An alternative source to promote phytonutrients in edible seeds. *Food Quality and Safety, 4*(3), 129–133. https://doi.org/10.1093/fqsafe/fyz043

Islam, M. J. (2017). *Light and temperature effects on sprout yield and its proximate composition and vitamin C content in Lignosus and Mung beans*. Journal of the Bangladesh Agricultural University. https://www.academia.edu/42945897/Light_and_temperature_effects_on_sprout_yield_and_its_proximate_composition_and_vitamin_C_content_in_Lignosus_and_Mung_beans.

Jeong, H., Sung, J., Yang, J., Kim, Y., Jeong, H. S., & Lee, J. (2018). Effect of sucrose on the functional composition and antioxidant capacity of buckwheat (Fagopyrum esculentum M.) sprouts. *Journal of Functional Foods, 43*, 70–76. https://doi.org/10.1016/j.jff.2018.01.019

Kocic, H., Damiani, G., Stamenkovic, B., Tirant, M., Jovic, A., Tiodorovic, D., & Peris, K. (2019). Dietary compounds as potential modulators of microRNA expression in psoriasis. *Therapeutic Advances in Chronic Disease, 10*, Article 2040622319864805. https://doi.org/10.1177/2040622319864805

Le, T. N., Chiu, C.-H., & Hsieh, P.-C. (2020). Bioactive compounds and bioactivities of Brassica oleracea L. Var. Italica sprouts and microgreens: An updated overview from a nutraceutical perspective. *Plants, 9*(8). https://doi.org/10.3390/plants9080946. Article 8.

Li, J., Zhao, P., Tian, Y., Li, K., Zhang, L., Guan, Q., Mei, X., & Qin, Y. (2021). The anti-inflammatory effect of a combination of five compounds from five Chinese herbal medicines used in the treatment of COPD. *Frontiers in Pharmacology, 12*, 2781. https://doi.org/10.3389/fphar.2021.709702

Liu, H., Kang, Y., Zhao, X., Liu, Y., Zhang, X., & Zhang, S. (2019). Effects of elicitation on bioactive compounds and biological activities of sprouts. *Journal of Functional Foods, 53*, 136–145. https://doi.org/10.1016/j.jff.2018.12.019

Martinez, J. R., Paz, S. M. la, Puerta, R. D. la, Dolores García-Giménez, M., & Ángeles Fernández-Arche, M. (2020). Characterization of bioactive compounds in defatted hempseed (Cannabis sativa L) by UHPLC-HRMS/MS and anti-inflammatory activity in primary human monocytes. *Food & Function, 11*(5), 4057–4066. https://doi.org/10.1039/D0FO00066C

Mazepa, E., Malburg, B. V., Mógor, G., de Oliveira, A. C., Amatussi, J. O., Corrêa, D. O., Lemos, J. S., Ducatti, D. R. B., Duarte, M. E. R., Mógor, Á. F., & Noseda, M. D. (2021). Plant growth biostimulant activity of the green microalga *Desmodesmus subspicatus*. *Algal Research, 59*, Article 102434. https://doi.org/10.1016/j.algal.2021.102434

Mir, S. A., Farooq, S., Shah, M. A., Sofi, S. A., Dar, B. N., Hamdani, A. M., & Mousavi Khaneghah, A. (2021). An overview of sprouts nutritional properties, pathogens and decontamination technologies. *LWT, 141*, Article 110900. https://doi.org/10.1016/j.lwt.2021.110900

Motsa, M. M., Slabbert, M. M., van Averbeke, W., & Morey, L. (2015). Effect of light and temperature on seed germination of selected African leafy vegetables. *South African Journal of Botany, 99*, 29–35. https://doi.org/10.1016/j.sajb.2015.03.185

Navarro, Q. R., de Oliveira Corrêa, D., Behling, A., Noseda, M. D., Amano, É., Suzuki, R. M., & Ribas, L. L. F. (2021). Efficient use of biomass and extract of the microalga Desmodesmus subspicatus (Scenedesmaceae) in asymbiotic seed germination and seedling development of the orchid Cattleya warneri. *Journal of Applied Phycology, 33*(4), 2189–2207. https://doi.org/10.1007/s10811-021-02442-y

Rajković, M. G., Popović-Grle, S., Dugac, A. V., Rogić, D., Rako, I., Antolić, M. R., Beriša, M., & Rumora, L. (2018). PON1 gene polymorphisms in patients with chronic obstructive pulmonary disease. *Journal of Clinical Pathology, 71*(11), 963–970. https://doi.org/10.1136/jclinpath-2018-205194

Rehman, M., Fahad, S., Du, G., Cheng, X., Yang, Y., Tang, K., Liu, L., Liu, F.-H., & Deng, G. (2021). Evaluation of hemp (Cannabis sativa L.) as an industrial crop: A review. *Environmental Science and Pollution Research, 28*(38), 52832–52843. https://doi.org/10.1007/s11356-021-16264-5

Ribeiro, D. M., Zanetti, G. T., Julião, M. H. M., Masetto, T. E., Gelinski, J. M. L. N., & Fonseca, G. G. (2019). Effect of different culture media on growth of Chlorella sorokiniana and the influence of microalgal effluents on the germination of lettuce seeds. *Journal of Applied Biology & Biotechnology, 7*(1), 6–10. https://doi.org/10.7324/JABB.2019.70102

Ronga, D., Biazzi, E., Parati, K., Carminati, D., Carminati, E., & Tava, A. (2019). Microalgal biostimulants and biofertilisers in crop productions. *Agronomy, 9*(4). https://doi.org/10.3390/agronomy9040192. Article 4.

Szulc, J., Czaczyk, K., Dobrowolska, A., Gozdecka, G., & Błaszak, B. (2024). Elicitation as a process of enhancing bioactive compounds concentration in sprouts. *Processes, 12*(1). https://doi.org/10.3390/pr12010174. Article 1.

Tomé-Sánchez, I., Martín-Diana, A. B., Peñas, E., Bautista-Expósito, S., Frias, J., Rico, D., González-Maillo, L., & Martinez-Villaluenga, C. (2020). Soluble phenolic composition tailored by germination conditions accompany antioxidant and anti-inflammatory properties of wheat. *Antioxidants, 9*(5). https://doi.org/10.3390/antiox9050426. Article 5.

Wang, S., Luo, Q., & Fan, P. (2019). Cannabisin F from hemp (Cannabis sativa) seed suppresses lipopolysaccharide-induced inflammatory responses in BV2 microglia as SIRT1 modulator. *International Journal of Molecular Sciences, 20*(3), 507. https://doi.org/10.3390/ijms20030507

Werz, O., Seegers, J., Schaible, A. M., Weinigel, C., Barz, D., Koeberle, A., … Appendino, G. (July 2014). Cannflavins from hemp sprouts, a novel cannabinoid-free hemp food product, target microsomal prostaglandin E2 synthase-1 and 5-lipoxygenase. *PharmaNutrition, 2*(3), 53–60. https://doi.org/10.1016/j.phanu.2014.05.001

Xiang, N., Guo, X., Liu, F., Li, Q., Hu, J., & Brennan, C. S. (2017). Effect of light- and dark-germination on the phenolic biosynthesis, phytochemical profiles, and antioxidant activities in sweet corn (Zea mays L.) sprouts. *International Journal of Molecular Sciences, 18*(6). https://doi.org/10.3390/ijms18061246. Article 6.

Zhang, X., Bian, Z., Yuan, X., Chen, X., & Lu, C. (2020). A review on the effects of light-emitting diode (LED) light on the nutrients of sprouts and microgreens. *Trends in Food Science & Technology, 99*, 203–216. https://doi.org/10.1016/j.tifs.2020.02.031