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THE COMPOSITION, VOLATILE COMPOUNDS, ANTIMICROBIAL AND ANTIOXIDANT PROPERTIES OF THE EDIBLE INSECT *TENEBRIO MOLITOR* FED WITH CYANOBACTERIAL *SPIRULINA PLATENSIS* BIOMASS UNDER DIELECTRIC BARRIER DISCHARGE TREATMENT

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ABSTRACT

This is a first attempt to examine the antibacterial and antioxidant properties of the edible insect *Tenebrio molitor* (yellow mealworm beetle), which was fed *Spirulina platensis*. subjected to dielectric barrier discharge (DBD) treatment (at 10 minutes during 3, 7, 14, and 30 days). In total, seven groups of larvae were used: larvae not fed with *S. platensis*, larvae fed with 5, 10, and 20% non- DBD -infused *S. platensis*, and larvae fed with 5, 10, and 20% DBD -infused *S. platensis*. The results of the nutritional composition showed that the percentage of protein, fat, ash, fiber, and carbohydrates in a 20% concentration of *S. platensis* under DBD treatment increased by 1.30, 1.28, 1.28, 1.67, 1.27, and 1.93 times ($p \leq 0.05$). The antioxidant activity by the DPPH method also showed that the highest value belonged to the treatment with 20% DBD, which was 1.18 times the control ($p \leq 0.05$). Results of catalase, superoxide dismutase and glutathione Peroxidase showed that the highest levels of these enzymes belonged to the treatment with 20% DBD, which was 1.6, 1.26, and 2.019 times the control, respectively ($p \leq 0.05$). Furthermore, the results of the antibacterial analysis indicated a significant increase. The results of the analysis of volatile compounds showed the highest abundance belonged to palmitic acid, while the lowest value belonged to compounds of oleic acid, 9-octadecenoic acid, and stearic acid. The overall results showed that the combination of DBD and cyanobacteria presents a promising technology for enhancing food properties in the food industry.

Keywords: *Tenebrio molitor, Spirulina platensis*, nutritional value, yellow mealworm beetle, dielectric barrier discharge (DBD) treatment

INTRODUCTION

Tenebrio molitor (Coleoptera: Tenebrionidae), commonly known as the yellow mealworm, is widely used as feed for insectivorous animals and has attracted increasing attention as an alternative protein source. The species is characterized by a high nutritional value, with protein contents ranging from 50–60% and lipid contents of approximately 20–34% on a dry matter basis, alongside a balanced profile of essential amino acids **(De Marco *et al.*, 2015; Kröncke and Benning, 2023).** Owing to these properties, *T. molitor* has been extensively studied in animal nutrition and emerging food systems.

Recent studies have demonstrated that bioactive fractions derived from mealworms, particularly protein hydrolysates, may exhibit enhanced antioxidant and antimicrobial activities when compared with conventional protein concentrates. These effects are especially pronounced when larvae are reared on modified or enriched diets, including food by-products **(Flores *et al.*, 2020; Hong *et al.*, 2024; Kim *et al.*, 2023; Villanova *et al.*, 2024).** In practical applications, mealworm larvae are routinely incorporated into pet feed and have also been evaluated in pig and poultry diets, as well as in artificial feeding systems designed for mass rearing of beneficial organisms **(De Marco *et al.*, 2015; Grimmond *et al.*, 1994; Jin *et al.*, 2016; Meyer *et al.*, 2020; Ramos-Elorduy *et al.*, 2002).** Beyond animal feeding, insects constitute an important component of human diets in many regions, with an estimated two billion people consuming insects as part of traditional food practices **(van Huis and Tomberlin, 2017).**

The nutritional composition and growth performance of insects are influenced by multiple factors, including diet composition, developmental stage, sex, species, rearing conditions, and analytical methodology **(Kröncke and Benning, 2023).** Among these variables, diet plays a dominant role, underscoring the importance of developing effective artificial feeding strategies for insect production systems **(Ramos-Elorduy *et al.*, 2002).** Considerable research has therefore focused on optimizing diets for *T. molitor* to enhance biomass yield and shorten developmental time. Feeding larvae with diverse agricultural and food by-products has been shown to significantly affect survival, feed conversion efficiency, and growth duration **(Gasco *et al.*, 2023; Kępińska-Pacelik *et al.*, 2023; Kröncke and Benning, 2023; Van Huis and Oonincx, 2017).**

Among potential dietary supplements, *Arthrospira platensis* (hereafter referred to as *S. platensis*) represents a sustainable source of proteins and bioactive compounds with recognized physiological functions **(Fernandes *et al.*, 2023).** Extracts derived from *S. platensis* have been reported to possess antioxidant, antimicrobial, and potential anticancer activities, attributed to a diverse range of intracellular and extracellular metabolites **(de Marco Castro *et al.*, 2019; Villaró *et al.*, 2023).** These bioactive constituents include terpenols, sterols, polysaccharides, dibutenolides, peptides, and proteins, which have demonstrated bactericidal and bacteriostatic effects against various clinical and foodborne pathogens **(Maddiboyina *et al.*, 2023; Seghiri *et al.*, 2019).**

Maintaining the stability and functionality of such bioactive molecules during processing is a critical challenge in the development of *S. platensis*-based products **(Cassani *et al.*, 2020; Pina-Pérez *et al.*, 2018).** In response, increasing attention has been directed toward non-thermal processing technologies that are rapid, energy-efficient, and environmentally friendly. These approaches, typically applied over short exposure times, aim to enhance bioactive potential while preserving product quality in food and pharmaceutical applications **(Pina-Pérez *et al.*, 2022).**

Cold atmospheric plasma (CAP) is one such emerging technology and is generally described as a partially ionized gas composed of electrons, ions, radicals, and electromagnetic radiation, generated using gases such as air, helium, or argon. Plasma formation can be achieved through various energy sources, including radiofrequency, microwave, thermal, and electric or magnetic fields, resulting in configurations such as plasma jets, surface micro-discharge plasma (SMD), and dielectric barrier discharge plasma (DBD) **(Seifi *et al.*, 2024; Tolouie *et al.*, 2018; Von Woedtke *et al.*, 2019).** Owing to its versatility, CAP has been increasingly recognized as a promising technology for food processing and bioactive enhancement **(Abdo and Kopecki, 2024; Bernhardt *et al.*, 2019; Dubuc *et al.*, 2018).**

Microalgae production, including *S. platensis*, faces economic and environmental challenges related to water use and production costs. Modulation of growth

conditions, nutrient availability, and the application of novel technologies have therefore been explored to improve biomass productivity and protein concentration. CAP has been investigated as a tool to influence microbial metabolism, potentially enhancing photosynthetic activity and biomolecule synthesis, with treatment outcomes dependent on factors such as exposure duration, intensity, application mode, and device configuration **(Seifi *et al.*, 2024).** At the same time, the insect-producing industry seeks cost-effective and sustainable feeding strategies capable of supporting efficient growth and development across insect life stages. Designing species-specific diets that meet nutritional requirements is essential for maximizing larval biomass and reproductive performance **(Bingqian *et al.*, 2023; Sogari *et al.*, 2023).** In this context, combining cyanobacterial supplementation with CAP treatment represents a novel approach that may enhance the antimicrobial and antioxidant properties of edible insects. Therefore, the present study aimed to evaluate the antibacterial and antioxidant activities of *Tenebrio molitor* larvae fed *S. platensis* biomass subjected to dielectric barrier discharge (DBD) treatment.

MATERIAL AND METHODS

Culture conditions and biomass production of the *s. Platensis* and cap

The cyanobacterial strain *Spirulina platensis* was obtained from the Cyanobacteria Culture Collection (CCC) of the ALBORZ Herbarium at the Science and Research Branch of Islamic Azad University (Tehran, Iran). Cultivation was carried out in modified Zarrouk medium under controlled growth-room conditions. Cultures were maintained at 28 ±2 °C with continuous illumination at an intensity of 300 µmol m^{-2} s^{-1} for a period of 15 days, following previously established protocols **(Bhattacharya and Shivaprakash, 2005; Liu *et al.*, 2014)** (Figure 1a).
At the end of the cultivation period, the biomass was harvested by centrifugation at 4000 rpm for 10 min to remove residual medium and cellular debris. The collected biomass was subsequently passed through a 300-mesh sieve and rinsed repeatedly with distilled water to ensure complete removal of culture medium components. The washed biomass was then freeze-dried at −56 °C for 24 h using a laboratory freeze dryer. The resulting dried material was finely ground to obtain a uniform powder, which was used for subsequent experimental procedures.

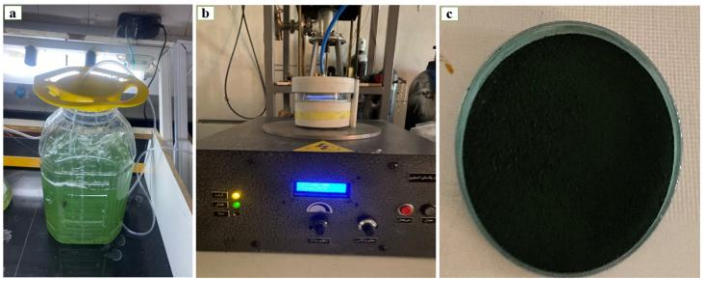

Figure 1 (a) Cyanobacterial culture of *S. platensis* during 15 days and (b and c) DBD treatment of activated biomass.

Dielectric barrier discharge (dbd) treatment of activated biomass

For dielectric barrier discharge (DBD) treatment, the algal suspension was prepared by mixing the culture medium with plasma-activated solution at a 1:1 (v/v) ratio. In parallel, argon-purified water was mixed at the same ratio with Zarrouk medium to maintain comparable nutrient availability across all experimental groups. This approach ensured uniform nutritional conditions during plasma exposure.
Plasma generation was performed using a DBD system operated at a power range of 25–300 kW and a frequency of 16 Hz. The activated S. platensis biomass was exposed to plasma for 10 min at different time points (3, 7, 14, and 30 days). Initial cyanobacterial cell densities were adjusted to 1×10^5 and 4×10^5 cells mL^{-1} prior to treatment.
During the exposure period, culture plates were covered with transparent lids and maintained under controlled incubation conditions, including constant temperature and illumination, without agitation. All treatments were carried out following the experimental setup described by **Noore *et al.* (2023)** (Figure 1b,c).

Preparation and cultivation of *Tenebrio molitor* larvae

Tenebrio molitor larvae were reared at the Insect Research Centre of the Zakaria Razi Laboratory Complex (Islamic Azad University, Science and Research Branch, Tehran, Iran). Rearing was conducted in plastic containers (60 × 40 cm; surface area 2000 cm^2) under controlled environmental conditions, including a temperature of 27 ±1 °C, relative humidity of 65 ±5%, and complete darkness **(Mohammadi *et al.*, 2022).**
Experimental larvae were obtained from adult beetles with an average age of four weeks. A total of eleven batches, each containing 250 g of beetles, were allowed to oviposit for eight days. Following oviposition, the eggs together with the commercial feed (Insectus, Mijten bv., Belgium) were transferred to fresh containers and maintained under identical environmental conditions. After two weeks, carrot pieces were introduced as a moisture source. At four weeks post-oviposition, frass was removed by sieving through a 0.5 mm mesh, and the remaining mixture of larvae and feed was gently homogenized.
After three consecutive generations of laboratory rearing, larvae were harvested and used for subsequent experimental analyses **(Hamerman, 2016; Van Huis *et al.*, 2013)** (Figure 2).

Figure 2 The preparation process of *Tenebrio molitor* larvae. (a) Cultivation in germinator, (b and c) Feeding the larvae up to three generations, (d) collection of reared larvae.

Feeding *tenebrio molitor* larvae with *s. Platensis* powder treated with dbd treatment in the fourth generation (for 21 days)

Larvae obtained from the third generation were randomly selected and allocated to experimental groups corresponding to DBD-treated and non-treated feeding conditions. Each group consisted of 80 larvae with comparable body size to minimize variability among treatments. In total, 49 plastic containers were prepared, each containing 80 larvae and the appropriate feeding medium, including both control and DBD-treated groups.
Prior to feeding, larvae were subjected to a fasting period of approximately 48 h. After starvation, the larvae were fed once every three days over the experimental period. Diets were formulated by supplementing the basal feed with S. platensis powder at concentrations of 5%, 10%, and 20%. To maintain moisture, each diet was mixed with 100 g of carrot pieces. Control groups received the same amount of carrot and basal diet without S. platensis, ensuring equal total food volume across all treatments. All feeding procedures were conducted following established insect-rearing protocols **(Van Huis *et al.*, 2013)** (Figure 3).

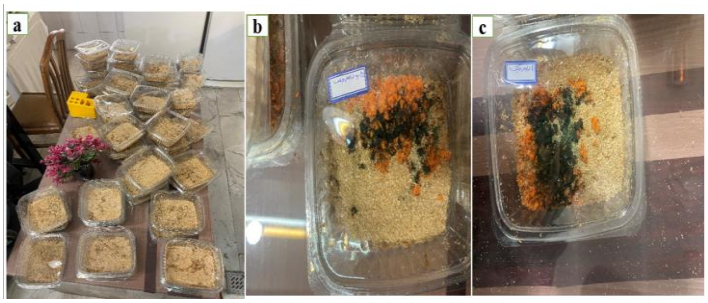

Figure 3 Groups prepared from larvae fed during thirty days. (a) Preparation of larvae before feeding with *S. platensis* powder, (b and c) Feeding larvae with *S. platensis* powder under DBD treatment (5 % and 10 %).

A total of seven experimental groups were established for subsequent analyses: (I) control larvae receiving no S. platensis supplementation; (II) larvae fed diets containing 5% S. platensis without DBD treatment; (III) larvae fed 10% S. platensis without DBD treatment; (IV) larvae fed 20% S. platensis without DBD treatment; (V) larvae fed 5% S. platensis subjected to DBD treatment for 10 min; (VI) larvae fed 10% S. platensis subjected to DBD treatment for 10 min; and (VII) larvae fed 20% S. platensis subjected to DBD treatment for 10 min.
At the end of the feeding period, larvae were separated from frass and residual substrate by sieving and rinsed thoroughly with distilled water. Cleaned larvae were subsequently freeze-dried at −55 °C under a pressure of 50 Pa using a laboratory-scale freeze dryer (Scanvac CoolSafe Pro SL12a02, Labogene, Allerød, Denmark). The dried samples were ground into a fine flour using a Multiquick System 100 blender (BRAUN, Frankfurt, Germany). The resulting powders were sealed in airtight zipper bags and stored at −20 °C until further analyses were performed.

Analysis of nutritional composition

Crude protein content was quantified using the Kjeldahl nitrogen determination method, applying a nitrogen-to-protein conversion factor of 6.25 **(Yu *et al.*, 2021; Boulos *et al.*, 2020).** Lipid content was measured by Soxhlet extraction according to the protocols of the Association of German Agricultural Analytic and Research Institutes, using petroleum benzine as the extraction solvent **(Forschungsanstalten, 2013).**
Ash content of the insect flour was determined following the AOAC official method 942.05 **(Horwitz and Latimer, 2000).** Crude fiber content was analyzed in accordance with the procedures described by the Association of German

Agricultural Analytic and Research Institutes **(Forschungsanstalten, 2013).** Carbohydrate content was calculated by difference, subtracting the sum of protein, fat, ash, and fiber from 100%. All nutritional components were expressed as percentages on a fresh weight basis.

Antioxidant activity

Antioxidant capacity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. The analysis was performed in 96-well microplates (Greiner Bio-One International GmbH, Germany), with each well containing 100 µL of DPPH solution (165 µM prepared in methanol; Sigma-Aldrich) and 100 µL of the test sample. Sample extracts were prepared by incubating 0.2 g of lyophilized material in 5 mL of a methanol/water mixture (1:5, v/v) for 30 min.
Following sample addition, the reaction mixtures were incubated in the dark at 30 °C for 30 min. Absorbance was subsequently measured at 517 nm using a UV–Vis microplate spectrophotometer (BioTek, USA). Antioxidant activity was expressed as µg mL^{-1}, and calculations were performed according to the method described by **Rajauria *et al.* (2013).**

$$\text{Radical scavenging activity (\%)} = 100\times \left[1-\left(\frac{(AS-AC)}{AB}\right)\right]$$

in which: Ac: absorbance of control, AS: absorbance of sample, AB: Absorption rate of the blank

Catalase (CAT)

Catalase (CAT; EC 1.11.1.6) activity was determined using a reaction system composed of 50 mM potassium phosphate ($K–PO_4$) buffer adjusted to pH 7.0, distilled water, 12.5 mM hydrogen peroxide (H_2O_2), and 10 g of powdered sample. Enzymatic activity was monitored spectrophotometrically by measuring absorbance at 540 nm, following the procedure described by **Heikal *et al.* (2012).**

Superoxide dismutase (SOD)

Superoxide dismutase (SOD; EC 1.15.1.1) activity was determined using a colorimetric assay based on the formation of formazan. The reaction mixture consisted of 50 mM potassium phosphate ($K–PO_4$) buffer (pH 7.8), 0.1 mM ethylenediaminetetraacetic acid (EDTA), 75 mM nitroblue tetrazolium (NBT), riboflavin (7×10^{-6} M), and 10 g of powdered sample.
Following incubation, absorbance was recorded at 560 nm. A negative control containing all reagents except the cell-free extract was exposed to fluorescent light and used as the reference for activity calculation. Enzyme activity was expressed as U min^{-1} mg^{-1} protein, in accordance with the method described by **Halliwell and Foyer (1978).**

Glutathione peroxidase (GP_X)

Glutathione peroxidase (GPx; EC 1.11.1.9) activity was measured using a coupled enzymatic assay. The reaction mixture consisted of 50 mM potassium phosphate ($K–PO_4$) buffer (pH 7.0), sodium ethylenediaminetetraacetic acid (Na_2EDTA), 0.28 µM NADPH, 13 µmol of reduced glutathione (GSH), and 10 g of powdered sample.
In this system, GPx catalyzes the reduction of organic hydroperoxides, generating oxidized glutathione. The oxidized form is subsequently converted back to reduced glutathione by glutathione reductase (GR) in the presence of NADPH. Oxidation of NADPH to $NADP^+$ results in a decrease in absorbance at 340 nm, which was monitored spectrophotometrically. The rate of decline in absorbance at 340 nm was directly proportional to GPx activity. Enzyme activity was expressed as nmol min^{-1} mg^{-1} protein, following the method described by **Hopkins and Tudhope (1973).**

Antimicrobial activity

Antimicrobial activity was evaluated using an agar well diffusion assay with minor modifications to previously reported methods **(de la Fuente-Salcido *et al.*, 2012; Al-Thubiani *et al.*, 2018).** Fresh Tryptic Soy Agar (TSA) was prepared, cooled to 40–50 °C, and 25 mL was poured into sterile Petri dishes previously inoculated with 115 µL of bacterial suspension (1×10^9 cells mL^{-1}). The tested microorganisms included Gram-positive strains Staphylococcus aureus (PTCC 1112), Bacillus subtilis (PTCC 1023), and Bacillus cereus (PTCC 1015), as well as Gram-negative strains Escherichia coli (PTCC 1047), Pseudomonas aeruginosa (PTCC 1310), and Salmonella typhi (PTCC 1609). All bacterial strains were obtained from laboratory stock cultures.
After agar solidification, wells measuring 6 mm in diameter and depth were aseptically created using sterile cork borers. Aliquots of 90 µL of each sample were added to the wells and allowed to diffuse at room temperature for 30 min prior to incubation. Plates were then incubated at 37 °C for 24 h. Antimicrobial activity was assessed by measuring the diameter of inhibition zones (mm) surrounding each well.
Standard antibiotic discs were used as positive controls for comparison. Tetracycline, oxacillin, and vancomycin discs were applied for Gram-positive bacteria, while sulfamethoxazole–trimethoprim (SXT), gentamicin, and ciprofloxacin discs were used for Gram-negative bacteria. All assays were performed in triplicate, and results were reported as mean values.

GC-MS analysis

Volatile compounds were analyzed using a gas chromatography system (Agilent Technologies 7890B GC, Waldbronn, Germany) coupled to a triple quadrupole mass spectrometer (Agilent Technologies 7010-MS). Helium was used as the carrier gas at a constant flow rate of 1.5 mL min^{-1}. Separation was achieved on an HP-Innowax capillary column (30 m × 250 µm i.d., 0.25 µm film thickness; Agilent Technologies Inc., Santa Clara, CA, USA).
Samples were injected in splitless mode using a split/splitless injector maintained at 300 °C, with a purge flow of 50 mL min^{-1} and a purge time of 1.5 min. A single taper liner was employed, and the injection volume was set to 1 µL. The oven temperature program began at 40 °C and was held for 3 min, followed by a temperature increase to 265 °C at a rate of 25 °C min^{-1} with a holding time of 12.5 min. A second ramp of 10 °C min^{-1} was applied to reach 270 °C, which was maintained for 2 min. The total chromatographic run time was 27 min, including a solvent delay of 4.5 min.
Electron impact ionization was applied at an energy of 70 eV. Mass spectra were acquired in full-scan mode over the range of 40–700 amu. Data acquisition, processing, and spectral deconvolution were performed using MassHunter software (Agilent Technologies Inc., Santa Clara, CA, USA). Compound identification was achieved by comparison with the NIST11 mass spectral library, and additional spectral interpretation for polystyrene oligomers was based on previously published data **(Tsochatzis *et al.*, 2020).**

Statistical analysis

All statistical analyses were performed using SPSS software (version 26; SPSS Inc., Chicago, IL, USA). Experimental data are reported as mean values accompanied by the standard error of the mean (SEM). Differences among experimental groups were evaluated using one-way analysis of variance (ANOVA). Statistical significance was defined at a probability level of $P \leq 0.05$.

RESULTS

The results of measuring protein, fat, ash, fiber and carbohydrates

The results showed that with the increase in the concentration of *S. platensis*, the percentage of protein, fat, ash, fiber, and carbohydrates increased with a significant difference. In addition, DBD treatment on *S. platensis* biomass with different concentrations had a significant effect on the amount of protein, fat, ash, fiber, and carbohydrates. According to the results, the percentage of protein, fat, ash, fiber, and carbohydrates in a 20% concentration of *S. platensis* under DBD treatment increased by 1.30, 1.28, 1.28, 1.67, 1.27, and 1.93 times ($p \leq 0.05$) (Figure 4).

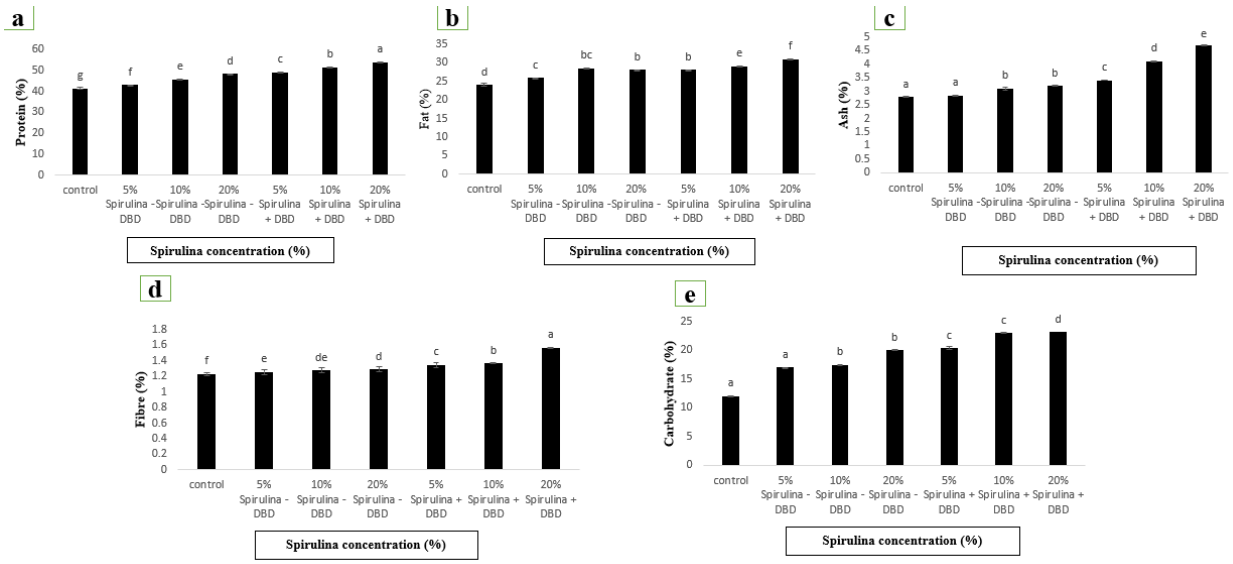

Figure 4 Results of measuring protein, fat, ash, fiber and carbohydrate (%) of larvae in the control and different feeding treatments with different concentrations of *S. platensis* treated and untreated *DBD*.

Measurement of antioxidant activity by DPPH method

The results demonstrated a significant increase in antioxidant percentage as the concentration of *S. platensis* increased. The larvae fed with *S. platensis* without DBD treatment and those not fed with *S. platensis* had significantly lower antioxidant levels than those treated with DBD. The highest amount of antioxidants with a significant difference belonged to the treatment with 20% DBD, which was 1.18 times the control ($p \leq 0.05$) (Figure 5).

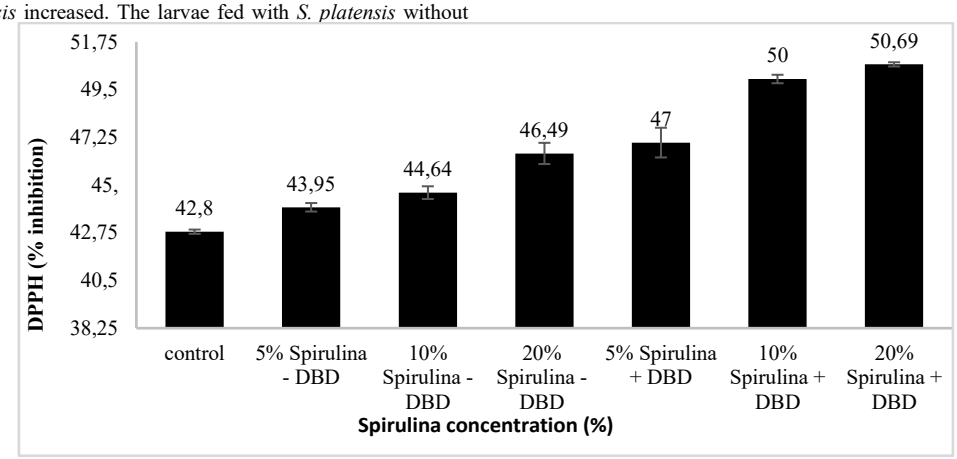

Figure 5 The results of the antioxidant percentage of larvae in the control and different feeding treatments with different concentrations of *S. platensis* treated and untreated DBD

Measurement of CAT, SOD AND GP_X activity

The results showed that with increasing the concentration of *S. platensis*, the amount of CAT, SOD and GP_x enzymes gradually increased with a significant difference. The amount of these enzymes in the larvae fed with *S. platensis* without DBD treatment and not fed with *S. platensis* was significantly lower than the DBD -treated ones. The highest levels of CAT, SOD and GP_x enzymes with a significant difference belonged to the treatment with 20% DBD treatment, which was 1.6, 1.26, and 2.019 times the control, respectively ($p \leq 0.05$) (Figure 6).

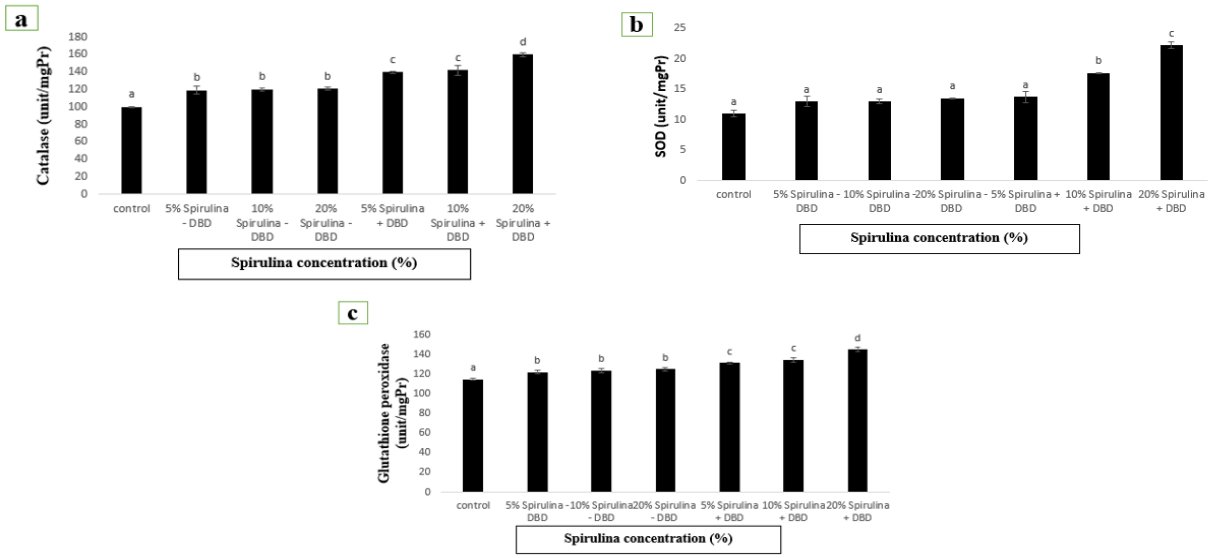

Figure 6 Results of CAT, SOD and GP_x of larvae in control and different feeding treatments with different concentrations of *S. platensis* treated and untreated with DBD

MEASUREMENT OF ANTIMICROBIAL ACTIVITY

The growth inhibition diameter of *Bacillus subtilis* (PTCC 1023), *Bacillus cereus* (PTCC 1015), *Pseudomonas aeruginosa* (PTCC 1310), *Salmonella typhi* (PTCC 1609), *Staphylococcus aureus* (PTCC 1112), and *Escherichia coli* (PTCC 1047) showed a gradual increase with a significant difference as the concentration of *S. platensis* increased. The growth inhibition diameter of the larvae fed with *S. platensis* without DBD treatment and not fed with *S. platensis* was significantly lower than the DBD -fed treatments. The highest growth inhibition diameter in *Bacillus subtilis* (PTCC 1023), *Bacillus cereus* (PTCC 1015), and *Pseudomonas aeruginosa* (PTCC 1310) with a significant difference belonged to the 20% DBD treatment, which was 5.25, 4.25, and 2.4 compared to the control, respectively ($p \leq 0.05$) (Figure 7 and 8).

The results of the diameter of inhibition of growth of *Salmonella typhi* (PTCC 1609) and *Escherichia coli* (PTCC 1047) also showed that the highest diameter of inhibition of growth with a significant difference belonged to the treatment with 20% and 10% DBD, which were 5.8 and 5.5 times compared to the control for *Salmonella* sp. and 4.07 and 4 times for *Escherichia* sp ($p \leq 0.05$) (Figure 7 and 8).

The results of the growth inhibition diameter of *Staphylococcus aureus* (PTCC 1112) also showed that the highest growth inhibition diameter with a significant difference belonged to the 20%, 10%, and 5% DBD treatments, which were 2.6, 2.6, and 2 times compared to the control, respectively ($p \leq 0.05$) (Figure 7 and 8).

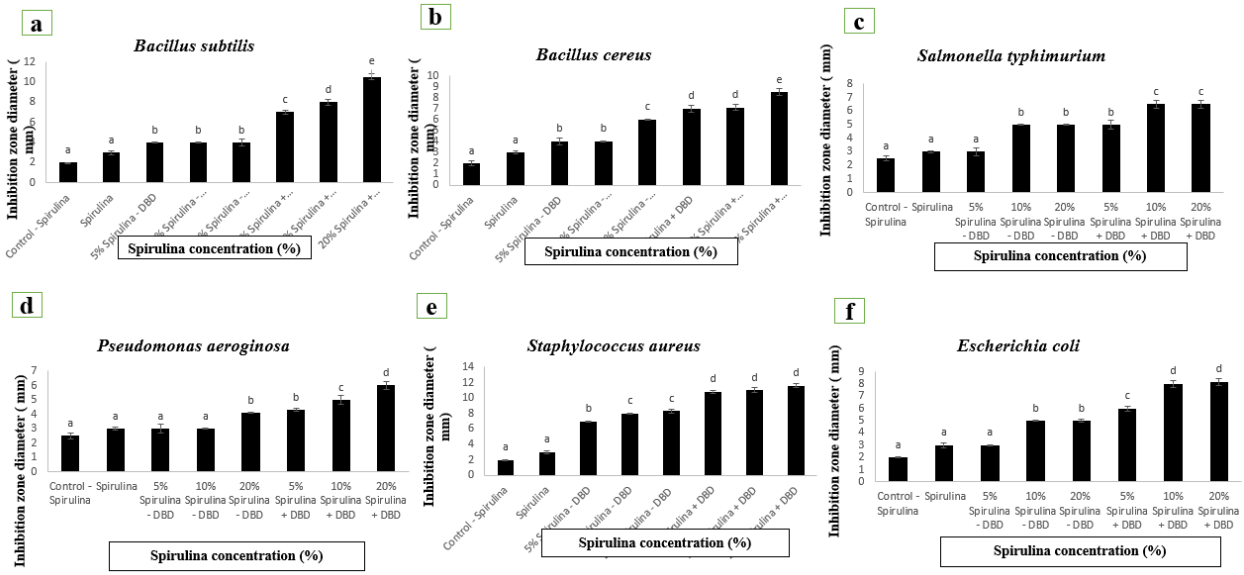

Figure 7 Growth inhibition diameter of *Bacillus subtilis* (PTCC 1023), *Bacillus cereus* (PTCC 1015) *Pseudomonas aeroginosa* (PTCC 1310), *Salmonella typhi* (PTCC 1609), *Staphylococcus aureus* (PTCC 1112) and *Escherichia coli* (PTCC 1047) larvae in control and different feeding treatments with different concentrations of *S. platensis* treated and untreated with DBD.

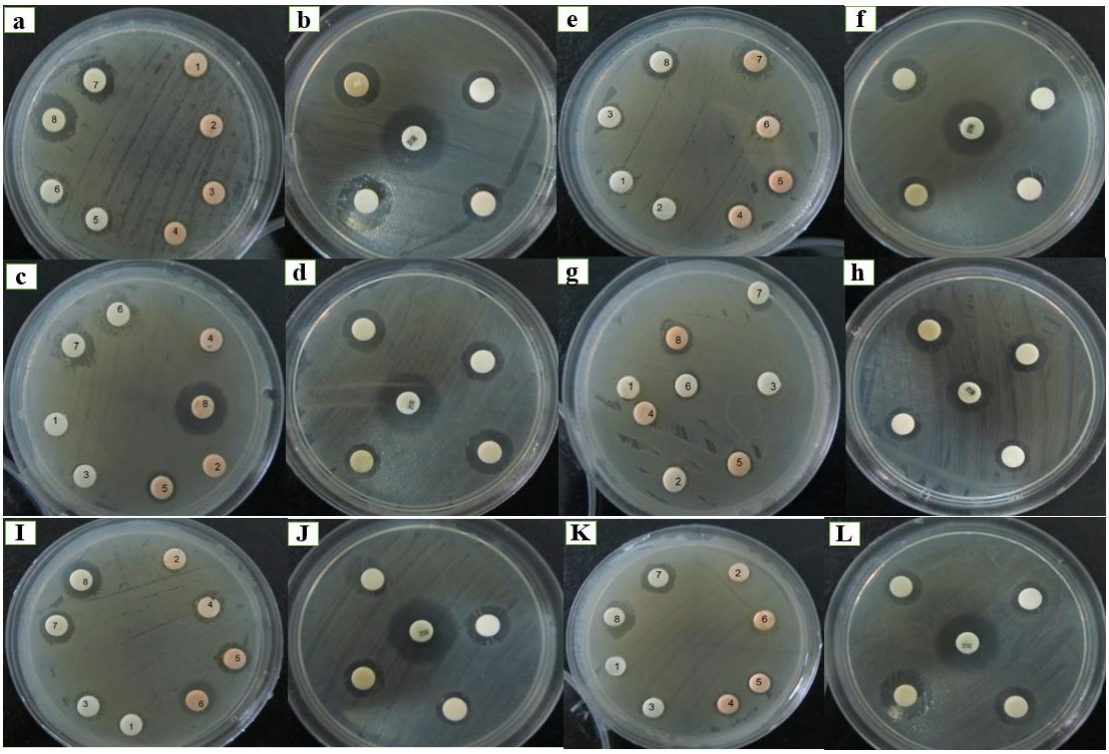

Figure 8 The results of the growth inhibition diameter of larvae in the control and different feeding treatments with different concentrations of *S. platensis* treated and untreated with DBD. (a) *Bacillus subtilis* (PTCC 1023), (c) *Bacillus cereus* (PTCC 1015), (e) *Salmonella typhi* (PTCC 1609), (g) *Pseudomonas aeroginosa* (PTCC 1310), (I) *Staphylococcus aureus* (PTCC 1112), (k) *Escherichia coli* (PTCC 1047), (b, d, f, h, j, and l) different replicates of the extract of different replicates of the larval extract fed with 20% *S. platensis* powder under DBD treatment. *1: Larva extract not fed with *S. platensis* 2: *S. platensis* extract alone 3: Larva extract fed with 5% *S. platensis* without DBD 4: Larva extract fed with 10% *S. platensis* without DBD 5: Larva extract fed with 20% *S. platensis* without DBD 6: Larva extract fed with *S. platensis* powder under DBD treatment (5 %). 7: Larva extract fed with *S. platensis* powder under DBD treatment (10 %).

Measuring the volatile compounds by gas chromatography-mass spectrometry (GC/MS) analysis

The results of analyzing the volatile compounds of the samples with the help of GC-MS in Tables 1 and 2 showed that about 21 compounds were found in the extract of larvae not fed with *S. platensis*. While in samples fed with *S. platensis* under DBD treatment and without it, only 8 compounds of Lauric acid, Palmitic acid, 9,12-Octadecadienoic acid, Oleic Acid, 9-Octadecenoic acid, Stearic acid, Tranylcypromine, pentafluorobenzoyl, and 1,1,1,3,5,5,5-Heptamethyltrisiloxan were found. Examining the percent abundance of the compounds revealed that it was higher in all feeding treatments compared to the control; additionally, it was significantly higher under DBD treatment at different concentrations than in treatments without DBD. The highest and lowest abundance percentages in larval extract fed with 5% *S. platensis* without DBD belong to palmitic acid and oleic acid. The highest and lowest amounts of palmitic acid and 9-octadecenoic acid were found in the extract of larvae that were fed 10% *S. platensis* without DBD treatment. The highest and lowest amounts of palmitic acid and 9-octadecenoic acid were found in the extract of larvae that were fed 20% *S. platensis* without DBD treatment. Palmitic acid and stearic acid had the highest and lowest abundance percentages in the extract of larvae fed with *S. platensis* under 5% DBD treatment. Palmitic acid and stearic acid had the highest and lowest abundance percentages in the extract of larvae fed with *S. platensis* under 10% DBD treatment. Palmitic acid and stearic acid had the highest and lowest abundance percentages in the extract of larvae fed with *S. platensis* under 20% DBD treatment.

Table 1 The results of volatile compounds extracted from GC/MS samples treated and untreated DBD.

(a) Larvae not fed with *S. platensis*			
Compound	**RT/min**	**Area**	**Abundance (%)**
Lauric acid	29.23	4928974	0.93%
4-Methyl-7-methoxyisatin	30.03	242300927	45.67%
2-(4-METHYLPHENYLTHIO)-5-METHYLPHEN	30.33	5373297	1.01%
Myristic acid	33.94	2522517	0.48%
3-Methyl-1-phenylpyrrolidine-2,5-dione	35.47	4821418	0.91%
2-Hydroxy-4-methoxybenzaldehyde	38.39	33339265	6.28%
4-cyclopropyl-2,3,3a,4,5,6-hexahydro-1H-indole	38.57	46874073	8.83%
Palmitic acid	38.72	13964481	2.63%
1-(p-sul-phophenyl)-3-methyl-5-pyrazolone	39.94	4406918	0.83%
6H-Purin-6-one, 1,7-dihydro-	40.49	3234948	0.61%
1,2-dioxo-3,8-dihydr	40.73	6169095	1.16%
3-Hepten-2-one, 3-propyl	41.05	7964958	% 1.50
Dimethyl 4,4'-methylenebis(3-methoxy-2-naphthoate)	41.7	3659620	0.69%
9,12-Octadecadienoic acid, methyl ester	42.22	2058275	5.76%
Oleic Acid	42.38	9343497	1.76%
9-Octadecenoic acid	42.46	4685193	0.88%
Stearic acid	42.87	3646664	0.69%
Tranylcypromine, pentafluorobenzoyl	45.10	3790711	% 14.87
Tranylcypromine, pentafluorobenzoyl	45.65	3171839	% 12.44
1,1,1,3,5,5,5-Heptamethyltrisiloxan	45.77	1201727	% 4.71
Tranylcypromine, pentafluorobenzoyl ester	46.17	1220147	% 4.79
(b) Larvae extract fed with 5% non- DBD -infused *S. platensis*			
Compound	**RT/min**	**Area**	**Abundance (%)**
Lauric acid	29.17	2936241	8.22%
Palmitic acid	38.72	8551807	23.93%
9,12-Octadecadienoic acid, methyl ester	42.22	1558275	6.1%
Oleic Acid	42.29	1143497	1.6%
9-Octadecenoic acid	42.4	1096778	1.13%
Stearic acid	42.89	1271210	4.68%
Tranylcypromine, pentafluorobenzoyl	53.67	1664131	6.66%
1,1,1,3,5,5,5-Heptamethyltrisiloxan	54.27	1326073	5.31%

(c) Larvae extract fed with 10% non- DBD -infused *S. platensis*			
Compound	**RT/min**	**Area**	**Abundance (%)**
Lauric acid	29.17	1836241	9.11%
9,12-Octadecadienoic acid, methyl ester	40.15	858200	7.16%
Oleic Acid	41.15	925453	% 7.4
Stearic acid	42.12	1523820	% 8.87
Palmitic acid	42.7	1998029	55.11%
9-Octadecenoic acid	42.9	596778	2.18%
1,1,1,3,5,5,5-Heptamethyltrisiloxan	45.73	749810	% 6.27
Tranylcypromine, pentafluorobenzoyl	54.39	1070780	7.89%
(d) Larvae extract fed with 20% non- DBD -infused *S. platensis*			
Compound	**RT/min**	**Area**	**Abundance (%)**
Lauric acid	29.17	1536241	9.55%
Palmitic acid	38.66	2543858	50.56%
9,12-Octadecadienoic acid, methyl ester	42.22	1308275	9.43%
9-Octadecenoic acid	42.35	987645	3.4%
Oleic Acid	42.38	1713497	9.7%
Stearic acid	42.89	1671210	9.68%
1,1,1,3,5,5,5-Heptamethyltrisiloxan	45.73	1004810	% 7.27
Tranylcypromine, pentafluorobenzoyl	46.57	1134262	% 8.93
(e) Larvae fed with 5% DBD -infused *S. platensis*			
Compound	**RT/min**	**Area**	**Abundance (%)**
Lauric acid	29.17	1536241	10.22%
Palmitic acid	38.68	3096570	44.68%
Oleic acid	42.10	767118	% 6.25
9,12-Octadecadienoic acid, methyl ester	42.22	2058275	10.6%
Stearic acid	42.41	633907	% 5.26
9-Octadecenoic acid	42.4	1096778	10.13%
1,1,1,3,5,5,5-Heptamethyltrisiloxan	45.73	849810	% 7.27
Tranylcypromine, pentafluorobenzoyl	53.91	96338	8.93%
(f) Larvae fed with 10% DBD -infused *S. platensis*			
Compound	**RT/min**	**Area**	**Abundance (%)**
Lauric acid	29.17	2936241	19.56%
Palmitic acid	38.68	3067003	29.74%
9,12-Octadecadienoic acid, methyl ester	42.22	2058275	12.74%
Oleic Acid	42.38	2343497	13.76%
9-Octadecenoic acid	42.4	2696778	16.23%
Stearic acid	42.89	1671210	6.17%
1,1,1,3,5,5,5-Heptamethyltrisiloxan	45.73	1949810	% 12.27
Tranylcypromine, pentafluorobenzoyl	54.15	1551391	9.53%
(g) Larvae fed with 20% DBD -infused *S. platensis*			
Compound	**RT/min**	**Area**	**Abundance (%)**

Lauric acid	29.17	10880622	28.72%
Palmitic acid	38.66	8456011	22.32%
9,12-Octadecadienoic acid, methyl ester	42.19	2095887	15.98%
9-Octadecenoic acid	42.35	7316821	19.31%
Oleic Acid	42.38	9343497	19.6%
Stearic acid	42.89	1671210	9.8%
1,1,1,3,5,5,5-Heptamethyltrisiloxane	45.73	3649810	% 15.27
Tranylcypromine, pentafluorobenzoyl	53.47	1022945	12.70%

Table 2 Comparison of volatile compounds extracted from GC/MS samples treated and untreated DBD.

Compound	**Changes**	**5%+ DBD**	**10%+ DBD**	**20%+ DBD**	**Control**	**5%- DBD**	**10%- DBD**	**20%- DBD**	**Changes**
	Abundance (%)								
Lauric acid	Increase	10.22%	19.56%	28.72%	0.93%	8.22%	9.11%	9.55%	Increase
Palmitic acid	Increase	44.68%	0.74%	22.32%	2.63%	23.93%	55.11%	50.56%	Increase
9,12-Octadecadienoic acid, methyl ester	Increase	10.6%	12.74%	15.98%	5.76%	6.1%	7.16%	9.43%	Increase
Oleic Acid	Increase	% 6.25	13.76%	19.6%	0.69%	1.6%	% 7.4	9.7%	Increase
9-Octadecenoic acid	Increase	10.13%	16.23%	19.31%	0.88%	1.13%	2.18%	4.4%	Increase
Stearic acid	Increase	% 5.26	6.17%	9.8%	0.69%	4.68%	% 8.87	9.68%	Increase
Tranylcypromine, pentafluorobenzoyl ester	Increase	8.93%	9.58%	12.70%	% 4.79	6.66%	7.89%	% 8.93	Increase
1,1,1,3,5,5,5-Heptamethyltrisiloxane	Increase	% 7.27	% 12.27	% 15.27	% 4.71	5.31%	% 6.27	% 7.27	Increase

DISCUSSION

Cold atmospheric plasma (CAP) has emerged as a promising tool in food processing due to its ability to modify nutritional and microbiological characteristics without the use of high temperatures. Given the growing interest in edible insects and microalgae as sustainable food resources, understanding how CAP interacts with *Tenebrio molitor* larvae and *Spirulina platensis* biomass is particularly relevant. In this context, the present study explored how the combination of *S. platensis* supplementation and dielectric barrier discharge (DBD) treatment influences the antimicrobial and antioxidant properties of *T. molitor* larvae.

Previous studies show that changes in rearing diet can significantly influence insect nutritional composition, including protein, lipid, ash, fiber, and carbohydrate contents **(Sánchez-Muros *et al.*, 2014).** Previous studies have demonstrated that modifying feed composition can substantially influence the nutritional profile of insects, including protein, lipid, ash, fiber, and carbohydrate contents **(Sánchez-Muros *et al.*, 2014).** In parallel, *S. platensis* has attracted increasing attention in animal nutrition due to its high protein content, antioxidant capacity, antimicrobial activity, and potential to enhance growth performance and immune function. Its incorporation into various food matrices, such as pasta, snacks, and cereal-based products, has consistently been shown to improve nutritional quality, particularly with respect to protein, lipid, and mineral content **(Lucas *et al.*, 2018; Tańska *et al.*, 2017; Koli *et al.*, 2022; El-Hameed** *et al.*, 2018). These findings support the use of *S. platensis* as a functional dietary component capable of enhancing the nutritional value of both conventional foods and alternative protein sources.

In the present study, increasing dietary levels of *S. platensis* resulted in higher protein, fat, ash, fiber, and carbohydrate contents in *T. molitor* larvae compared with non-supplemented controls. These effects were further amplified when *S. platensis* biomass was subjected to DBD treatment, indicating that plasma processing enhanced the nutritional impact of the cyanobacterial supplement. Similar compositional values have been reported for mealworm larvae used in livestock and poultry feed formulations, supporting the applicability of enriched larvae as a high-value feed ingredient **(Ostadi and Matofi, 2020).**

The observed increases in antioxidant capacity and enzyme activities (CAT, SOD, and GPx) with higher *S. platensis* concentrations and DBD treatment are consistent with the known antioxidant composition of cyanobacteria. Compounds such as phycocyanin, carotenoids, and phenolic constituents present in *S. platensis* are likely contributors to the enhanced antioxidant responses observed in larvae **(Wu *et al.*, 2005; El-Desoky *et al.*, 2013).** Comparable effects have been reported in animal feeding studies, including broiler chickens supplemented with *S. platensis*, which exhibited elevated antioxidant enzyme activity **(Park *et al.*, 2018).** These findings suggest that dietary enrichment combined with plasma treatment may enhance oxidative stress resistance in edible insects.

The antimicrobial effects observed in this study are likely attributable to the combined influence of *S. platensis* bioactive compounds and DBD treatment. Cyanobacterial metabolites such as peptides, polysaccharides, and lipid-associated molecules have previously been reported to exhibit bactericidal and bacteriostatic activity against foodborne and clinical pathogens **(Seghiri *et al.*, 2019; Maddiboyina *et al.*, 2023).** The enhanced inhibition zones observed at higher *S. platensis* concentrations, particularly under DBD treatment, suggest a synergistic interaction between dietary enrichment and plasma processing rather than a concentration-dependent effect alone.

The results indicated that the growth inhibition diameter of *Bacillus subtilis* (PTCC 1023), *Bacillus cereus* (PTCC 1015), *Salmonella typhi* (PTCC 1609), *Pseudomonas aeruginosa* (PTCC 1310), *Staphylococcus aureus* (PTCC 1112), and *Escherichia coli* (PTCC 1047) increases progressively with higher concentrations of *S. platensis*, demonstrating a significant difference. The 20% DBD treatment exhibited the highest growth inhibition diameter, demonstrating a significant difference. In contrast to our study, **María Consuelo Pina-Pérez *et al.* (2022)** examined the efficacy of CP on the microbial activity of *S. platensis* against *Salmonella enterica Serovar Typhimurium*, utilizing Caenorhabditis elegans as an animal model. The findings indicated that CP treatment enhances the biological properties of *S. platensis* **(Pina-Pérez *et al.*, 2022). Mahshid Sharifi Moghaddam *et al.* (2024)** studied the inactivation of *Bacillus subtilis* spores by phycoerythrin extracted from cyanobacterium *Desmonostoc* sp under CP. They showed that phycoerythrin under DBD plasma treatment decreased the microbial index by 0.82. Additionally, applying DBD plasma for 3 minutes at °C resulted in the inactivation of spores by 1.45 times up to the 30th day **(Moghaddam *et al.*, 2024). Flores *et al.* (2020)** investigated the antimicrobial properties of *Ulomoides dermestoides* and *Tenebrio molitor*. The findings indicated that these insects possess beneficial antimicrobial properties against *Shigella* and *Bacillus* bacteria **(Flores *et al.*, 2020).**

Analysis of volatile profiles using GC–MS demonstrated clear differences between larvae fed *S. platensis* and non-supplemented controls, with a reduced number of

dominant compounds detected in enriched samples. In the samples containing *S. platensis*, both treated and untreated DBD, only eight compounds of Lauric acid, Palmitic acid, 9,12-Octadecadienoic acid, Oleic acid, 9-Octadecenoic acid, Stearic acid, Tranylcypromine, pentafluorobenzoyl, and 1,1,1,3,5,5,5-Heptamethyltrisiloxane were identified. Analysis of the percent abundance of the compounds indicated that all feeding treatments exhibited higher levels than the control. Furthermore, the abundance was significantly greater in the DBD -treated groups across various concentrations compared to the groups without DBD treatment.

CONCLUSION

The growing focus on healthy foods has led to research on functional foods, with *S. platensis* being a nutrient-dense microalgae with phytochemical compounds suitable for functional products. Surface cold atmospheric pressure plasma is a cost-effective method for sterilizing food and pharmaceutical matrices. This study aimed to enhance the antimicrobial and antioxidant properties of the yellow cockroach, *Tenebrio molitor,* as a food substitute and incorporate edible insects into the food industry. The larvae treated with DBD showed increased levels of protein, fat, ash, fiber, carbohydrates, antioxidant activity, antimicrobial activity, and volatile compounds. The antioxidant activity by the DPPH method also showed that the highest value belonged to the treatment with 20% DBD. Results of catalase, superoxide dismutase and glutathione Peroxidase showed that the highest levels of these enzymes belonged to the treatment with 20% DBD. Furthermore, the results of the antibacterial analysis indicated a significant increase. The results of the analysis of volatile compounds showed the highest abundance belonged to palmitic acid, while the lowest value belonged to compounds of oleic acid, 9-octadecenoic acid, and stearic acid. The overall results showed that the combination of CP and cyanobacteria presents a promising technology for enhancing food properties in the food industry.

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