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CROP SCIENCE

Effect of the seed coating with biomass of *Dunaliella salina* on early plant growth and in the secondary metabolites content of *Coriandrum sativum*

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Abstract: The environmental and health risks associated with the application of synthetic chemical inputs in agriculture increased the demand for technologies that allow higher performance and quality of vegetable crops by implementing synergistic materials with the principles of sustainability. In this work, the seed coating with the biomass of *Dunaliella salina* incorporated in a bioplastic film of *Manihot esculenta* (cassava) was evaluated as an initial growth and secondary compounds stimulator of *Coriandrum sativum* (coriander) plants. The obtained results demonstrated that the coating stimulated an increase in the germination percentage (28.75%) and also in concentration of bioactive compounds, such as the six-fold increment of caffeic acid (13.33 mg 100 g^{-1}). The carbohydrates, lipids, and proteins present in the microalgae biomass seem to be responsible for these increments once they are known for providing energy to the seedling development and coordinating the secondary metabolites synthesis. As conclusion, we consider the coating with biomass of *D. salina* an alternative for crop improvement that contributes to the development of sustainable agricultural practices.

Key words: biomass, coating, coriander, *Dunaliella salina*, germination percentage, secondary metabolites.

INTRODUCTION

Seeds represent one of the most important raw materials in agriculture, and their markets are projected to exceed US$ 130 billion by 2022 (FAO 2018). The main reason of this growth comes from the use of chemical inputs of synthetic origin that, despite increasing germination performance, can cause negative effects on the environment and human health (Chandini et al. 2019). Due to the risks associated with those inputs, sustainable technological strategies are increasingly gaining more relevance in agriculture.

The employment of methods, such as seed coating, application of biofertilizers, biostimulants, and nanoparticles, have generated a significant increase in agricultural productivity (Chandrika et al. 2019, Salcedo et al. 2020, Venkatachalam et al. 2017). Among the aforementioned techniques, it is worth mentioning the coating, which allows a gradual and close to the root zone availability of nutrients to the vegetable. This technique has been carried out through the use of vegetable seeds due to their small sizes (Pedrini et al. 2017).

Coriander is one of the most common herbs employed in Brazilian cuisine, an annual plant of the family Apiaceae (Rashed & Darwesh 2015). This herb is constantly referred to as a "medicinal plant" owing to its gathering of pharmacological properties, such as anti-inflammatory and antioxidant activities. These properties are due to the presence of secondary metabolites such as the ellagic, gallic, chlorogenic, caffeic, coumaric, *trans*-cinnamic, vanillic, and ferulic acids, in addition to quercetin and kaempferol (Dua et al. 2014, Laribi et al. 2015). Therefore, many studies have been developing procedures that allow an increment in the biosynthesis of those products (Parić et al. 2017, Meng et al. 2019). However, there is not enough exploration of coating techniques for this purpose.

Even though the coating technique offers countless advantages, it may trigger the delay of the initial germination phase and restrict the gas exchange between the seed and the external environment (Pedrini et al. 2017). Therefore, the materials used in the coating must attend functionality and applicability requirements, which contribute to vegetable emergence.

Microalgae are renewable sources of a great variety of substances with high added value, such as polyunsaturated fatty acids, polysaccharides, vitamins, and amino acids (Tinoco et al. 2015). Among the microalgae species that hold a high content of these compounds in their constitution, it is worth emphasizing the *Dunaliella salina*. This species belongs to the Chlorophyta phylum and is eukaryotic, photosynthetic, unicellular, and devoid of rigid cell walls, thereby accumulating organic substances in its biomass (Oren 2005).

Due to the high added value of the microalgae biomass, it has been applied to the formulation of biofertilizers (Chiaiese et al. 2018). However, in literature there are few studies employing this biomass as a coating material since its rheological characteristics hamper its incorporation in the seed cover tissue. As a consequence, the vegetable performs an incomplete absorption of the biomass constituents. As a result, the application of bioplastic films of vegetal origin as stabilizers of biomass in the coating has become common, allowing a greater profit from those nutrients (Chandrika et al. 2019).

In this work, it was evaluated the implementation of the biomass of *D. salina* incorporated in the *Manihot esculenta* (cassava) biofilm as inputs of the seed coating technique in order to stimulate the development of *Coriandrum sativum* plants. This becomes possible as compounds, such as carbohydrates, lipids, and proteins, present in the biomass can activate a set of biochemical responses, enhancing not only the initial growth but also the production of secondary metabolites by the plant.

MATERIALS AND METHODS

Plant material

Organic coriander seeds (*Coriandrum sativum*) were commercially obtained from ISLA Sementes Ltda., Brazil.

Microalgae and culture medium

The strains of *Dunaliella salina* were provided by the Banco de Microalgas Iracema Nascimento (BMIN) in the biology institute of the Universidade Federal da Bahia (UFBA), Salvador, Brazil. The obtained inoculants were cultivated in 1 L Erlenmeyer flasks filled with modified Artificial Seawater (ASW) solution (Millero 1996), which consists of distilled water and chloride salts (g L^{-1}) added in the proportions of 1.16 $CaCl_2$, 30.07 NaCl, 5.20 $MgCl_2$, and 0.72 KCl (pH adjusted to 7.5 with HCl). To supplement the medium, 0.1 mL of vitaminic solution and 1 mL of Conway medium were added (Walne 1979). The microalgae growth

was promoted by continuous illumination with fluorescent lamps (40.5 μE m^{-2} s^{-1}) with a 24-hour photoperiod and constant aeration through injection of atmospheric air (Ores et al. 2016). The medium was maintained at room temperature (25°C). The cell concentration measurements were performed through an optical microscope (TNB-01B-Opton, Brazil) and hemocytometer (Zarei et al. 2016).

Obtention of Microalgae biomass

The biomass obtention procedure was performed when the maximum cell concentration was reached. The pretreatment of the cells with NaOH (g L^{-1} of microalgae solution) as well as its mechanical stirring for 3 min (3 rpm) and 15 min (1 rpm) allowed its separation from the supernatant. The vacuum filtration was carried out through Whatman® grade 4 qualitative filter paper to obtain biomass (Andrade & Filho 2014). The filtered product was dried in a vacuum oven (Olidef CZ, Brazil) at 60 °C for 24 h and pulverized in a mortar before its incorporation in the bioplastic film.

Production of bioplastic films

The bioplastic films were produced employing the casting method (Perotto et al. 2018). The initial step consisted in continuously heating and rotating (60°C; 1 rpm) a solution containing 20 g of cassava starch dispersed in distilled water. Subsequently, glycerin (USP grade, Synth, Brazil) was added to the mixture (Chiumarelli & Hubinger 2012). For the films with microalgae, the powdered biomass was homogenized with glycerin and the polymerization reaction occurred via base catalysis with addition of NaOH (1 mol L^{-1}). To perform the seed coating, the temperature and rotation conditions were maintained at 200°C and 5 rpm, respectively.

Treatment groups and seed coating

Coriander seeds were separated into three treatment groups: the uncoated, Control Group (CT); the coated with bioplastic film, Blank (BR); seeds coated with microalgae biomass incorporated in bioplastic film, Microalgae Biopolymer (BM). The groups with coating were prepared through the modified polymerization method (Giminéz-Sampaio & Sampaio 1994), which allowed the addition of 1.04 mg ± 0.23 of bioplastic film around each seed. The resulting material was dried through evaporation at room temperature (25°C) for 24 h.

Cultivation conditions and growth parameters definition

Coriander seeds were germinated in expanded polyethylene sheets containing sterile silica and incubated in a growth chamber for 15 days at 25°C under continuous illumination (40.5 μE m^{-2} s^{-1}) with a 12h light/12h dark photoperiod and daily irrigation. Thereafter, their seedlings were transplanted into 10.7 cm diameter plastic pots containing organic-matter rich substrate (Biomix, Substrato Mudas e Plantio Orgânico, São Paulo, Brazil). This substrate has in its composition: coconut fiber; crushed pine bark composted with the addition of organic macronutrients and micronutrients. Its physicochemical characteristics include pH 6.2 and 45% humidity. The plants were harvested after 15 days of being transferred to the pots.

In the development of the cultivation of the plants, the Germination Rate (GR) was calculated according to the equation: GR = Σ (number of seeds that germinated in a given time/time or day) (Maguire 1962). The seed was considered germinated when the radicles emerged. After plant harvesting, the Plant Height (PHe) as well as the Root Length (RL) were measured through a Vernier caliper (Marbeg, Brazil) and the Germination Percentage (G%) was determined

according to the following ratio: G% = (total of germinated seeds/total of planted seeds) × 100 (Chauhan et al. 2019).

Methanol extracts of coriander samples

Before the extract preparations, the plants were dried in a lyophilizer (Liotop – K202) at -42°C and 0.025 mbar. The resulting materials were homogenized in a domestic food processor and sieved through a Nylon sieve (300 µm). The extracts were prepared in triplicate by adding 0.5 g of the obtained sample, 30 mL of methanol HPLC grade, and 100 µL of hydrochloric acid 1% v/v. Then, the mixing was carried out for 30 minutes in an orbital shaker (Fisatom, Brazil), at 300 rpm and 25°C (Santos et al. 2017). The extracts were filtered and concentrated on a rotary evaporator (Fisatom, Brazil) at 15 rpm and 60°C. The obtained material was resuspended in 1.5 mL of methanol HPLC grade and stored in microtubes at -20°C. The extracts were filtered through polytetrafluoroethylene (PTFE) syringe filters (0.45 µm) for the HPLC analyses.

Total polyphenol content (TPC) determination

The total polyphenol content was determined through the modified method of Singleton & Rossi (1965). In short, 500 µL of the Folin-Ciocalteu reagent was added to 100 µL of the obtained extract, 400 µL of 7.5% (w/v) sodium carbonate, and 4 mL of ultrapure water. After 30 minutes, the samples were read at 760 nm in UV-Vis spectrophotometer (SP-22 Biospectro, Brazil). The obtained results were compared with a gallic acid standard curve (0.5 to 6.0 mg L^{-1}) and expressed as gallic acid equivalents per 100 g dry weight (mg GAE 100 g^{-1} DW). All samples were prepared in triplicate.

Total flavonoid content (TFC) determination

The total flavonoid content was quantified through colorimetric assay (Meda et al. 2005, Fan et al. 2015) with adaptations, which employed aluminum chloride ($AlCl_3$) as complexing agent. To prepare the solution, 3 mL of aluminum chloride (2.0% in methanol), 100 µL of the sample, and 6.9 mL of methanol HPLC grade were added to a 15 mL centrifuge tube. After 30 minutes, the samples were read at 415 nm in UV-Vis spectrophotometer. The results were determined through a quercetin standard curve (0 to 20 mg L^{-1}) and expressed in quercetin equivalents per 100 g dry weight (mg QE 100 g^{-1} DW). All samples were prepared in triplicate.

DPPH radical scavenging assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH, Merck, Germany) radical scavenging activity was determined according to the modified Brand-Williams et al. (1995) method. In order to perform the assay, 2.4 mg of DPPH were diluted in 100 mL of HPLC grade methanol and transferred to an amber glass vial. Subsequently, 3.9 mL of the prepared DPPH solution were added to 100 µL of the extract. After 11 minutes (measured by the kinetic test), readings were taken at 515 nm in a UV-Vis spectrophotometer. The results were defined according to the µmol Trolox equivalent antioxidant capacity per 100 g dry weight (µM TEAC 100 g^{-1} DW). All solutions were prepared in triplicate and in the absence of light.

Determination and quantification of secondary metabolites

The analysis of bioactive compounds was carried out through High-Performance Liquid Chromatography (HPLC) with a Shimadzu equipment (Shimadzu Scientific Instruments, Japan), which consists of a photodiode array detection (PDA) system (SPD-20A, Shimadzu), high-pressure quaternary pump (LC-20AD, Shimadzu), degasser vacuum, and high-pressure injector manual valve (20 µL injection loop). The LC-System software was used to control the

equipment and acquire data. The determination of secondary metabolites was carried out using the method that was developed and validated by Santos et al. (2017). The separation was performed through the "Licrhorspher®" RP-18 (Agilent) column (5,0 µm, 4.6 × 250 mm) at a temperature of 40 °C and a flow rate of 1.0 mL min^{-1}. The mobile phase consisted of (A) 1.0% acetic acid (v/v) and (B) methanol HPLC grade, eluted with the programmed gradient as follows: (A) 0-5 min, 100%; (A) 5-10 min, 70%; (A) 10-15 min, 10%; (A) 15-17 min, 70%; (A) 17-25 min, 100%. The compounds were identified according to the following relations between wavelength and retention time: ellagic acid (260 nm; 13.45 min); gallic acid (280 nm; 7.04 min); *trans*-cinnamic acid (280 nm; 14.98 min); coumaric acid (310 nm; 11.34 min); chlorogenic and caffeic acids (330 nm; 11.26 min).

Statistical analysis

Assays were performed with all treatments, each containing four repetitions of 20 seeds (a total of 80 seeds per treatment). The data gathered in the analysis of growth parameters was submitted to the Kruskal-Wallis test with post hoc comparisons of Dunn's test ($p < 0.05$) to evaluate the statistical difference between treatments. Both tests were performed through the Paleontological Statistics Software (PAST) 4.01 (Hammer et al. 2001) and expressed as mean ± standard deviation (SD). The data acquired through spectrophotometry was submitted to the Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA). Both analyses were performed through the STATISTICA 7.0 software. The values obtained through chromatography and spectrophotometry were subjected to the Multivariate Analysis of Variance (MANOVA) to analyze variation among treatments, followed by the Tukey test to assess the statistical difference between treatments, both expressed as mean ± SD.

RESULTS AND DISCUSSION

Incorporation of *D. salina* biomass in the coating promotes growth in coriander plants

To evaluate the effect of the coating with microalgae biomass in coriander plants, analyses were conducted to measure the effects in plant growth parameters. The parameters G%, PHe, and RL demonstrated that the incorporation of biomass influenced coriander growth. However, parameters, such as GR, did not present significant differences among the treatments (Figure 1a), which demonstrates that the coating did not suppress the initial germination phase. These results contrast with most recent studies (Qiu et al. 2020).

In agreement with those results, the G% did not indicate a statistically significant difference when comparing the control group with the coating with only bioplastic film (Figure 1b). On the other hand, when biomass was incorporated in the biopolymer for the coating, the results indicated a significant increase (28.75%), which demonstrates the synergistic effect between the used materials and the efficiency of *D. salina* as a promoter of seed development. In a similar study, the biomass of *Selenastrum capricornutum* and *Chlorella sorokiniana* was applied as an input to seed coating. Nevertheless, it resulted in germination percentages equivalent to those of their control group (Montanhim et al. 2014).

The PHe and RL analyses indicated that there was a significant difference between the treatment groups and the source of data variability being the BM treatment (Figures 1c and 1d). Recent studies achieved resembling benefits in plant height and root length when applying algae extracts in tomato and lettuce species (Salcedo et al. 2020, Torres et al. 2018).

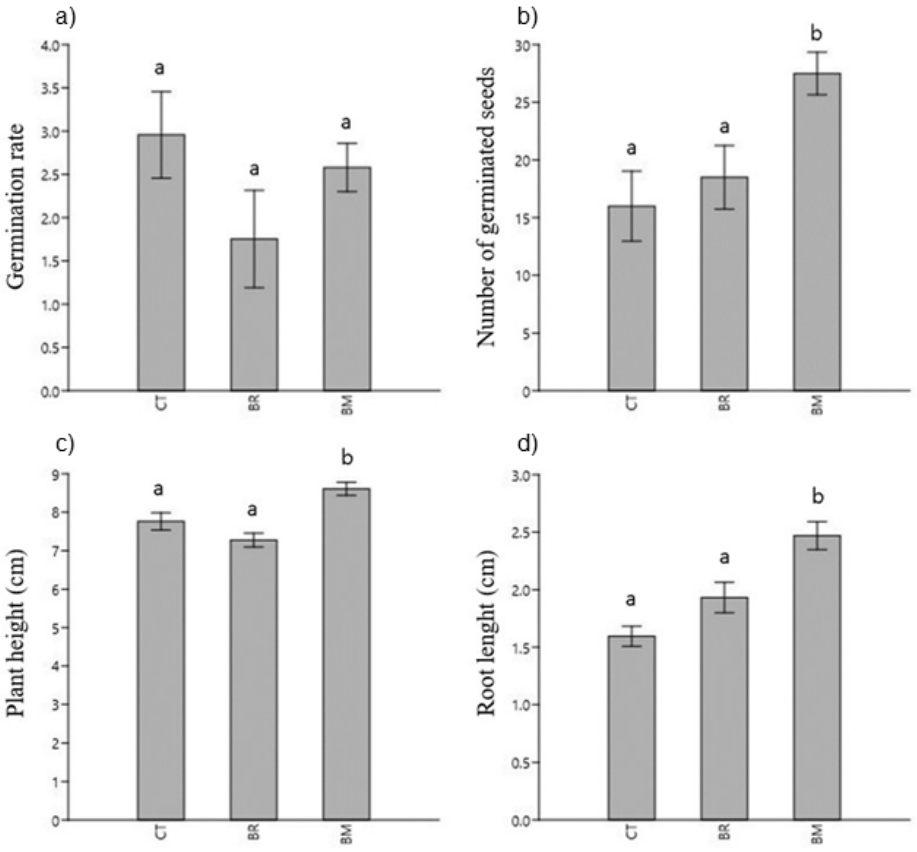

Figure 1. Effect of seed coating in Germination Rate (a), Germination Percentage (b), Plant Height (c), and Root Length (d). Control Group (CT), Blank (BR), and Microalgae Biopolymer (BM). The values were displayed with mean ± SD. Significant differences between samples ($p < 0.05$), measured by the Kruskal-Wallis test and Dunn's test, are indicated by distinct superscript letters (a and b), while non-significant differences are indicated by the same superscript letters.

This influence has been related to the presence of organic stimulants in biomass, such as polysaccharides, fatty acids, vitamins, amino acids, and phytohormones (Chiaiese et al. 2018). Different microalgae species, including the *D. salina*, are known to show 20 to 40% of carbohydrates and 8 to 15% of lipids in their organic compositions, and these compounds are essential nutrients to the development and germination of plants (Ferreira & Borghetti 2013, Pandey et al. 2015).

The phenolic compounds are secondary metabolites which play an important role in inhibiting the oxidation of various substrates. To evaluate whether the microalgae biomass can stimulate those compounds, analyses were performed for the determination of TPC and TFC, and to measure its influence in antioxidant activity, the DPPH analysis was carried out.

The phenolic and flavonoid content increased by approximately 3.5 and 5.0 times, respectively, when the biomass was incorporated in the coating (Table I). On the other hand, the radical DPPH showed a different behavior as it achieved reduced concentrations in coated treatments. This occurred since the presence of samples with a higher content of phenolic compounds contributed to reduce more effectively the DPPH radical (Pereira et al. 2013). Thus, the treatment group with the highest phenolic content (BM) exhibited 51.07% of radical DPPH in the medium while the control group had 86.48%, indicating that the coating with microalgae promoted a greater antioxidant capacity against free radicals.

Previous studies indicate that the application of plant hormones also causes an expressive increase in the phenolic compounds and antioxidant activity of species, such as

Table I. Effect of the seed coating in Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and radical scavenging activity (DPPH) in coriander plants.

Determinations	CT	BR	BM
TPC (mg GAE 100 g^{-1} DW)	92.11^{d} ± 2.52	264.37^{c} ± 0.63	325.07^{b} ± 2.80
TFC (mg QE 100 g^{-1} DW)	118.69^{d} ± 3.19	190.59^{c} ± 1.29	579.06^{a} ± 3.54
DPPH (µM TEAC 100 g^{-1} DW)	86.48^{a} ± 0.51	82.33^{b} ± 2.16	51.07^{c} ± 0.19

Control Group (CT), Blank (BR), Dry Weight (DW), and Microalgae Biopolymer (BM). The values represent the means of the triplicates ± SD. Significant differences between samples ($p < 0.05$), measured by the MANOVA and Tukey test, are indicated by distinct superscript letters (a, b, c and d), while non-significant differences are indicated by the same superscript letters.

Vitis vinifera and *Mentha piperita*. According to them, this increase occurred due to the fact that the plant hormones stimulated the enzymatic activity responsible for the synthesis of phenolic compounds (Parić et al. 2017, Meng et al. 2019). Likewise, *D. salina* contains compounds in its constitution, such as amino acids, that compose 30 to 50% of its biomass and can act in the synthesis of plant secondary metabolites (Ferreira & Borghetti 2013, Pandey et al. 2015).

Chemometric analyses confirmed the influence of microalgae biomass in the secondary metabolism of coriander plants

In order to confirm the correlation between the microalgae biomass and the increase of phenolic compounds in coriander, the PCA and HCA were applied. The PCA method demonstrated this relation through the First Principal Component (PC1) and Second Principal Component (PC2), which described 99.93% of the total variance. The eigenvalues for PC1 and PC2, were respectively, 2.71 and 0.28. Based on the Kaiser criterion, eigenvalues higher than one are considered reliable (Kaiser 1960), which demonstrates that the PC1 had a more influence on the data variance. The score plots (Figure 2) indicate these influences, where the first principal component provided discrimination between the phenolic compounds (TPC and TFC) and the radical scavenging activity (DPPH).

Table II shows the factor responsible for separating those variables based on the distribution of the samples in the first principal component. As displayed in the table, the BM treatment had more weight in the separation of variables, while the BR treatment exhibited less weight but contributed the most in the second principal component. This process occurred due to the high concentration of phenolic compounds and antioxidant activity in the BM treatment (Table I). As can be seen in Figure 3, the BM sample is clustered on the negative portion of the PC1, similar to the TPC and TFC variables, while the CT and BR samples are located in the positive portion of the PC1, corresponding to the DPPH variable.

In accordance, the HCA analysis confirmed tendencies observed in previous results through the creation of two clusters. As shown in Figure 4, the treatments that did not contain microalgae biomass (CT and BR) built a cluster, while the treatment coated with biomass (BM) was arranged separately from the others in the dendrogram, thus forming another cluster. This confirms that the microalgae biomass was a prominent factor to the differentiation of samples by increasing the concentration of phenolic compounds.

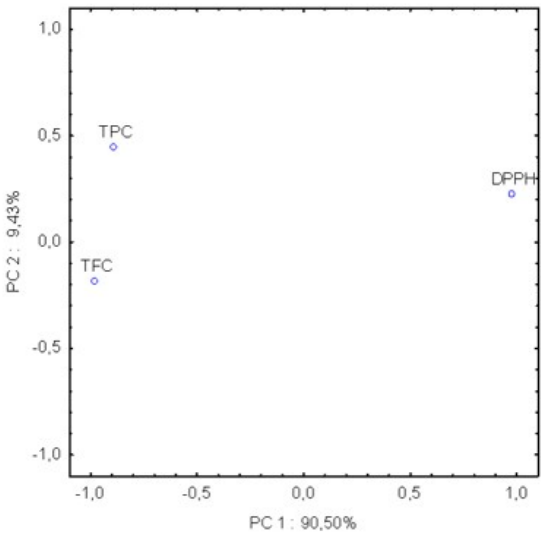

Figure 2. Score plots. Plots (PC1 × PC2) of the treatments means, obtained through spectrophotometry. Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and radical scavenging activity (DPPH).

D. salina enhances compounds with high pharmacological and agricultural potential

The plant secondary metabolism has been increasingly highlighted due to its ability to generate compounds with high pharmacological and agricultural potential, such as the secondary metabolites. The LC-DAD was used to detect and quantify the bioactive compounds that were stimulated by the microalgae biomass. The compounds that were more influenced by the microalgae biomass were respectively: the caffeic (13.33 mg 100 g^{-1}), gallic (4.42 mg 100 g^{-1}), coumaric (3.65 mg 100 g^{-1}), and chlorogenic (14.23 mg 100 g^{-1}) acids (Table III). Previous studies have shown the potential of these acids as antioxidant, analgesic, anti-inflammatory, and anticancer agents (Laribi et al. 2015). Further, analyses have been carried out to determine the antibacterial activity of these compounds against pathogenic strains (Fu et al. 2016).

Among the compounds with proven antibacterial activity, it is worth mentioning the gallic and coumaric acids, which showed high concentrations in the BM treatment while contents below the limit of detection were evidenced in the control group (Supplementary Material – Figure S1 and S2). The increase of metabolites with antibacterial activity can be an alternative to enhance the protection of plants against agricultural pathogens. Other metabolites, such as the caffeic acid, also demonstrated the capability to inhibit the development of pathogenic bacteria (Lengai et al. 2020). This compound was significantly influenced by the microalgae biomass, as its concentration increased by 6 times when compared to the control group (Figure S3). Likewise, substances such as the chlorogenic acid also had expressive increases (Figure S3). Besides that, the concentrations of the *trans*-cinnamic and ellagic acids, which were shown as high in the control group, remained similar in the BM treatment (Figure S1 and S4). Thus, demonstrating that the microalgae biomass can maintain the production of bioactive compounds, and significantly increase those that are less present in coriander crops. Therefore, the increase in secondary metabolites can benefit pharmacological and agricultural practices, as it provides an alternative to the use of conventional pesticides that bring impacts to the environment and human health.

Table II. Factorial coordinates of the variables.

Samples	PC1	PC2
CT	1.68	-0.48
BR	0.46	0.73
BM	-2.11	-0.36

Control Group (CT), Blank (BR), Microalgae Biopolymer (BM), First Principal Component (PC1), Second Principal Component (PC2).

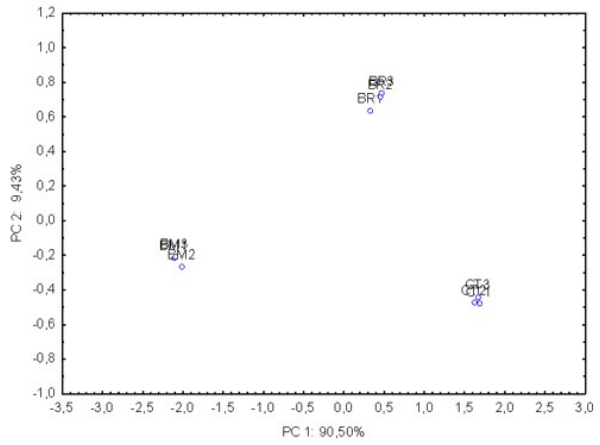

Figure 3. Loading plots. Plots (PC1 × PC2) of the mean of the treatments obtained through spectrophotometry. Control Group (CT), Blank (BR), and Microalgae Biopolymer (BM).

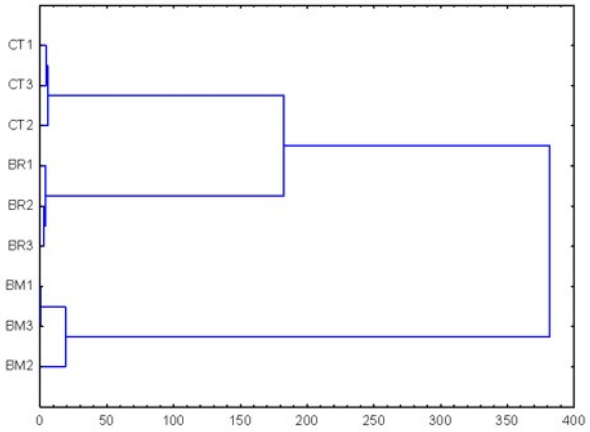

Figure 4. Dendrogram of the treatments means, obtained through spectrophotometry. Control Group (CT), Blank (BR), and Microalgae Biopolymer (BM).

Table III. Effect of incorporating *D. salina* biomass in the coating on the concentration of secondary metabolites (mg 100 g^{-1} DW) in coriander.

Secondary Metabolites	CT	BR	BM
Gallic acid	<LOD	<LOD	4.42^{e} ± 0.09
***Trans*-cinnamic acid**	18.75^{a} ± 0.09	0.25^{ab} ± 0.01	22.79^{a} ± 0.23
Chlorogenic acid	5.69^{c} ± 0.02	0.12^{c} ± 0.02	14.23^{b} ± 0.06
Caffeic acid	2.23^{d} ± 0.37	0.13^{c} ± 0.01	13.33^{c} ± 0.11
Coumaric acid	<LOD	0.17^{bc} ± 0.04	3.65^{f} ± 0.10
Ellagic acid	15.81^{b} ± 0.18	0.32^{a} ± 0.02	22.29^{b} ± 0.10

Control Group (CT), Blank (BR), Microalgae Biopolymer (BM), and Below the Limit of Detection (<LOD). The values represent the averages of the triplicates ± SD. Significant differences between samples ($p < 0.05$), measured by the MANOVA and Tukey test, are indicated by distinct superscript letters (a, b, c, d, e and f), while non-significant differences are indicated by the same superscript letters.

CONCLUSIONS

The incorporation of microalgae biomass in the coating significantly influences coriander growth and its secondary metabolism. The vegetable growth stimulus was verified in the increase of the germinative percentage, plant height, and root length. Accordingly, in the secondary metabolism, this was identified through an increase in the content of bioactive compounds, such as the caffeic and gallic acids, which have a high pharmacological and agricultural potential. These results can be led due to the presence of organic compounds in microalgae biomass, such as carbohydrates, lipids, and proteins, that trigger biochemical responses in plant metabolism, thereby increasing vegetal growth and synthesis of bioactive compounds. Additional studies can be carried out on the characterization of microalgae biomass to quantify the compounds responsible for influencing the development of coriander.

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SUPPLEMENTARY MATERIAL

Figures S1-S4

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Yasmin B.T. Fonseca and Nicole M. Almeida planned and designed the research, conducted the experiments, performed the analyses and wrote the manuscript. Jamile C. Caldas and Isaac M.J. Silva helped with the spectrophotometric assays, the HPLC, PCA, and HCA analyses. Fernando L.B. Moutinho and Jamile C. Caldas supervised the research. Fernando L.B. Moutinho, Gabriel N. Morais, Valéria B. Riatto and Walter N.L Santos revised the manuscript. All authors read and approved the final manuscript.