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Biostimulant effect of hydrolyzed *Spirulina* (Ficocyan®) on bioactive compounds in pigmented corn from Jalisco, Mexico

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ABSTRACT

The biostimulant effect of *Spirulina* (*Arthrospira maxima*) on various agricultural crops is well-documented, demonstrating improvements in yield, productivity, and product quality. However, limited research has explored its impact on bioactive compound stimulation, and no studies to date have examined its effect on pigmented corn cultivars high in anthocyanins. Mexico harbors a diverse array of pigmented maize varieties rich in anthocyanin content, with antioxidant activity, which decreases oxidative stress preventing degenerative diseases. In this study, the biostimulant effect of commercial hydrolyzed *Spirulina* (Ficocyan®) was evaluated on polyphenols, flavonoid, anthocyanins contents, and antioxidant capacity (DPPH and ABTS assays) in three maize cultivars (black, red and purple). The application of *Spirulina* significantly influenced polyphenol and flavonoid of red variety ($P < 0.05$). Conversely, anthocyanins were affected in the black variety. Regarding antioxidant capacity, the DPPH assay revealed no significant differences due to *Spirulina* treatment; however, the ABTS assay showed a difference in antioxidant capacity for red and purple varieties treated with *Spirulina*.

1. INTRODUCTION

Currently, biofertilizers and biostimulants are applied in diverse agricultural crops to reduce the problems associated with excessive application of mineral fertilizers, such as soil fertility degradation and environmental pollution. Biostimulants have been shown to improve soil structure, water retention, and microbial activity. In plants, they stimulate growth, enhance seed germination, and elevate metabolic processes. Furthermore, biostimulants contribute to improved photosynthesis, nutrient assimilation, and resilience to abiotic stress, leading to higher yields, better crop quality, and enhanced overall plant functionality. Consequently, biostimulants have become a valuable tool in optimizing agricultural productivity [1-3].

The application of microalgae as biofertilizers and biostimulants has emerged as a natural, environmentally sustainable, biodegradable, and cost-effective strategy, with no reported risks to human or animal health [4]. Microalgal species, particularly those from the genus Nostoc, have been employed by their capacity of fix atmospheric nitrogen and enhance phosphorus bioavailability through solubilization and mobilization, resulting mayor yields in several crops [5]. Similarly, *Spirulina* was tested as a biofertilizer for maize, utilizing its nutrient-dense biomass, which is rich in bioavailable micronutrients, to enhance crop productivity [6].

The biostimulant properties of *Spirulina* (Arthrospira maxima) are attributed to carbohydrates and other compounds [3]. Hydrolyzed *Spirulina* has also been utilized as a biostimulant in lettuce (*Lactuca sativa*), where its hydrolyzed proteins release polyamines and amino acids that act as growth promoters [7,8]. These hydrolyzed proteins are essential in activating biochemistry processes to promote plant and root growth, enhance yield, influence soil microbiota composition, optimize water and nutrient uptake, and alleviate the effects of abiotic stress [9].

Hydrolyzed proteins of both animal and plant origins, including those derived from microalgae, increased carbon and nitrogen metabolism by enhancing nitrogen uptake [10]. However, the *Spirulina* studie's on maize, in terms of enhancing bioactive compounds with health benefits, remain largely unexplored. Existing studies on *Spirulina* in maize have primarily focused on agronomic parameters. The present investigation is the first to evaluate the impact of *Spirulina* as biostimulants on the bioactive compounds in pigmented maize varieties rich in anthocyanins.

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Corn is an ancient culture originating from Mexico and has been a staple food since pre-Hispanic times [11]. It is the most widely cultivated crop worldwide, appreciated for its high yield and versatility. The origin, domestication, and diversity of this crop is Mexico, with a harvested area of 1,021,501 ha, yield of 6.818 t/ha, and harvests of 6,964,201 t [12]. Among the 220 recognized maize landraces, 59 are native to Mexico, including more than 45 pigmented varieties [13].

The pigmentation in maize is predominantly attributed to anthocyanins, primarily cyanidin, the most abundant type, along with peonidins and pelargonidins, depending on the specific variety [14-18]. Anthocyanins, classified within the flavonoid group, are renowned for to have significant effect benefits, largely because to their potent scavenge free radical properties, and activate key enzymes involved in cellular oxidative stress management [19,20].

Anthocyanins have attracted considerable attention by sensory attributes, in addition to an extensive antioxidant activity. They have been shown to inhibit the growth of *Helicobacter pylori*, a bacterium linked to duodenal ulcers and gastric cancer, and to prevent conditions such as diabetic retinopathy, glaucoma, retinitis pigmentosa, and cataracts. Moreover, anthocyanins enhance neuro-cognitive functions and are DNA protector [21-23]. They also prevent cardiovascular diseases, arthritis, and cancer and are hypoglicemics [24-26].

Given these health benefits, investigating the bioactive compounds in pigmented maize with antioxidant activity and preventive properties against degenerative diseases holds significant relevance. The purpose of this research was to evaluate a biostimulant based on hydrolyzed *Spirulina* on levels of polyphenols, flavonoids, anthocyanins, and antioxidant activity of three corn varieties.

2. MATERIALS AND METHODS

2.1. Plant Material and Treatments

Maize seeds of black, purple, and red varieties from the western maize landrace from the Valles region, Jalisco, were sown in the experimental field of the University Center of Biological and Agricultural Sciences UDG. Sowing was conducted on June 14, 2022, using a completely randomized block design with four plots of 20 m^2 (4 m long × 5 m wide), each containing six furrows measuring 4 m in length and 0.8 m in width and separated by 1 m of distance each experimental units and replicates. One plot was designated for controls, comprising black control (BC), red control, and purple control maize without *Spirulina* application (two per furrow). The remaining three plots were sown with each color variety individually-black treatment, purple treatment, and red treatment.

A solution of 5 mL/L of commercial hydrolyzed *Spirulina* (Ficocyan®) diluted in water was applied to seedlings at emergence (June 23, 2022) and subsequently every month until fruiting (October 21, 2022) for a total of four applications. This dosage and frequency of application have been tested to increase yield up to 12% in unpigmented corn in previous studies. Physicochemical analyses were performed on the soil used in the experiment [Table 1].

2.2. Sample Preparation

Grains from each treatment were harvested separately, dehydrated inside a stove to 45°C, then ground with a Krups mill (Model GX410011V) fitted with a No. 60 sieve.

Table 1: Physicochemical properties of the soil used for planting the experimental maize varieties.

Determination	Results	
pH	6.5	
Electrical conductivity (ds/m)	0.31	
Organic matter (%)	3.01	
Texture	**Sandy loam**	
Sand (%)	44.92	
Silt (%)	36.42	
Clay (%)	15.44	
Macroelements	**ppm**	
Nitrogen (NO_3)	11	
Phosphorus	18	
Potassium (acetate)	473	
Calcium (acetate)	2,073	
Magnesium (acetate)	321	
Microelements	**ppm**	
Copper	1.1	
Manganese	4.5	
Zinc	1.3	
Iron	6.7	
Cations	**meq/100g**	**Percentage**
Sodium	0.53	3.6
Potassium	1.21	8.2
Calcium	10.37	70.2
Magnesium	2.64	17.9

2.3. Extract Preparation

The extracts for analysis were made by homogenizing 0.5 g of flour from each treatment and control with 10 mL of a mixture of 80% methyl alcohol and 20% water, then subjected to sonication for 20 min using a Branson 3510 sonicator (power: 100 W, 42 kHz), followed by filtration and concentration with an R-215 rotary evaporator (Büchi).

2.4. Polyphenols Analysis

From each triplicate, 1 mL of extract was combined with 9 mL of water and 1 mL of Folin-Ciocalteu 2 N. Five min past, 10 mL of a 7% Na_2CO_3 solution was added, and the mixture was diluted to 25 mL with water. Samples remained 90 min before being read at 750 nm in a JENWAY 6320D spectrophotometer. Phenol content was determined using gallic acid at different concentrations (mg GAE)/g of dry weight [27].

2.5. Flavonoids Analysis

One mL from each extract by triplicate was taken to blend in 4 mL of high-performance liquid chromatography (HPLC)-grade water and 0.3 mL of 5% $NaNO_2$ solution. Passing 5 min, 0.3 mL of 10% $AlCl_3$ solution was added. Then to 6 min, 2 mL of 1 M NaOH solution was added and diluted to 10 mL with water. Samples were mixed in vortex, and it was read at 510 nm in a JENWAY 6320D spectrophotometer. Flavonoids were determined with (+)-catechin to different concentrations (mg EC)/g [27].

2.6. Anthocyanin Quantification

For this analysis, aliquots of 200 µL were taken by triplicate and added to 1800 µL of sodium acetate buffer (pH 4.5), while another 200 µL was combined with 1800 µL of potassium chloride buffer (pH 1.0). The mixtures were thoroughly vortexed and read at 510 nm and 700 nm in a UV-VIS JENWAY 6320D spectrophotometer [28]. Final absorbance was estimated using the formula:

$$A = (A_{510}–A_{700}) \text{ at pH } 1.0–(A_{510}–A_{700}) \text{ at pH } 4.5.$$

The content was expressed as mg C3G/g and calculated with the formula:

$$TAC = A \times MW \times DF \times 1000/\varepsilon \times 1$$

TAC = Total anthocyanins content
A = Final absorbance
MW = Molecular weight of cyanidin-3-glucoside (449.2 g/moL)
DF = Dilution factor utilized
ε = Molar absorptivity (26,900 L/cm/mol)

2.7. Determination of Antioxidant Capacity

Two techniques to evaluate the antioxidant capacity of samples were used, with slight modifications. Phenolic extracts (0.2 mL) were mixed with 3.3 mL of DPPH (60 µM) solution in 96-well microplates. After 30 min, the absorbance was read at 510 nm in a Mca. Instruments advanced microwave radiometer (AMR-100) microplate reader [29].

The second technique employed was the ABTS assay. A 7.4 mM ABTS solution was prepared and mixed in 1 mL of 2.6 mM potassium persulfate ($K_2S_2O_8$) solution. Then, at 12 h to allow for radical formation, and subsequently tuned to absorbance to 0.7 at 734 nm. From this mixture, 3.3 mL was combined with 0.2 mL of the extract in 96-well microplates. Absorbance was read at 734 nm using a Mca. Instruments AMR-100 microplate reader after incubation in the dark for 2 h. Results from both techniques were expressed as millimoles of Trolox equivalents/g [29].

2.8. Statistical Analysis

The results were processed with an analysis of variance and Tukey's test for mean comparisons. Statistical analyses were performed using Stat Graphics 19 software.

3. RESULTS

3.1. Polyphenols

Polyphenol revealed a rise in red maize variety with *Spirulina*, of 84.2 mg GAE/g in comparison to 75 mg GAE/g in untreated sample. In the black variety, the treated sample exhibited a slightly higher polyphenol content (83.4 mg) in comparison to the control (82.8), without difference. Conversely, the purple variety showed a negative effect, as untreated seeds contained significantly higher polyphenol levels (85) in comparison to treated seeds (76.6) [Table 2].

3.2. Flavonoids

Regarding flavonoid content, an increase was observed in the red maize variety treated with *Spirulina*, with 253.5 mg EC/g compared to 91.6 mg EC/g in the untreated sample. Similarly, the treated purple variety exhibited higher flavonoid content (136.2 mg EC/g) compared to the untreated sample (81.0 mg EC/g). In the black maize variety, the treated sample showed a slightly higher flavonoid content (106.4 mg EC/g) compared to the control (94.6 mg EC/g) without difference [Table 2].

3.3. Anthocyanins

The *Spirulina* treatment significantly increased anthocyanin content in the black maize variety, with treated samples exhibiting 67.9 mg C3GE/g compared with 23.6 mg C3GE/g in the BC; no differences were observed with other treatments. In the purple maize variety, the treated samples exhibited slightly higher anthocyanin content (35.0 mg C3GE/g) in comparison to the control (30.2 mg C3GE/g). In red maize variety, comparable levels of total anthocyanins were observed between the treated and control samples [Figure 1].

3.4. Antioxidant Activity

The the use of DPPH assay showed no effect of *Spirulina* treatment. In black maize variety, the control sample exhibited a significantly higher antioxidant activity (1690.7 µmoL TE/g) in comparison to the treated sample (1438.1 µmoL TE/g). Similarly, in red variety, the control disclosed slightly major antioxidant activity in comparison to the treated sample (1490.0 vs. 1404.4 µmoL TE/g sample), without difference. In the purple variety, the treated sample exhibited higher antioxidant activity (1593.1 µmoL TE/g) in comparison to the control (1374.3 µmoL TE/g sample) without difference statistically ($P > 0.05$).

In contrast, ABTS assay revealed differences in red and purple maize varieties treated with *Spirulina*. The treated red variety exhibited a mayor antioxidant activity of 1210.1 µmoL TE/g in comparison to 861.8 µmoL TE/g in control, while the treated purple variety showed effect significant of 1127.0 µmoL TE/g compared to 742.7 µmoL TE/g

Table 2: Polyphenol and flavonoid content in black, purple, and red maize from the western maize landrace of the Valles region, Jalisco, under the effect of hydrolyzed *Spirulina*.

Polyphenols (mg GAE/g sample)			Flavonoids (mg CE/g sample)	
Sample	**Average±SD**	**VC (%)**	**Average±SD**	**VC (%)**
BC	82.8±3.15^{a}	3.81	94.6±5.2^{bc}	5.5
RC	75±3.52^{b}	4.7	91.6±11.0^{bc}	12.0
PC	85±2.1^{a}	2.46	81.0±11.0^{c}	13.5
BT	83.4±2.8^{a}	3.4	106.4±16.8^{bc}	15.8
RT	84.2±1.47^{a}	1.75	253.5±42.62^{a}	16.8
PT	76.6±4.13^{b}	5.38	136.2±47.8^{b}	35.1

BC: Black control, RC: Red control, PC: Purple control, BT: Black treatment, RT: Red treatment, PT: Purple treatment.
Means with the same letter indicate no significant difference (P>0.05).

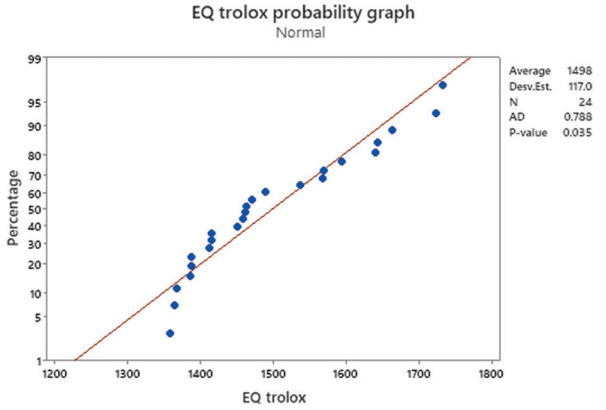
Table 3: Antioxidant activity measured by DPPH and ABTS assays (µmol TE/g sample) in pigmented maize from the Valles region, Jalisco, under the effect of hydrolyzed *Spirulina*.

Sample	Average±SD DPPH (µmol TE/g sample)	VC (%)	Average±SD ABTS (µmol TE/g sample)	VC (%)
BC	1690.7±43.8^{a}	2.6	1139.7±26^{ab}	2.3
RC	1490±33.4^{c}	2.2	861.7±28^{c}	3.2
PC	1374.32±15.3^{d}	1.1	742.7±31.1^{d}	4.2
BT	1438.1±26.8^{b}	1.8	814.2±37.2^{c}	4.6
RT	1404.4±36.1^{cd}	2.6	1210.1±3.2^{a}	0.3
PT	1593.1±33.3^{cd}	2.1	1127.0±18.8^{b}	1.7

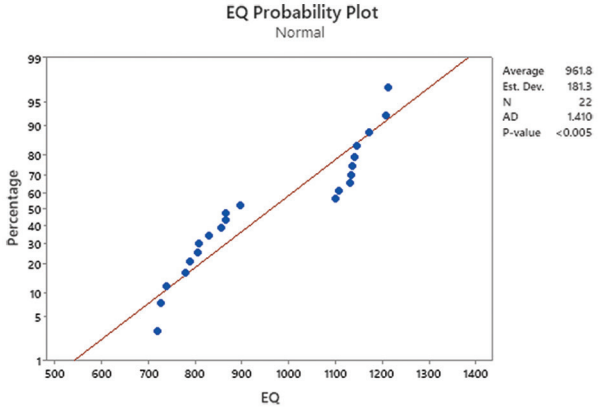
BC: Black control, RC: Red control, PC: Purple control, BT: Black treatment, RT: Red treatment, PT: Purple treatment.
Means with the same letter indicate no significant difference (P>0.05).

Table 4: Normality (Shapiro-Wilk) and homogeneity of variance (Levene's test) by DPPH and ABTS assays.

Tests		
DPPH		
Method	**Test statistics**	***P*-value**
Multiple comparisons	-	0.326
Levene	1.00	0.447



ABTS		
Method	**Test statistics**	***P*-value**
Multiple comparisons	-	0.543
Levene	0.41	0.838



in control. However, BC sample demonstrated mayor antioxidant activity than the treated black sample, of 1139.7 µmoL TE/g and 814.2 µmoL TE/g sample, respectively [Table 3]. In table 4 it can be seen normality (Shapiro-Wilk) and homogeneity of variance (Levene's test) by DPPH and ABTS assays

4. DISCUSSION

Polyphenol content in pigmented maize has been widely studied. Previous research [14,29,30] reported lower polyphenol values for purple maize varieties compared to the purple variety analyzed in this study (85 mg GAE/g). Similarly, Francavilla and Joye [31] documented lower polyphenol levels for black maize varieties (45.7–54.4 mg GAE/g) compared to our black variety (82.8 mg GAE/g), as well as lower values for purple (7–34) and red varieties (61.15). Red varieties of the Mixtec race, as reported by Herrera-Sotero *et al*. [26], also exhibited lower polyphenol levels (32.7–37.3 mg GAE/g). However,

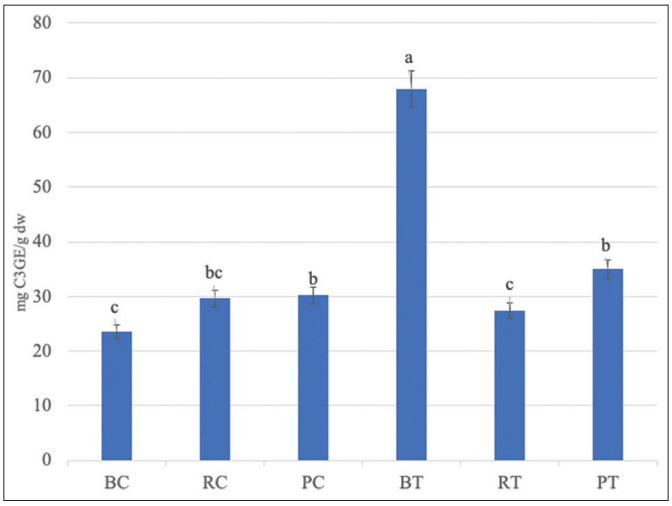


Figure 1: Anthocyanin content (mg C3GE/g dry weight) in pigmented maize from the western maize landrace of Jalisco under the effect of hydrolyzed *Spirulina*. BC: Black control, RC: Red control, PC: Purple control, BT: Black treatment, RT: Red treatment, PT: Purple treatment. Means with the same letter indicate no significant difference ($P > 0.05$).

the polyphenol content observed in the varieties analyzed in this study (75–85 mg GAE/g) was lower compared to other pigmented maize varieties, such as those reported with 340.0 mg GAE/g and *Zea mays* var. *Ceratina*, which exhibited 183.43 mg GAE/g [32,33].

Anthocyanin content varies depending on maize variety and pigmentation, as highlighted by Francavilla and Joye [31]. A study on 15 maize genotypes from northeastern Mexico reported anthocyanin levels ranging from 1.38 mg C3G/g in white maize to 85.9 mg C3G/g in purple maize [30,32]. The untreated varieties analyzed in this study fall within this range, with anthocyanin content values of 23.6–30.2 mg C3G/g.

Secondary metabolites are influenced by interactions of edaphoclimatic factors. Variations in latitude, altitude, and season alter the amount of solar radiation received, which affects anthocyanin content. This is attributed to the effect of sunlight on the phenylpropanoid biosynthetic pathways and the accumulation of anthocyanin [34]. Similarly, soil conditions, low pH, and elevated electrical conductivity, combined with elevated levels of organic matter, especially nitrogen, have been shown to stimulate the synthesis of total phenols and anthocyanins in pigmented Mexican landrace accessions [35].

This is partially attributed to the carbon-to-nitrogen (C:N) balance, which may facilitate carbon allocation to the synthesis of phenolic compounds. However, the underlying mechanisms remain poorly understood in maize landraces [34]. Furthermore, agricultural management practices significantly influence maize production parameters. Unfortunately, limited studies have investigated the impact of sowing date and phosphorus-potassium-nitrogen (P-K-N) fertilization on pigmented maize landraces [34].

Our landraces were cultivated under consistent edaphoclimatic conditions, including summer climate and soil with an optimal pH and nitrogen, phosphorus, and potassium levels close to the recommended standards. Therefore, the results obtained could be attributed to the effect of hydrolyzed *Spirulina*, consistent with findings reported for other biostimulants derived from microalgal extracts [36].

The untreated maize varieties exhibited mayor anthocyanin

content in comparison to pigmented maize studied by several authors [14,17,26,32]. Previous studies reported total anthocyanin values fluctuated from 0.01 to 7.54 mg ECG/g and 0.99–3.79 mg C3G/g in blue maize, 0.265–1.439 mg C3G/g in purple maize, 0.76–1.20 mg C3G/g in black maize, and 0.025–6.96 mg C3G/g in red maize. Similarly, other authors [15,37,38] stated anthocyanin values of 16.0, 3.2, and 5.0, respectively, in purple maize values lower than those observed in the untreated maize varieties analyzed in this study.

It has also been reported that for the purple maize variety, C3G is the mayor anthocyanin [37,38], who reported concentrations of 1.0–2.9 mg/g. However, anthocyanin of our maize variety was lower than reported for purple maize from China (55.8 mg C3G/100 g) [39]. In contrast, Peruvian black maize exhibited a higher anthocyanin content (48.50 mg C3G/g) [40] compared to the untreated black maize variety in this study. Notably, this value was surpassed by the *Spirulina* -treated black maize variety analyzed here, which exhibited an anthocyanin content of 67.9 mg C3G/g.

The combination of anthocyanins, such as cyanidin and delphinidin, and their derivatives has been reported to induce black pigmentation in plants [41]. Therefore, black maize varieties may contain higher levels of these anthocyanins, potentially stimulated by *Spirulina* hydrolysate. This hypothesis could be confirmed through HPLC of individual anthocyanins. The observed increase in these specific anthocyanins may result from interaction between genotypic traits and the abiotic factors discussed previously.

Reports on antioxidant activity in pigmented maize varieties are limited. Francavilla and Joye [31] reported DPPH values of 1400, 2200, and 2700 µmoL TE/g samples in purple, black, and red maize varieties, respectively, while Rodriguez-Salinas *et al*. [29] found a value of 1875.70 µmoL TE/g in purple maize. These values are higher than those observed in both treated and untreated maize varieties analyzed in this study for the same colors. However, the DPPH values in our red maize samples exceed those reported by Rodriguez-Salinas *et al*. (1127.70–1875.70 µmoL TE/g) and Herrera-Sotero *et al*. (10.5–12.7 µmoL TE/g) [26,29] for red maize.

Similarly, Harakotr *et al*. [30] reported ABTS values of 94.6 µmoL TE/g in *Z. mays* var. cera, which are significantly lower than those observed in this study (742.7–1210.1 µmoL TE/g). In contrast, Rodriguez-Salinas *et al*. [29] reported higher ABTS values, ranging from 2826.90 to 4263.90 µmoL TE/g in purple maize varieties.

Different techniques have been reported to evaluate the antioxidant capacity, which is determined by diverse factors. DPPH evaluate antioxidants that directly react with DPPH radicals, by finding a proton-donor substrate like an antioxidant, the radical is caught, and the absorbance decreases. DPPH is the principal radical used to measure antioxidant capacity in phenolic compounds [42].

ABTS methods are used by flavonoids and phenolic acids and are more specific than DPPH radicals. Scavenging takes place by electron donation. ABTS is oxidized to its radical cation, and ABTS+, is intensely colored [43].

However, few studies on biostimulants have focused on enhancing active compounds in maize. Most research has examined the use of biostimulants derived from macroalgae extracts to improve yield parameters [4].

Hydrolyzed *Spirulina* releases polyamines such as spermidine, putrescine, and spermine, which are hormone-like natural compounds capable of controlling biochemical and physiological functions in plants. These effects are likely mediated by the influence on gene and enzyme interactions, leading to improvements in crop quality and chemical composition, including polyphenol content [10,44].

This study focused on the stimulating effect of *Spirulina* as a promoter of bioactive compound production, with results showing considerable variability depending on the maize variety. A increase in polyphenol was shown in red maize variety in comparison with untreated sample, while no significant differences were detected in the black and purple varieties. Further investigation into the individual phenolic composition of these varieties is necessary to better understand these differences.

Anthocyanins are synthesized by plants, as from the phenylpropanoid pathway, the phenylalanine is the precursor. This mechanism is well known; however, there are few studies on the mechanism of action on the effect biostimulants in plants. Because of this, is important to investigate the biochemical and molecular pathways involved in the mechanism of action [45]. Only it has been reported genes encoding anthocyanin biosynthetic enzymes cloned from maize, snapdragon, and petunia. The first flavonoid biosynthetic gene isolated was Chalcone Synthase from parsley [46].

It is probable that the *Spirulina* hydrolysate stimulated the production of other non-anthocyanin phenolic compounds or carotenoids in the red maize variety, which may contribute to its coloration and are present in lower amounts in the black and purple maize varieties. Therefore, it is essential to conduct an analysis of individual phenolic compounds, as purple maize kernels are generally richer in anthocyanins compared to red ones [47].

Similar findings have been reported with biostimulants from seaweed extracts and hydrolyzed bean protein in sage (*Salvia officinalis* L.), which increased the yield of essential oils. However, other compounds, including total phenols, rosmarinic acid, and antioxidant capacity, were lower in treated plants compared to untreated ones [48].

The use of commercial biostimulants, such as AgroGain from red alga *Kappaphycus alvarezii*, has been reported to enhance growth parameters in maize plants. These biostimulants promote chlorophylls, carotenoids, sugars, amino acids, phenols (47% increase), and flavonoids (51% increase) by improving microbial counts in soil. In addition, they enrich metabolic pathways implicated in the assimilation of nitrogen, sulfur, and phosphorus [49]. These results are comparable to those observed in the red and purple maize varieties analyzed in this study.

Regarding anthocyanins, limited research has investigated the effect of biostimulants on maize. One notable study reported that using *Ascophyllum nodosum* algae extract enhanced maize plant growth and induced the accumulation of metabolites, including anthocyanins, by modulating genes [50].

Similarly, in *Phaseolus vulgaris* var. *Toska*, the application of *Ecklonia* maxima seaweed extracts increased phenol and anthocyanin levels. However, antioxidant activity by ABTS assay was lower compared to untreated samples [51]. This effect is attributed to the composition of *E. maxima* extract, which contains phytohormones, spermine and putrescine, brassinosteroids, phlorotannins, and abscisic acid, all of which contribute to the production of secondary metabolites [52].

5. CONCLUSION

Our results demonstrated a positive effect of hydrolyzed *Spirulina* on bioactive compounds in the evaluated maize landraces. Specifically,

the red variety exhibited an increase in polyphenol and flavonoid content, likely due to the stimulation of other phenolic compounds by the *Spirulina* hydrolysate. The purple variety showed a significant increase in flavonoid content, while the black variety experienced the greatest enhancement in anthocyanin content following *Spirulina* treatment. Antioxidant capacity, by the DPPH assay, showed no significant differences in the treatments. In contrast, the ABTS assay revealed positive effects in the purple and red varieties. These findings underscore the potential of natural biostimulants like *Spirulina* to promote bioactive compounds in traditional Mexican foods, such as pigmented maize, in an environmentally sustainable manner. Notably, this is the first study to report such effects. Furthermore, these findings may contribute to the production of compounds with preventive properties against degenerative diseases, such as anthocyanins.

6. ACKNOWLEDGMENTS

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7. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to the conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be authors as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

8. FUNDING

There is no funding to report.

9. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

10. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

11. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

12. PUBLISHER'S NOTE

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