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Research Article

Use of Crucifer Botanical Extracts as Biostimulants in Melon and Maize

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Abstract

Background and Objective: Modern agriculture faces biotic and abiotic stresses and requires new biostimulation tools to improve crop performance. This study evaluated the biostimulant potential of hydroalcoholic extracts from cruciferous foliage (cabbage, broccoli, and cauliflower) on melon and maize. **Materials and Methods:** Extracts were prepared by 72 hrs maceration in water:ethanol or water:methanol (1:1 v/v) and characterized for total phenols, flavonoids, proteins, and antioxidant capacity. Concentrates were diluted 1:300 and applied to crops by seed priming (24 hrs) or foliar spraying. Distilled water and a commercial biostimulant (Bionare, Germinare) served as controls. Germination, root and plumule length, stem diameter, biomass, and chlorophyll content were measured. Data were analyzed using Shapiro-Wilk normality tests, followed by ANOVA with Fisher's LSD ($p \leq 0.05$) for normally distributed data and the Kruskal-Wallis test for non-normal data, using InfoStat software. **Results:** Ethanolic extracts contained higher metabolite concentrations and stronger antioxidant activity, with cabbage showing the highest phenolic content. Seed priming enhanced melon germination with methanolic broccoli (39.54%), ethanolic cabbage (35.6%), and the commercial control (28%). In maize, germination accelerated with methanolic cauliflower (9.6%) and methanolic broccoli (6.4%). Foliar sprays mainly stimulated root growth: In melon, ethanolic cabbage exceeded the absolute control by 43% and the commercial control by 21.68%, whereas methanolic cabbage matched the commercial control and surpassed the absolute control by 32.34%. Height and biomass were largely unaffected, but total phenols and chlorophyll increased. **Conclusion:** Biostimulant efficacy depended on both the extraction solvent and plant biomass, with ethanolic cabbage extracts showing the most consistent positive effects.

Key words: Biostimulation, metabolites, seed priming, foliar spray, plant extracts

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Agriculture is among the oldest practices that human beings have used to subsist; this practice has evolved and adapted according to the needs that have arisen over time¹. Among the most important needs that have motivated its evolution are food security, environmental deterioration, soil salinization and the overexploitation of soils and exposure to various types of stress². All of these processes are accelerated by climate change, which together have caused crop yields to be unfavorably affected. This adaptability has led to the search for alternatives to new production systems that reduce stress and improve the yield, production and quality of crops³. One of the techniques used to improve the growth and production of plants is biostimulation, which is defined as the application of substances or microorganisms that, when applied in low amounts, are capable of modifying the metabolism and gene expression of plants to induce tolerance to biotic and abiotic stress, in addition to improving the nutritional efficiency, yield and quality of crops^{4,5}. Currently, seven categories of biostimulants have been identified: humic and fulvic acids, amino acid and peptide mixtures, algae extracts, botanical extracts, chitosans and biopolymers, inorganic compounds, fungi and beneficial bacteria and beneficial elements⁴.

Botanical extracts are commonly used in agricultural practice. These compounds are obtained by extracting soluble substances from the biomass of the plant and are usually used as phytosanitary products, since plants contain active compounds called allelochemicals that are used in the sustainable management of crops^{4,6}. Among the botanical extracts are extracts produced from cruciferous biomass, which are highly rich in phytochemicals, including phenolics, flavonoids, antioxidants, proteins, vitamins, and glucosinolates⁷. These phytochemicals provide resistance to biotic stress and antifungal activity^{8,9}. Similarly, responses to the growth, productivity and quality of crops have been observed and are not directly related to the suppression of pathogens, which suggests that they may be related to the biostimulation of the plant. Taking into account the biostimulant action of phytochemicals extracted from cruciferous biomass^{4,6}, the objective of this study was to evaluate and compare the effects of water:ethanol and water:methanol extracts (1:1 v/v) of cabbage, broccoli and cauliflower on the germination and early growth of melon and corn seedlings and to identify the extractant-composition-response relationships between the extracts and the seedlings.

MATERIALS AND METHODS

Study area and duration: The study was conducted from November 2023 to October 2024 in the Laboratory of Physiology, a greenhouse and experimental area of the Department of Horticulture of Antonio Narro Autonomous Agrarian University, in Buenavista, Saltillo, Coahuila, Mexico (25° 23 36"N 101° 00 02"W / 25.3934, -101.0005).

Cruciferae biomass production: Plants of cabbage (*Brassica oleracea* var. *capitata*), broccoli (*Brassica oleracea* var. *italica*), and cauliflower (*Brassica oleracea* var. *botrytis*) were grown from November 2023 to March 2024 in a field with loamy clay soil under drip irrigation and fertigation with 100% Hoagland solution. Transplanting was performed in double rows, with 0.3 m between plants and rows, and 1.4 m between beds. Average temperature and relative humidity were 13°C and 47.6%, respectively.

Collection of waste and preparation of foliar flour: One day after harvesting broccoli, cauliflower, and cabbage, leaves from 30 randomly selected plants per species were manually collected. The samples were labeled, placed in polyethylene sacks, and sun-dried for 15 days in a greenhouse with shade mesh, covered with Tufflite IV® 6000-gauge milky white plastic with 30.92% PAR transmittance, turning the sacks every two days. The dried material was then ground with a mortar and passed through a 1.0 mm metal sieve to obtain foliar flour.

Production of botanical extracts: Ethanolic (EE) and methanolic (EM) extracts were obtained from foliar flour by maceration with agitation⁷. For each crucifer, 2.5 g of foliar flour were placed in 250 mL Erlenmeyer flasks with 62.5 mL of distilled water and 62.5 mL of ethanol or methanol (Jalmek® 99.5%). The mixtures were intermittently hand-shaken every 12 hrs for 72 hrs, then filtered through Whatman No. 1 paper and stored at 5°C in aluminum foil-covered glass flasks.

Quantification of metabolites present in the botanical extracts: The characterization of the metabolites was performed by quantifying the following metabolites:

- **Total phenols:** Were determined according to Yu and Dahlgren⁸. In this procedure, 50 µL of concentrated extract was mixed with 200 µL of Folin-Ciocalteu reagent, 500 µL of 20% Na₂CO₃, and 5 mL of distilled water; the mixture was vortexed and incubated in a water bath at 45°C for 30 min. Absorbance was read at 750 nm using a UV-Vis spectrophotometer (Thermo Scientific Model

G10S, Waltham, MA, USA). A gallic acid calibration curve (0.02-0.4 mg/mL) was used, and results were expressed as mg/L of total phenols

- **Total protein:** Determined by the Bradford method⁹. Fifty microliters of the concentrated extract was removed, and 1.5 mL of Bradford reagent was added. After 5 minutes, the absorbance was read at a wavelength of 595 nm in a UV-Vis spectrophotometer (Thermo Scientific Model G10S, Waltham, MA, USA). A calibration curve was constructed with standard bovine serum albumin (0.005-0.5 mg/mL). The results are expressed as mg/L of total protein
- **Hydrophilic antioxidant capacity (ABTS):** Was determined by the method of Re *et al.*¹⁰. On the basis of the decolorization of the ABTS radical cation. The radical was obtained from the reaction of ABTS at 7 mM with potassium persulfate at 2.45 mM (1:1 v/v) in the dark for 16 hrs and then diluted with 20% ethanol until an absorbance of 0.7 ± 0.01 was obtained at 754 nm. To determine the antioxidant capacity of the hydrophilic compounds, 20 mL of the concentrated extract and 980 L of the ABTS radical dilution were placed in a 2 mL tube, stirred for 5 seconds, and allowed to stand for 7 min in the dark. The absorbance was measured with a UV-Vis spectrophotometer (Genesis 10s UV-Vis, Thermo Scientific, Waltham, MA, USA) at 734 nm. The results are expressed in mg/L
- **Flavonoids:** Were determined following Zhishen *et al.*¹¹. A total of 250 μ L of concentrated extract was mixed with 75 μ L of 5% NaNO_2 . After 5 min, 150 μ L of 10% AlCl_3 , 500 μ L of 1 M NaOH, and 2.025 mL of distilled water were added. Absorbance was read immediately at 510 nm using a UV-Vis spectrophotometer (Thermo Scientific Model G10S, Waltham, MA, USA). Flavonoid content was quantified using a catechin standard curve and expressed as mg EC/L
- **Glutathione:** Was quantified following the spectrophotometric technique established by Moron *et al.*¹² by reaction with 5,5-dithio-bis-2-nitrobenzoic acid (DTNB). In a test tube, 0.48 mL of the concentrated extract, 2.2 mL of dibasic sodium phosphate (0.32 M Na_2HPO_4), and 0.32 mL of DTNB dye at 1 mM were added. It was mixed and read in a UV-VIS spectrophotometer at 412 nm. The values are reported in mg/L

Preparation of diluted extracts: To obtain usable biostimulants without causing phytotoxicity, the botanical extracts were diluted with distilled water. Each concentrated extract was diluted 1:300 (v/v) and stored in glass bottles at

3°C until use¹³. To select the dilution to be used in the subsequent evaluations, 1:100, 1:200, 1:300, and 1:500 (v/v) were compared; the 1:300 dilution showed the best response in terms of seed germination, so it was selected.

Petri dish evaluation of the botanical extracts of melon and corn seeds with seed priming:

Sixty seeds of melon (Lia®, Samini's, 2003) and maize (AN447®, UAAAN, 2023) were disinfected with 70% alcohol for 2 min, 1% sodium hypochlorite for 2 min, and distilled water for 2 min; they were then imbibed for 24 hrs in the corresponding diluted extract in light-protected glass containers at $22 \pm 2^\circ\text{C}$. Treatments included ethanolic and methanolic extracts from broccoli, cauliflower, and cabbage, as well as an absolute control (distilled water) and a commercial biostimulant (Bionare®, Germinare®), with six replicates of 10 seeds placed in Petri dishes containing moistened filter paper. The dishes were randomly arranged in a germination chamber at $24 \pm 2^\circ\text{C}$, 55% relative humidity, and darkness. After 50% radicle emergence, seedlings were exposed to continuous light (53 $\mu\text{mol}/\text{m}^2/\text{sec}$ PAR) for 10 days in maize and 15 days in melon, with moistening every 48 hrs using 2 mL of distilled water. Germination percentage and rate were recorded, and two post-germination evaluations were performed in melon (8 and 16 days) and maize (5 and 10 days), including fresh and dry weight.

Test of the extracts on container seedlings: This test was performed in two ways: (a) Pretreatment of the seed or seed priming with the extracts and subsequent growth of the seedlings, and (b) Germination of untreated seeds to obtain seedlings that received foliar spray with the extracts.

Test of botanical extracts in melon and corn seedlings treated with seed priming in containers:

As in seed priming, ethanolic and methanolic extracts from broccoli, cauliflower, and cabbage were evaluated against an absolute control (distilled water) and a commercial biostimulant (Bionare®, Germinare®). Disinfected melon and maize seeds were imbibed in each extract for 24 hrs and sown individually in 0.5 L polystyrene containers filled with peat moss. Each treatment consisted of 20 replicates (one plant per replicate), arranged in a randomized complete block design in a greenhouse with shade mesh, covered with Tufflite IV® 6000-gauge milky white plastic with 30.92% PAR transmittance. The experiment was conducted from September to October 2024 at an average temperature of $24 \pm 3^\circ\text{C}$. Plants were irrigated daily with 250 mL of tap water, and from true leaf emergence onward, 50% Steiner solution was applied by drenching twice daily. Seedling development was evaluated twice in melon

Table 1: Coefficients of the contrasts applied to the treatments

	Trt.	Ct.1	Ct.2	Ct.3
Absolute control (water)	1	0	0	-6
Commercial control (BIONARE)	2	0	0	0
EE. BROCCOLI	3	1	-1	1
EE. CABBAGE	4	1	2	1
EE. CAULIFLOWER	5	1	-1	1
EM. BROCCOLI	6	-1	-1	1
EM. CABBAGE	7	-1	2	1
EM. CAULIFLOWER	8	-1	-1	1

EE: Ethanolic extract, EM: methanolic extract and ct: Contrast

(14 and 28 days after emergence) and maize (10 and 20 days), measuring height, stem diameter, root length, and fresh and dry biomass of shoots and roots.

The contents of total phenols⁹ and total chlorophyll were quantified.

Total chlorophyll was determined following the methodology of Wellburn¹⁴. A total of 15 mg of lyophilized tissue was mixed with 1.25 mL of methanol in 2 mL Eppendorf tubes and incubated in darkness for 24 hrs at 20-25°C. The supernatant was then read at 666, 653, and 470 nm using a UV-Vis spectrophotometer (Genesis 10s UV-Vis, Thermo Scientific, Waltham, MA, USA). Results were expressed as mg/g dry weight.

Testing of botanical extracts by foliar spraying on melon and maize seedlings in containers: Ethanolic and methanolic extracts from broccoli, cauliflower, and cabbage were evaluated in melon and maize seedlings, compared with an absolute control (distilled water) and a commercial biostimulant (Bionare®, Germinare®). Seeds were sown individually in 0.5 L polystyrene containers filled with peat moss, with 20 replicates per treatment, and maintained under the same conditions described for the container seed-priming experiment. Treatments were applied as foliar sprays twice: When 50% of the plants showed the first true leaf and again 15 days later. Seedling development was evaluated twice using six seedlings per evaluation: Melon at 14 and 28 days, and maize at 10 and 20 days after emergence. Height, stem diameter, root length, and shoot and root fresh and dry biomass were recorded, while total phenols⁹ and total chlorophyll¹⁴ were quantified in the remaining eight seedlings.

Statistical analysis: The data obtained were subjected to Shapiro-Wilk normality tests to evaluate their distribution. An analysis of variance was performed on the data with a normal distribution, and means comparison tests were performed according to Fisher's LSD ($p \leq 0.05$). A Kruskal-Wallis test was performed on the data that did not show normality. The statistical analyses described above were performed in the statistical software Infostat.

Subsequently, a correlation analysis was performed on the variables evaluated with the statistical software Minitab v22.

Orthogonal contrasts: Orthogonal contrasts were performed to evaluate differences between treatments, considering the type of extractant, the culture of origin and the absolute control. The analysis was performed with the statistical software Infostat 2020 (Table 1).

RESULTS

Quantification of metabolites present in the botanical extracts: The contents of metabolites varied according to the extractant and biomass source (Table 2). Overall, ethanolic extracts showed the highest concentrations, particularly in cabbage, with values up to 115.34 mg/L for proteins, 113.98 mg/L for antioxidant capacity, and 108.48 mg/L for flavonoids. Glutathione also reached its maximum in ethanolic cauliflower (0.860 mM/L). In contrast, total phenolics showed similar ranges between extractants (210.2-263.2 mg/L) in ethanolic and 235.7-260.95 mg/L in methanolic extracts). Overall, ethanolic cabbage extract presented the highest metabolite content.

Petri dish evaluation of the botanical extracts of melon and corn seeds with seed priming: The application of ethanolic and methanolic extracts from broccoli, cauliflower, and cabbage to seeds improved germination in both species. In melon (Fig. 1), the greatest increases were observed with the commercial control (35.6%) and the ethanolic cabbage extract (28%), indicating a stronger response in this species. In maize (Fig. 2), the improvement was more moderate and occurred at early stages, where methanolic extracts of cauliflower and broccoli increased germination by 9.6 and 6.4% on day 4. Overall, the results indicate that botanical extracts enhance germination, with a more pronounced response in melon and an early effect in maize.

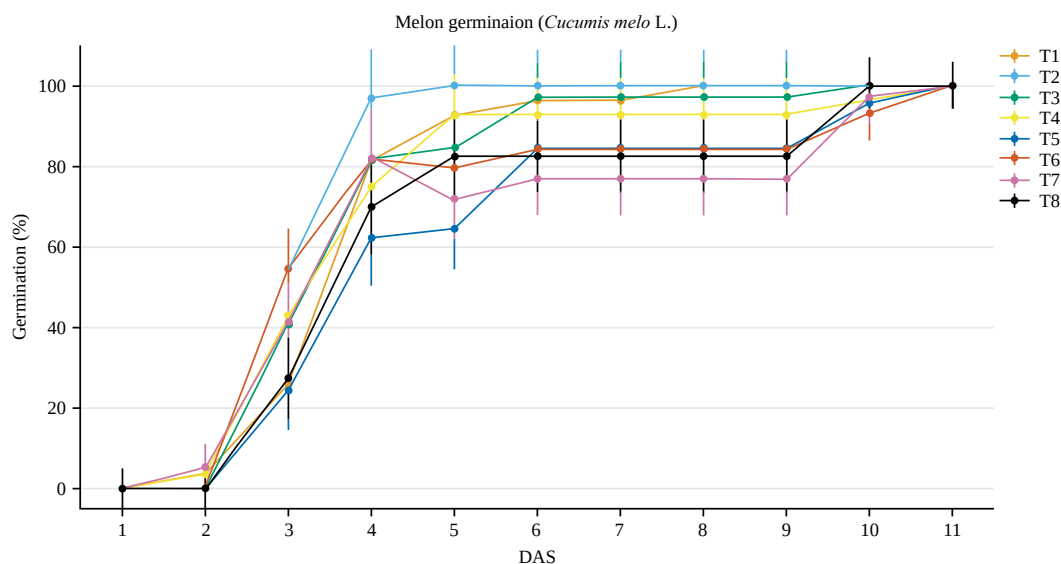


Fig. 1: Percentage of germination of melon seeds with seed priming. The mean values and standard errors are shown

DAS: Days after Sowing, T1: Absolute control, T2: Commercial control, T3: Broccoli ethanolic extract, T4: Cabbage ethanolic extract, T5: Cauliflower ethanolic extract, T6: Broccoli methanolic extract, T7: Cabbage methanolic extract and T8: Methanolic cauliflower extract

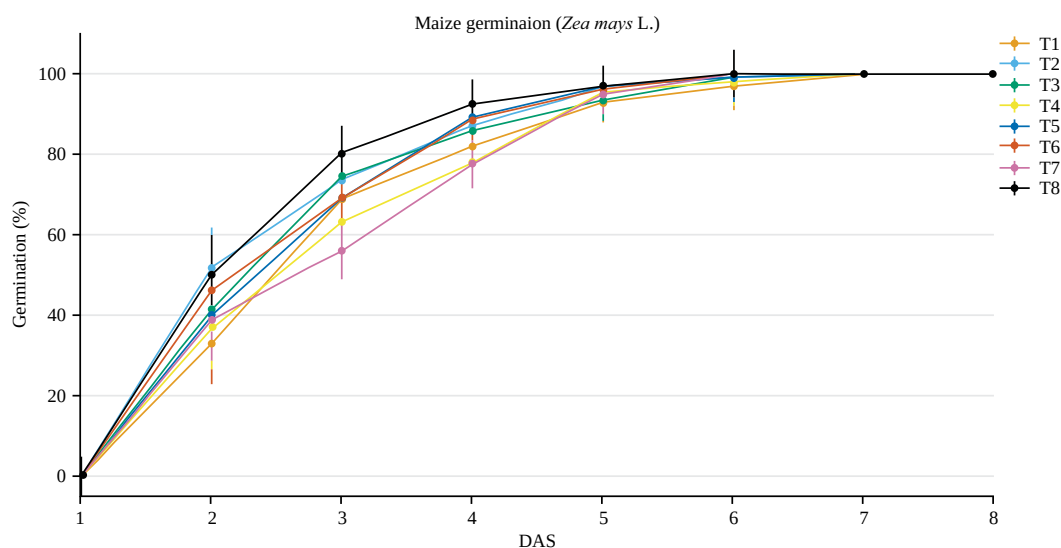


Fig. 2: Percentage of germination of maize seeds with seed priming. The mean values and standard errors are shown

DAS: Days after Sowing, T1: Absolute control, T2: Commercial control, T3: Broccoli ethanolic extract, T4: Cabbage ethanolic extract, T5: Cauliflower ethanolic extract, T6: Broccoli methanolic extract, T7: Cabbage methanolic extract and T8: Methanolic cauliflower extract

Table 2: Contents of total phenols, total proteins, antioxidant capacity, total flavonoids and glutathione obtained from ethanolic and methanolic extracts of broccoli, cauliflower and cabbage leaves

Type extract	Plant	Total phenols (mg/L)	Total protein (mg/L)	Hydrophilic antioxidant capacity (mg/L)	Total flavonoids (mg/L)	Glutathione (mM/L)
Ethanolic	Broccoli	224.45±8.50 ^c	106.25±5.60 ^b	93.71±3.98 ^b	105.83±7.75 ^a	0.638±0.01 ^c
	Cabbage	263.2±6.58 ^a	115.34±4.13 ^a	113.98±8.12 ^a	108.48±0.01 ^a	0.783±0.2 ^{ab}
	Cauliflower	210.2±5.59 ^b	104.57±3.48 ^b	110.95±5.47 ^a	116.95±3.32 ^a	0.860±0.01 ^a
Methanolic	Broccoli	235.7±8.48 ^{bc}	105.5±1.30 ^b	94.88±6.77 ^b	80.63±6.25 ^b	0.594±0.59 ^c
	Cabbage	260.95±8.99 ^a	103.93±5.81 ^b	77.80±9.10 ^b	74.75±3.07 ^b	0.752±0.02 ^b
	Cauliflower	241.45±7.67 ^b	97.06±4.36 ^c	81.69±10.23 ^b	85.18±6.28 ^b	0.741±0.01 ^b

Mean±Standard Deviation (SD) and Different letters represent significant differences according to the LSD test ($p \leq 0.05$)

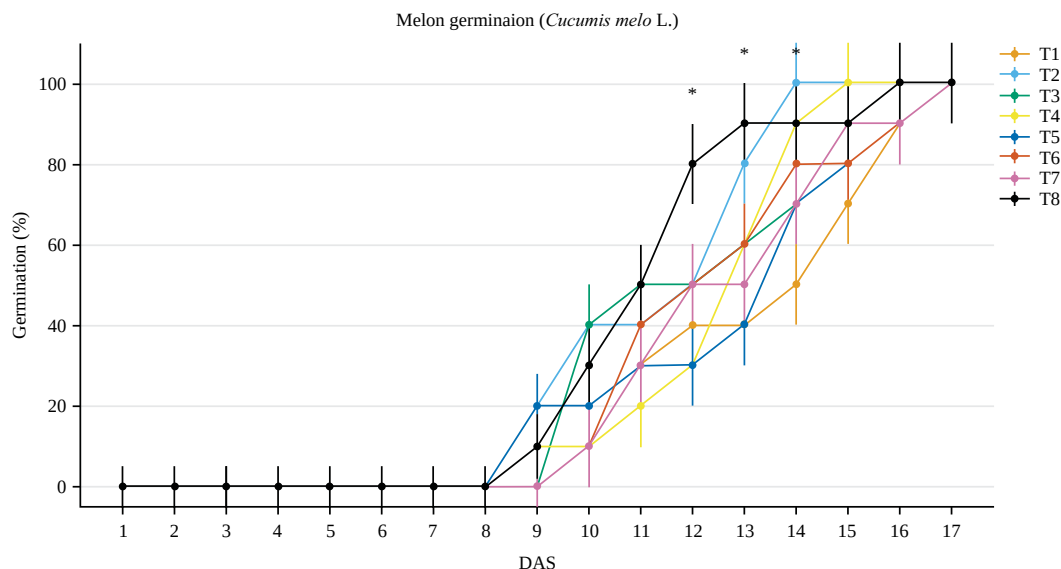


Fig. 3: Percentage of emergence of melon seeds with seed priming produced in the greenhouse

DAS: Days after sowing, T1: Absolute control, T2: Commercial control, T3: Broccoli ethanolic extract, T4: Cabbage ethanolic extract, T5: Cauliflower ethanolic extract, T6: Broccoli methanolic extract, T7: Cabbage methanolic extract and T8: Methanolic cauliflower extract

Table 3: Effects of the application of ethanolic and methanolic extracts obtained from broccoli, cauliflower and cabbage leaves on the growth of melon and corn seedlings

Cultivation	Treatment	Intermediate evaluation		Final evaluation		Fresh weight (g)**	Dry weight (g)**
		Plumule (mm)**	Radicle (mm)**	Plumule (mm)**	Radicle (mm)**		
Melon	Absolute control	12.20±2.65 ^d	49.01±9.61 ^a	26.70±12.31 ^e	63.24±19.88 ^d	1.48±0.46 ^a	0.07±0.03 ^a
	Commercial control	16.51±7.08 ^a	55.59±14.99 ^a	31.99±5.89 ^{ab}	75.74±18.04 ^{bcd}	1.55±0.91 ^a	0.11±0.05 ^a
	Ethanolic extract						
	Broccoli	14.26±6.19 ^{bc}	64.71±17.03 ^a	28.13±3.49 ^{de}	93.29±17.36 ^{ab}	1.88±0.76 ^a	0.08±0.02 ^a
	Cabbage	15.65±7.14 ^{ab}	62.79±13.33 ^a	33.23±4.53 ^a	94.97±18.22 ^a	1.62±0.87 ^a	0.07±0.09 ^a
	Cauliflower	13.52±6.36 ^{cd}	57.22±19.62 ^a	28.86±3.80 ^{cd}	80.07±11.13 ^{bc}	2.0±0.88 ^a	0.10±0.06 ^a
	Methanolic extract						
	Broccoli	15.91±6.58 ^{ab}	51.25±13.70 ^a	26.70±8.24 ^e	81.85±18.15 ^{ab}	1.97±0.70 ^a	0.09±0.04 ^a
	Cabbage	16.17±6.54 ^{ab}	57.60±15.84 ^a	30.50±8.93 ^{bc}	70.50±15.63 ^{cd}	1.33±0.28 ^a	0.13±0.07 ^a
	Cauliflower	15.32±5.82 ^{abc}	64.28±16.12 ^a	30.29±5.67 ^{bc}	77.46±18.58 ^{bcd}	1.72±0.55 ^a	0.08±0.04 ^a
	P value	0.0002	0.2039	0.0001	0.0005	0.3795	0.2184
	HP	22.69	26.51	13.1	29.77	28.73	30.73
Maize	Absolute control	16.48±3.29 ^a	17.51±1.14 ^c	21.43±4.28 ^c	22.77±1.48 ^d	0.98±0.32 ^a	0.35±0.09 ^a
	Commercial control	18.29±1.68 ^a	18.73±4.20 ^b	26.51±2.44 ^a	30.14±5.86 ^{ab}	1.18±0.99 ^a	0.41±0.18 ^a
	Ethanolic extract						
	Broccoli	18.01±1.90 ^a	20.14±2.37 ^{ab}	25.21±2.66 ^{ab}	28.19±3.31 ^{abc}	1.00±0.55 ^a	0.34±0.12 ^a
	Cabbage	18.13±2.04 ^a	22.06±3.50 ^a	25.38±2.86 ^{ab}	30.88±4.90 ^a	0.93±0.41 ^a	0.32±0.15 ^a
	Cauliflower	17.91±2.52 ^a	21.53±2.91 ^a	25.08±3.52 ^b	27.65±4.08 ^c	1.05±0.88 ^a	0.37±0.13 ^a
	Methanolic extract						
	Broccoli	18.03±1.92 ^a	21.00±3.29 ^{ab}	25.24±2.69 ^{ab}	29.40±4.61 ^{abc}	1.03±0.62 ^a	0.36±0.08 ^a
	Cabbage	17.63±0.87 ^a	20.63±3.23 ^{ab}	24.68±1.21 ^b	28.88±4.52 ^{abc}	0.90±0.23 ^a	0.32±0.07 ^a
	Cauliflower	17.87±1.13 ^a	19.74±3.42 ^b	25.02±1.58 ^b	27.64±4.79 ^{bc}	1.23±0.86 ^a	0.43±0.18 ^a
	P value	0.4289	0.0001	0.0003	0.0001	0.0827	0.1761
	HP	11.18	15.66	11.01	15.73	15.88	17.99

Mean±Standard Deviation (SD), Different letters represent significant differences (<0.05). *Data with a normal distribution underwent an analysis of variance, and means comparison tests were performed according to Fisher's LSD ($p \leq 0.05$) and **Data with a distribution without normal adjustment were analyzed using a Kruskal-Wallis test

Regarding seedling growth (Table 3), in melon, plumule length showed significant differences in both evaluations. At the final evaluation, the ethanolic cabbage extract stood out, with a 24.46% increase compared with the absolute control.

Radicle length did not show significant differences at the intermediate evaluation; however, at the final evaluation, the ethanolic cabbage and broccoli extracts increased radicle length by 50.17 and 47.52%, respectively. Likewise, the

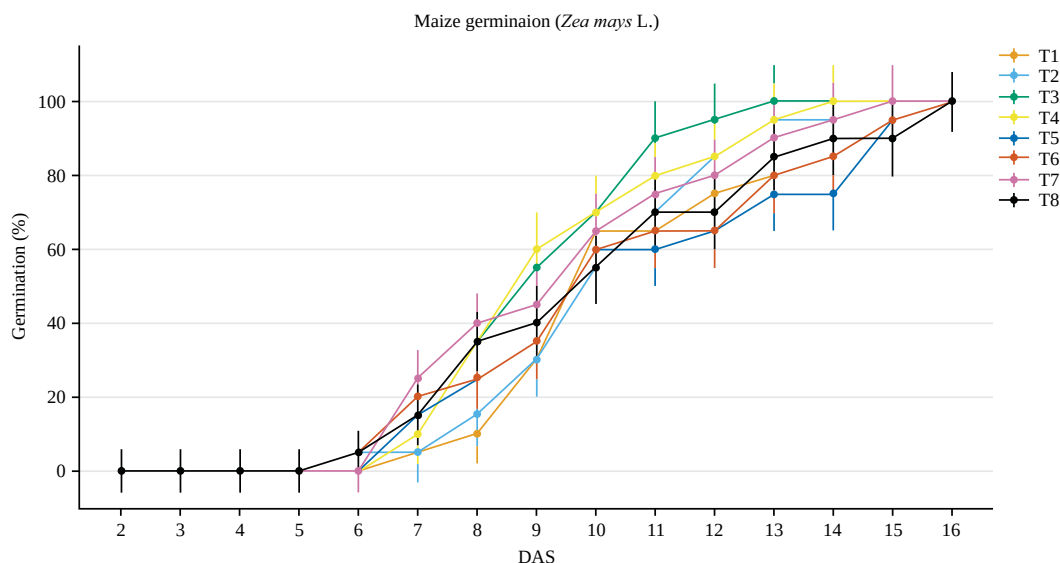


Fig. 4: Percentage of emergence of maize seeds with seed priming produced in the greenhouse

DAS: Days after sowing, T1: Absolute control, T2: Commercial control, T3: Broccoli ethanolic extract, T4: Cabbage ethanolic extract, T5: Cauliflower ethanolic extract, T6: Broccoli methanolic extract, T7: Cabbage methanolic extract and T8: Methanolic cauliflower extract

methanolic broccoli and ethanolic cauliflower extracts increased this variable by 29.43 and 26.61%, respectively. Fresh and dry weight did not show significant differences among treatments.

In maize, plumule length did not show significant differences at the intermediate evaluation, but significant differences were observed at the final evaluation, where the ethanolic cabbage extract and broccoli extracts showed increases close to 18% compared with the absolute control. Radicle length showed significant differences in both evaluations, with the ethanolic cabbage and cauliflower extracts standing out at the intermediate stage, increasing this variable by 25.99 and 22.96%, respectively. At the final evaluation, the ethanolic cabbage and methanolic broccoli extracts showed the greatest increases, with 35.62 and 29.12%, respectively. Fresh and dry weight did not show significant differences among treatments.

Test of botanical extracts in melon and corn seedlings treated with seed priming in containers.

In melon (Fig. 3), emergence began at 9 DAS and intensified at later stages. The ethanolic cabbage extract and the methanolic cauliflower extract were the most outstanding treatments, both showing 40% increases in germination. These values were clearly higher than those of the absolute control and close to the performance of the commercial control, indicating high efficiency in accelerating the process. In maize (Fig. 4), emergence was earlier and more dynamic, beginning on day 5. The ethanolic broccoli extract showed the best performance, reaching 100% emergence (25% higher

than the absolute control), while the ethanolic cabbage extract reached 95 (20% increase). Both treatments clearly outperformed the absolute control and showed similar or even superior performance compared to the commercial control at the final stage, while also accelerating germination during intermediate stages (20-30%).

Under seed priming in containers (Table 4), the clearest response was root growth. In melon, the response was mainly concentrated in root development. At the final evaluation, significant differences were observed in root length, with the methanolic extracts of cabbage, cauliflower, and broccoli showing the highest values. These treatments increased root length by 33.96, 32.93, and 23.05%, respectively, compared with the commercial control, with the methanolic cabbage extract showing the strongest effect. Stem diameter also responded positively at the intermediate stage, particularly with cabbage extracts, with increases of 16.66% (ethanolic) and 20% (methanolic). In contrast, plumule length and shoot biomass showed no relevant changes, indicating that the effect was mainly focused on the root system. In maize, the response was more variable but still centered on root growth and some physiological traits. At intermediate stages, radicle length showed general increases, with the ethanolic cabbage extract standing out (15.15%). At the final evaluation, the methanolic cauliflower extract showed the best overall performance, with increases of 17.53% in both root length and shoot fresh weight, making it the most effective treatment. Overall, biomass responses were limited, with more noticeable effects during early stages.

Table 4: Effects of botanical extracts applied with seed priming on the growth and development of the plumule, radicle, fresh weight and dry weight of melon and corn seedlings

Table 4. Effects of botanical extracts applied with seed priming on the growth and development of the primule, radicle, fresh weight and dry weight of melon and corn seedlings										
Intermediate evaluation										
Cultivation	Treatment	Root length (mm)*	Apex height (cm)**	Stem diameter (mm)*	Aerial fresh weight (g)*	Aerial dry weight (g)*	Fresh root weight (g)*	Dry root weight (g)**		
Melon	Absolute control	162.78±3.39 ^b	17.1±4.46 ^a	1.40±0.16 ^d	0.37±0.03 ^b	0.16±0.02 ^c	0.18±0.01 ^b	0.07±0.02 ^c		
	Commercial control	189.20±6.63 ^a	11.43±1.63 ^a	1.50±0.18 ^{cd}	0.46±0.02 ^b	0.25±0.1 ^a	0.25±0.02 ^a	0.16±0.02 ^a		
	Ethanolic extract									
	Broccoli	187.03±5.06 ^a	15.08±2.54 ^a	1.63±0.12 ^{abc}	0.45±0.03 ^b	0.23±0.02 ^{ab}	0.22±0.03 ^{ab}	0.13±0.02 ^{abc}		
	Cabbage	180.88±10.51 ^a	19.13±6.08 ^a	1.75±0.12 ^{ab}	0.45±0.03 ^b	0.21±0.01 ^{bcd}	0.21±0.05 ^{ab}	0.12±0.03 ^{cb}		
	Cauliflower	184.50±10.90 ^a	15.10±0.53 ^a	1.550.12 ^{bcd}	0.44±0.05 ^b	0.20±0.02 ^{bcd}	0.18±0.04 ^b	0.14±0.03 ^{ab}		
	Methanolic extract									
	Broccoli	177.80±3.56 ^a	15.63±3.91 ^a	1.480.17 ^{cd}	0.44±0.03 ^b	0.18±0.03 ^{de}	0.22±0.02 ^{ab}	0.14±0.01 ^{ab}		
	Cabbage	178.33±7.50 ^a	13.50±2.75 ^a	1.80±0.11 ^a	0.57±0.04 ^a	0.22±0.05 ^{abc}	0.25±0.03 ^a	0.14±0.02 ^{ab}		
	Cauliflower	178.43±9.76 ^a	17.13±2.62 ^a	1.680.05 ^{abc}	0.41±0.02 ^b	0.19±0.03 ^{cde}	0.22±0.02 ^{ab}	0.13±0.01 ^{abc}		
	p-value	0.0046	0.0753	0.0046	0.0308	0.0038	0.0911	0.017		
	HP	4.49	22.09	8.69	15.33	14.25	15.88	15.2		
Final evaluation										
Cultivation	Treatment	Root length (mm)*	Apex height (cm)*	Stem diameter (mm)*	Aerial fresh weight (g)*	Aerial dry weight (g)*	Fresh root weight (g)*	Root dry weight (g)*	Total chlorophyll (mg/g PS)	Total phenols (g/kg)
Melon	Absolute control	175.58±6.15 ^d	26.03±3.44 ^a	2.60±0.39 ^a	1.10±0.12 ^b	0.16±0.05 ^b	0.51±0.12 ^d	0.07±0.02 ^a	2.82±0.60 ^b	0.312±0.17 ^a
	Commercial control	216.85±16.8 ^{cd}	29.98±6.91 ^a	2.83±0.36 ^a	1.34±0.15 ^{ab}	0.20±0.04 ^{ab}	0.76±0.13 ^{cd}	0.07±0.02 ^a	3.95±0.53 ^{ab}	0.574±0.13 ^a
	Ethanolic extract									
	Broccoli	254.35±15.33 ^{abc}	29.55±3.04 ^a	2.78±0.43 ^a	1.36±0.20 ^{ab}	0.23±0.04 ^a	0.99±0.20 ^{bc}	0.08±0.04 ^a	3.98±0.49 ^{ab}	0.848±0.18 ^a
	Cabbage	231.55±16.81 ^{bc}	31.58±4.62 ^a	2.68±0.56 ^a	1.44±0.43 ^a	0.23±0.05 ^a	0.74±0.18 ^{cd}	0.09±0.02 ^a	2.98±0.03 ^b	0.657±0.08 ^a
	Cauliflower	178.23±18.72 ^d	28.60±7.64 ^a	2.55±0.41 ^a	1.11±0.09 ^{ab}	0.16±0.03 ^b	0.59±0.17 ^d	0.08±0.04 ^a	4.84±0.14 ^a	0.715±0.24 ^a
	Methanolic extract									
	Broccoli	266.85±19.97 ^{ab}	30.50±3.06 ^a	2.60±0.22 ^a	1.42±0.20 ^{ab}	0.23±0.03 ^a	1.17±0.26 ^{ab}	0.09±0.03 ^a	4.73±1.10 ^a	0.608±0.08 ^a
	Cabbage	290.50±16.01 ^a	30.90±6.43 ^a	2.80±0.35 ^a	1.44±0.22 ^a	0.25±0.02 ^a	1.44±0.33 ^a	0.11±0.02 ^a	3.98±1.13 ^{ab}	0.366±0.28 ^a
	Cauliflower	288.28±15.40 ^a	32.18±5.81 ^a	2.60±0.29 ^a	1.34±0.26 ^{ab}	0.22±0.06 ^{ab}	1.14±0.45 ^{ab}	0.11±0.02 ^a	4.20±0.43 ^a	0.448±0.23 ^a
	p-value	0.0001	0.8157	0.9466	0.2528	0.0461	0.0003	0.3527	0.0179	0.1649
	HP	13.48	18.05	14.61	17.81	21.22	27.93	29.85	17.04	8.28
Intermediate evaluation										
Cultivation	Treatment	Root length (mm)*	Apex height (cm)**	Stem diameter (mm)*	Aerial fresh weight (g)**	Aerial dry weight (g)**	Fresh root weight (g)*	Dry root weight (g)**		
Maize	Absolute control	27.28±2.17 ^c	90.33±13.43 ^b	1.90±0.34 ^b	0.30±0.12 ^c	0.04±0.13 ^{cd}	0.51±0.02 ^b	0.07±0.01 ^{bc}		
	Commercial control	32.08±2.25 ^a	145.75±15.23 ^{ab}	2.38±0.33 ^a	0.38±0.05 ^{bc}	0.06±0.08 ^{ab}	0.60±0.007 ^{ab}	0.09±0.01 ^{ab}		
	Ethanolic extract									
	Broccoli	28.83±2.02 ^c	150.33±18.52 ^{ab}	2.33±0.17 ^a	0.55±0.13 ^a	0.07±0.08 ^a	0.84±0.02 ^a	0.10±0.01 ^a		
	Cabbage	28.25±0.77 ^c	141.15±7.42 ^{bc}	2.05±0.24 ^{ab}	0.35±0.10 ^{bc}	0.03±0.05 ^d	0.54±0.01 ^b	0.05±0.01 ^c		
	Cauliflower	31.70±1.54 ^{ab}	145.18±10.71 ^b	2.33±0.23 ^a	0.48±0.10 ^{ab}	0.05±0.12 ^{bc}	0.73±0.01 ^{ab}	0.08±0.02 ^{abc}		
	Methanolic extract									
	Broccoli	29.23±1.45 ^{bc}	136.65±7.48 ^{bc}	2.03±0.18 ^{ab}	0.33±0.09 ^c	0.04±0.05 ^{cd}	0.55±0.01 ^b	0.07±0.01 ^{bc}		
	Cabbage	32.68±1.94 ^a	166.38±4.28 ^a	2.30±0.14 ^a	0.53±0.06 ^a	0.06±0.08 ^{ab}	0.82±0.007 ^a	0.09±0.01 ^{ab}		
	Cauliflower	33.90±1.75 ^a	155.40±3.28 ^{ab}	2.30±0.29 ^a	0.38±0.05 ^c	0.04±0.14 ^{cd}	0.67±0.008 ^{ab}	0.06±0.02 ^{bc}		
	p-value	0.0001	0.0043	0.0776	0.0031	0.0003	0.0128	0.0046		
	HP	5.89	15.18	11.13	22.16	19.55	23.69	19.47		
Final evaluation										
Cultivation	Treatment	Root length (mm)**	Apex height (cm)**	Stem diameter (mm)**	Aerial fresh weight (g)**	Aerial dry weight (g)**	Fresh root weight (g)*	Dry root weight (g)**	Total chlorophyll (m/g PS)	Total phenols (g/kg)
Maize	Absolute control	33.50±1.11 ^c	21.13±1.99 ^c	2.40±0.29 ^{cd}	0.55±0.09 ^b	0.07±0.01 ^c	0.80±0.15 ^d	0.10±0.02 ^d	4.90±0.08 ^d	0.737±0.10 ^b
	Commercial control	40.45±1.38 ^b	25.38±0.96 ^a	2.68±0.09 ^{bc}	0.90±0.27 ^a	0.11±0.03 ^a	1.83±0.54 ^a	0.23±0.07 ^a	5.24±0.08 ^d	0.810±0.20 ^a
	Ethanolic extract									
	Broccoli	39.50±1.55 ^b	23.40±1.26 ^{ab}	2.83±0.22 ^{ab}	0.65±0.10 ^b	0.08±0.01 ^{bc}	1.13±0.34 ^{bcd}	0.14±0.04 ^{bcd}	5.58±0.22 ^c	0.879±0.11 ^a
	Cabbage	46.58±3.07 ^a	24.03±0.46 ^{ab}	2.78±0.25 ^b	0.70±0.16 ^{ab}	0.09±0.02 ^{abc}	1.38±0.36 ^{abc}	0.17±0.05 ^{bc}	6.68±0.12 ^a	1.012±0.08 ^a
	Cauliflower	37.63±1.16 ^b	21.20±1.68 ^c	2.30±0.18 ^d	0.60±0.08 ^b	0.08±0.01 ^c	1.05±0.13 ^{cd}	0.13±0.02 ^{cd}	6.81±0.16 ^a	1.008±0.14 ^a
	Methanolic extract									
	Broccoli	39.18±1.25 ^b	20.53±2.15 ^c	2.38±0.41 ^{cd}	0.53±0.17 ^b	0.07±0.02 ^c	0.95±0.35 ^{cd}	0.12±0.04 ^{cd}	5.33±0.17 ^d	1.564±0.05 ^a
	Cabbage	39.25±2.05 ^b	22.28±1.15 ^{bc}	2.33±0.09 ^{cd}	0.88±0.05 ^a	0.09±0.01 ^{abc}	1.43±0.16 ^{abc}	0.18±0.02 ^{abc}	5.89±0.11 ^b	1.075±0.17 ^a
	Cauliflower	39.55±5.40 ^b	25.05±1.14 ^a	3.15±0.12 ^a	0.87±0.12 ^a	0.11±0.01 ^{ab}	1.58±0.38 ^{ab}	0.20±0.04 ^{ab}	5.90±0.10 ^b	0.935±0.05 ^a
	p-value	0.0001	0.0002	0.0003	0.003	0.0058	0.0031	0.0032	0.0001	0.0001
	HP	6.41	6.33	9.24	20.8	21.2	26.09	25.88	2.41	8.45

Mean ± standard deviation (SD); different letters represent significant differences (<0.05). * Data with a normal distribution underwent an analysis of variance, and means comparison tests were performed according to Fisher's LSD (p<0.05) and **Data with a distribution without normal adjustment were analyzed using a Kruskal-Wallis test

For biochemical traits, melon showed moderate changes, with the ethanolic cauliflower extract standing out by increasing total chlorophyll by 71.63%, followed by the methanolic broccoli (67.73%) and methanolic cauliflower

(48.94%) extracts, while total phenols remained relatively stable and comparable to the controls. In maize, the response was more pronounced, where the ethanolic cauliflower extract showed the highest increase in chlorophyll (38.98%), followed

Table 5: Correlation analysis among root length, apex height, stem diameter, fresh apex weight (FAW), dry apex weight (DAW), fresh root weight (FRW), dry root weight (DRW), total phenols, and chlorophyll in melon seedlings pretreated by seed priming

	Root length	Apex height	Stem diameter	FAW	DAW	FRW	DRW	Total phenols
Apex height	0.372*							
Stem diameter	0.073	0.022						
FAW	0.401*	0.240	0.556*					
DAW	0.468*	0.209	0.336	0.821*				
FRW	0.753*	0.127	0.242	0.649*	0.668*			
DRW	0.337	0.060	-0.041	0.128	0.361	0.436		
Total phenols	0.096	-0.044	-0.026	0.085	0.036	0.071	-0.057	
Chlorophyll	0.130	0.268	-0.173	0.028	-0.084	-0.170	-0.068	-0.472

FAW: Fresh apex weight, DAW: Dry apex weight, FRW: Fresh root weight, DRW: Dry root weight. (Spearman, $p \leq 0.05$)*

Table 6: Correlation analysis among root length, apex height, stem diameter, fresh apex weight (FAW), dry apex weight (DAW), fresh root weight (FRW), dry root weight (DRW), total phenols, and chlorophyll in maize seedlings pretreated by seed priming

	Root length	Apex height	Stem diameter	FAW	DAW	FRW	DRW	Total phenols
Apex height	0.454*							
Stem diameter	0.426*	0.562*						
FAW	0.101	0.441*	0.535*					
DAW	0.101	0.441*	0.535*	1.000*				
FRW	0.166	0.464*	0.506*	0.821*	0.821*			
DRW	0.166	0.464*	0.506*	0.821*	0.821*	0.100*		
Total phenols	0.557*	0.359	0.359	-0.004	-0.004	0.104	0.104*	
Chlorophyll	0.287*	0.206*	0.162*	-0.004	-0.363	-0.246	-0.246	0.414

FAW: Fresh apex weight, DAW: Dry apex weight, FRW: Fresh root weight, DRW: Dry root weight. (Spearman, $p \leq 0.05$)*

by the ethanolic cabbage extract (36.33%); additionally, the methanolic broccoli extract stood out for the highest increase in total phenols (112.21%), indicating a greater biochemical activation in this species.

Correlation analysis supported these patterns: In melon (Table 5), root length was linked to apex height ($p = 0.37$), shoot biomass ($p = 0.47$), and especially fresh root weight ($p = 0.753$), with no association with phenols or chlorophyll. In maize (Table 6), relationships were more integrated, with apex height and stem diameter ($p = 0.562$) associated with biomass ($p = 0.44$), and root length linked to growth ($p = 0.54$), phenols ($p = 0.557$), and chlorophyll ($p = 0.287$), indicating coordinated responses.

Testing of botanical extracts by foliar spraying on melon and maize seedlings in containers: In melon seedlings treated by foliar spraying (Table 7), the response was mainly concentrated in root growth and biochemical adjustments at the final stage. The ethanolic cabbage extract stood out as the most effective treatment for root length, with a 75.61% increase compared with the absolute control, followed by the ethanolic cauliflower and methanolic cabbage extracts, with increases of 57.09 and 47.80%, respectively. At the biochemical level, the ethanolic cabbage extract increased total chlorophyll by 28.22%. Regarding total phenols, the methanolic broccoli extract showed the highest increase, with 226.51%, followed by the ethanolic cabbage and ethanolic cauliflower extracts, with increases of 111.27 and 110.44%, respectively.

In maize (Table 7), the response was more variable but with marked effects on biomass and physiological traits. Root growth showed moderate increases, particularly with the methanolic cabbage extract (24.46%) and the ethanolic cabbage extract (20.52%). However, the strongest effects were observed in shoot biomass, where the ethanolic cauliflower and broccoli extracts promoted substantial increases in fresh weight (320-325%) and especially in dry weight. Additionally, total chlorophyll increased by up to 38.98%, while total phenols reached increases of up to 65.13%, mainly with ethanolic extracts.

Correlation analysis showed species-specific patterns. In melon (Table 8), apex height was positively correlated with root length ($p = 0.439$), fresh aerial biomass ($p = 0.457$), dry aerial biomass ($p = 0.547$), and dry root weight ($p = 0.464$). Total phenols were not associated with growth variables but were correlated with chlorophyll content ($p = 0.434$). In maize (Table 9), apex height was associated with root length ($p = 0.525$), fresh aerial biomass ($p = 0.391$), dry aerial biomass ($p = 0.381$), fresh root weight ($p = 0.591$), and dry root weight ($p = 0.570$). In this species, stem diameter was also positively correlated with fresh aerial biomass ($p = 0.357$), dry aerial biomass ($p = 0.341$), fresh root weight ($p = 0.455$), and dry root weight ($p = 0.460$), while total phenols showed a strong correlation with chlorophyll content ($p = 0.848$).

Contrasts: Orthogonal contrasts revealed effects of the extractant, species, and application method. Overall, ethanol was more effective than methanol (Table 10), increasing

Table 7: Effects of spraying with botanical extracts on the growth and development of the plumule, radicle, fresh weight and dry weight of melon and maize seedlings produced in the greenhouse

		Intermediate evaluation								
Cultivation	Treatment	Root length (mm)*	Apex height (cm)*	Stem diameter (mm)**	Aerial fresh weight (g)*	Aerial dry weight (g)**	Fresh root weight (g)**		Dry root weight (g)**	
Melon	Absolute control	148.4±7.59 ^c	17.70±2.57 ^a	1.43±0.05 ^a	0.31±0.07 ^c	0.08±0.04 ^d	0.23±0.06 ^c		0.08±0.01 ^a	
	Commercial control	205.10±6.28 ^a	19.18±5.94 ^a	1.55±0.06 ^a	0.47±0.09 ^{ab}	0.16±0.03 ^{abc}	0.57±0.10 ^{ab}		0.17±0.11 ^a	
	Ethanolic extract									
	Broccoli	186.05±14.55 ^{ab}	23.20±3.46 ^a	1.60±0.08 ^a	0.420.05 ^{bc}	0.09±0.04 ^d	0.43±0.06 ^{bc}		0.16±0.11 ^a	
	Cabbage	204.85±16.25 ^a	19.78±4.08 ^a	1.53±0.09 ^a	0.48±0.06 ^{ab}	0.13±0.06 ^{bcd}	0.47±0.04 ^{ab}		0.12±0.03 ^a	
	Cauliflower	202.80±16.15 ^a	20.68±3.51 ^a	1.68±0.27 ^a	0.47±0.16 ^{ab}	0.13±0.1 ^{bcd}	0.43±0.10 ^{ab}		0.11±0.08 ^a	
	Methanolic extract									
	Broccoli	170.33±13.64 ^{bc}	22.10±9.44 ^a	1.70±0.14 ^a	0.59±0.12 ^a	0.20±0.02 ^a	0.59±0.09 ^a		0.14±0.06 ^a	
	Cabbage	184.75±14.67 ^{ab}	22.75±4.71 ^a	1.63±0.12 ^a	0.47±0.07 ^{ab}	0.18±0.14 ^{ab}	0.55±0.10 ^{ab}		0.14±0.06 ^a	
	Cauliflower	177.33±18.45 ^b	19.18±3.69 ^a	1.58±0.17 ^a	0.390.05 ^{bc}	0.1±0.02 ^{cd}	0.41±0.11 ^{bc}		0.11±0.05 ^a	
	p-value	0.0002	0.67	0.0973	0.0064	0.01	0.0095		0.2547	
	HP	8.42	24.89	7.59	18.7	27.83	28.06		28.23	
Final Evaluation										
Cultivation	Treatment	Root length (mm)*	Apex height (cm)*	Stem diameter (mm)*	Aerial fresh (mm)*	Aerial dry weight (g)*	Fresh root weight (g)**	Root dry weight (g)**	Total chlorophyll (mg/g PS)	Total phenols g/kg
Melon	Absolute control	184.10±9.78 ^d	30.60±2.19 ^b	2.65±0.40 ^a	1.18±0.13 ^a	0.17±0.01 ^b	0.55±0.24 ^a	0.14±0.01 ^b	5.28±0.28 ^{cd}	0.479±0.04 ^c
	Commercial control	253.20±19.41 ^{bc}	36.35±3.37 ^{ab}	2.88±0.46 ^a	1.48±0.36 ^a	0.24±0.01 ^{ab}	1.01±0.15 ^a	0.19±0.008 ^a	5.22±0.20 ^d	0.737±0.26 ^b
	Ethanolic extract									
	Broccoli	219.70±12.11 ^{cd}	37.03±4.94 ^{ab}	2.78±0.21 ^a	1.43±0.30 ^a	0.23±0.10 ^{ab}	0.93±0.18 ^a	0.19±0.02 ^a	5.43±0.52 ^c	0.710±0.13 ^{bc}
	Cabbage	323.30±16.36 ^a	41.73±5.59 ^a	2.85±0.13 ^a	1.46±0.27 ^a	0.27±0.05 ^a	0.84±0.20 ^a	0.19±0.03 ^a	6.77±0.53 ^a	1.012±0.32 ^a
	Cauliflower	289.20±10.08 ^{ab}	33.15±2.66 ^b	2.93±0.23 ^a	1.50±0.24 ^a	0.23±0.09 ^{ab}	0.870.23 ^a	0.18±0.04 ^{ab}	6.68±0.09 ^a	1.008±0.09 ^a
	Methanolic extract									
	Broccoli	258.55±19.71 ^{bc}	31.85±7.97 ^b	3.00±0.14 ^a	1.46±0.21 ^a	0.19±0.02 ^{ab}	0.71±0.28 ^a	0.16±0.02 ^{ab}	5.17±0.52 ^d	1.564±0.13 ^a
	Cabbage	272.10±17.36 ^{abc}	36.03±1.82 ^{ab}	2.68±0.26 ^a	1.500.08 ^a	0.22±0.06 ^{ab}	0.85±0.23 ^a	0.17±0.04 ^{ab}	6.02±0.10 ^b	1.075±0.21 ^a
	Cauliflower	188.20±16.98 ^d	31.03±0.38 ^b	2.83±0.13 ^a	1.38±0.09 ^a	0.24±0.04 ^{ab}	0.96±0.05 ^a	0.18±0.05 ^{ab}	5.97±0.48 ^b	0.635±0.28 ^{bc}
	p-value	0.0002	0.0269	0.7632	0.8173	0.4573	0.2706	0.3349	0.0001	0.0001
	HP	15.07	12.96	11.06	20.8	28.59	26.43	17.16	1.55	8.47
Intermediate evaluation										
Cultivation	Treatment	Root length (mm)*	Apex height (cm)**	Stem diameter (mm)*	Aerial fresh weight (g)**	Aerial dry weight (g)**	Fresh root weight (g)*	Dry root weight (g)**		Dry root weight (g)**
Maize	Absolute control	26.40±9.78 ^e	14.03±2.19 ^c	2.18±0.40 ^{cd}	0.42±0.13 ^c	0.03±0.01 ^c	0.55±0.24 ^d	0.06±0.01 ^d		0.06±0.01 ^d
	Commercial control	33.60±19.41 ^b	16.95±3.37 ^{ab}	20.20±0.45 ^{bcd}	0.53±0.33 ^{abc}	0.05±0.01 ^{bc}	0.80±0.15 ^{abc}	0.07±0.009 ^{abc}		0.07±0.009 ^{abc}
	Ethanolic extract									
	Broccoli	30.80±14.41 ^{bcd}	15.73±4.94 ^{ab}	2.43±0.20 ^{bc}	0.50±0.29 ^{bc}	0.05±0.09 ^{bc}	0.85±0.18 ^a	0.11±0.02 ^a		0.11±0.02 ^a
	Cabbage	30.75±13.11 ^{bcd}	15.65±5.59 ^{ab}	2.08±0.13 ^d	0.49±0.27 ^c	0.03±0.05 ^c	0.53±0.20 ^d	0.05±0.02 ^d		0.05±0.02 ^d
	Cauliflower	28.00±10.08 ^e	14.80±2.66 ^{bc}	2.58±0.33 ^b	0.72±0.26 ^a	0.06±0.01 ^{bc}	0.83±0.22 ^{ab}	0.09±0.03 ^{ab}		0.09±0.03 ^{ab}
	Methanolic extract									
	Broccoli	31.98±15.71 ^{bc}	18.80±7.97 ^{ab}	2.33±0.14 ^{bcd}	0.52±0.22 ^{bc}	0.06±0.02 ^{bc}	0.63±0.21 ^{bcd}	0.05±0.01 ^d		0.05±0.01 ^d
	Cabbage	37.93±12.36 ^a	20.21±1.82 ^a	2.90±0.26 ^a	0.65±0.07 ^{ab}	0.07±0.06 ^a	0.88±0.23 ^a	0.10±0.03 ^a		0.10±0.03 ^a
	Cauliflower	29.53±9.98 ^{cd}	14.93±1.38 ^{bc}	2.17±0.13 ^{cd}	0.48±0.08 ^c	0.05±0.04 ^b	0.60±0.05 ^{cd}	0.5±0.05 ^d		0.5±0.05 ^d
	p-value	0.0001	0.0007	0.0001	0.0229	0.0150	0.0037	0.0139		0.0139
	HP	6.55	8.79	8.38	23.11	21.37	19.53	22.38		
Final evaluation										
Cultivation	Treatment	Root length (mm)**	Apex height (cm)**	Stem diameter (mm)**	Aerial fresh weight (g)**	Aerial dry weight (g)**	Fresh root weight (g)*	Dry root weight (g)**	Total chlorophyll (mg/g PS)	Total phenols g/ kg
Maize	Absolute control	34.13±1.31 ^e	19.30±0.38 ^d	2.18±0.09 ^c	0.40±0.08 ^d	0.02±0.007 ^d	0.78±0.17 ^d	0.09±0.02 ^d	4.90±0.06 ^d	1.821±0.30 ^b
	Commercial control	43.88±1.03 ^a	22.80±0.72 ^a	2.98±0.24 ^a	1.35±0.05 ^{abc}	0.14±0.008 ^{ab}	1.93±0.14 ^a	0.28±0.01 ^a	5.24±0.11 ^d	2.553±0.12 ^{ab}
	Ethanolic extract									
	Broccoli	38.18±3.83 ^d	20.03±0.80 ^d	2.33±0.15 ^{bc}	1.68±0.08 ^{ab}	0.19±0.009 ^a	1.63±0.13 ^{ab}	0.22±0.01 ^{ab}	5.58±0.10 ^c	2.70±0.081 ^{ab}
	Cabbage	41.13±1.61 ^b	20.05±1.70 ^d	2.48±0.13 ^{abc}	1.43±0.11 ^{abc}	0.12±0.02 ^{ab}	0.85±0.19 ^{cd}	0.11±0.02 ^{cd}	6.68±0.05 ^a	2.906±0.54 ^a
	Cauliflower	38.98±2.60 ^d	21.95±1.23 ^{abc}	2.57±0.15 ^{abc}	1.70±0.08 ^a	0.21±0.01 ^a	1.30±0.10 ^{bc}	0.16±0.01 ^{bc}	6.81±0.11 ^a	3.008±0.25 ^a
	Methanolic extract									
	Broccoli	38.58±2.03 ^d	20.13±0.40 ^{cd}	2.50±0.27 ^{abc}	0.83±0.09 ^{cd}	0.07±0.009 ^{cd}	1.32±0.07 ^{bc}	0.18±0.01 ^{bc}	5.33±0.09 ^d	2.208±0.08 ^a
	Cabbage	42.48±1.35 ^{ab}	22.30±3.24 ^{ab}	2.65±0.25 ^{ab}	0.78±0.02 ^{cd}	0.09±0.04 ^{cd}	1.68±0.13 ^{ab}	0.20±0.02 ^{ab}	5.89±0.10 ^b	2.804±0.57 ^a
	Cauliflower	39.10±0.53 ^{cd}	20.73±0.39 ^{abc}	2.83±0.15 ^a	1.03±0.01 ^{bcd}	0.11±0.05 ^{bc}	1.33±0.11 ^{bc}	0.18±0.01 ^{bc}	5.90±0.06 ^b	2.875±0.35 ^a
	p-value	3.53	6.04	10.42	26.08	24.2500	25.12	21.45	0.0001	0.0001
	HP	0.0012	0.0045	0.0064	0.0015	0.0041	0.0007	0.0019	1.55	5.13

Mean±Standard Deviation (SD), different letters represent significant differences (<0.05). *Data with a normal distribution underwent an analysis of variance, and means comparison tests were performed according to Fisher's LSD ($p \leq 0.05$) and **Data with a distribution without normal adjustment were analyzed using a Kruskal-Wallis test and Different letters represent significant differences (<0.05)*

Table 8: Correlation analysis among root length, apex height, stem diameter, fresh apex weight (FAW), dry apex weight (DAW), fresh root weight (FRW), dry root weight (DRW), total phenols, and chlorophyll in melon seedlings treated with extract spraying

	Root length	Apex height	Stem diameter	FAW	DAW	FRW	DRW	Total phenols
Apex height	0.439*							
Stem diameter	0.290	0.130						
FAW	0.333	0.457*	0.309					
DAW	0.390*	0.547*	0.246	0.518*				
FRW	0.057	0.312	0.285	0.508*	0.371*			
DRW	0.143	0.464*	-0.012	0.291	0.625*	0.344		
Total phenols	0.200	0.155	0.002	0.137	0.466	-0.030	0.272	
Chlorophyll	0.234	0.032	-0.171	0.015	0.067*	0.010	0.022	0.434*

FAW: Fresh apex weight, DAW: Dry apex weight, FRW: Fresh root weight, DRW: Dry root weight. (Spearman, $p \leq 0.05$)*

Table 9: Correlation analysis among root length, apex height, stem diameter, fresh apex weight (FAW), dry apex weight (DAW), fresh root weight (FRW), dry root weight (DRW), total phenols, and chlorophyll in maize seedlings treated with extract spraying

	Root length	Apex height	Stem diameter	FAW	DAW	FRW	DRW	Total phenols
Apex height	0.525*							
Stem diameter	0.313	0.215						
FAW	0.279	0.391*	0.357*					
DAW	0.279	0.381*	0.341*	0.981*				
FRW	0.330	0.591*	0.455*	0.408*	0.408*			
DRW	0.330	0.570*	0.460*	0.408*	0.415*	1.000*		
Total phenols	0.081	-0.216	0.178	0.115	0.115	-0.002	-0.002	
Chlorophyll	-0.002	-0.290	0.102	0.169	0.169	0.105	0.105	0.848*

FAW: Fresh apex weight, DAW: Dry apex weight, FRW: Fresh root weight, DRW: Dry root weight. (Spearman, $p \leq 0.05$)*

Table 10: Orthogonal contrasts comparing ethanol and methanol for the evaluated variables

Evaluation in melon	Results ethanol (+) vs methanol (-)	Evaluation in maize	Results ethanol (+) vs methanol (-)
<i>In vitro</i> assay of melon seedlings treated by seed priming	(+) Plumule ($p = 0.002$) (+) DW ($p = 0.0031$) (+) Radicle ($p = 0.0079$)	<i>In vitro</i> assay of maize seedlings treated by seed priming	No differences were found
Melon seedlings treated by seed priming	(-) Root length ($p = 0.001$) (-) FRW ($p = 0.001$) (-) Chlorophyll ($p = 0.045$)	Maize seedlings treated by seed priming	(+) Chlorophyll ($p = 0.001$)
Melon seedlings treated with extract spraying	(+) Root length ($p = 0.021$) (+) Apex height ($p = 0.0268$) (+) DAW ($p = 0.017$)	Maize seedlings treated with extract spraying	(+) FAW ($p = 0.0001$) (+) DAW ($p = 0.001$) (+) Chlorophyll ($p = 0.0001$) (+) Total phenols ($p = 0.0042$)

DW: Dry weight, FAW: Fresh apex weight, DAW: Dry apex weight, FRW: Fresh radicle weight, DRW: Dry radicle weight, Only contrasts with statistically significant results are shown. Signs (+/-) indicate the coding of the corresponding results and the direction of the effect

Table 11: Orthogonal contrasts comparing cabbage vs. broccoli and cauliflower for the evaluated variables.

Evaluation in melon	Results Cabbage (+) vs broccoli and cauliflower (-)	Evaluation in maize	Results Cabbage (+) vs broccoli and cauliflower (-)
<i>In vitro</i> assay of melon seedlings treated by seed priming	No differences were found	<i>In vitro</i> assay of maize seedlings treated by seed priming	(+) DW ($p = 0.0157$)
Melon seedlings treated by seed priming	(+) Root length ($p = 0.001$) (-) Total phenols ($p = 0.0032$)	Maize seedlings treated by seed priming	(+) Root length ($p = 0.0014$) (+) Chlorophyll ($p = 0.0001$)
Melon seedlings treated with extract spraying	(+) Root length ($p = 0.001$) (+) Apex height ($p = 0.0082$) (+) Chlorophyll ($p = 0.0165$)	Maize seedlings treated with extract spraying	(+) Root length ($p = 0.0003$) (+) DRW ($p = 0.0046$) (+) Chlorophyll ($p = 0.0001$)

DW: Dry weight, FAW: Fresh apex weight, DAW: Dry apex weight, FRW: Fresh radicle weight, DRW: Dry radicle weight. Only contrasts with statistically significant results are shown. Signs (+/-) indicate the coding of the corresponding results and the direction of the effect

plumule length ($p = 0.002$), radicle length ($p = 0.0079$), and dry weight ($p = 0.0031$) in melon, as well as root length ($p = 0.021$), apex height ($p = 0.0268$), and biomass under foliar spraying. In maize, ethanol increased shoot biomass ($p = 0.0001-0.001$), chlorophyll ($p = 0.0001$), and

phenols ($p = 0.0042$). In contrast, methanol was more effective in melon under seed priming, improving root length and fresh root weight ($p = 0.001$), as well as chlorophyll ($p = 0.045$). Cabbage extracts generally outperformed those from broccoli and cauliflower (Table 11), particularly for root length

Table 12: Orthogonal contrasts comparing the absolute control vs. botanical extracts for the evaluated variables

Evaluation in melon	Results AC (-) vs BE (+)	Evaluation in maize	Results AC (-) vs EB (+)
<i>In vitro</i> assay of melon seedlings	(+) Plumule (p = 0.0001)	<i>In vitro</i> assay of maize seedlings	(+) Plumule (p = 0.0001)
treated by seed priming	(+) Radicle (p = 0.005)	treated by seed priming	(+) Radicle (p = 0.0001)
Melon seedlings treated by seed priming	(+) Root length (p = 0.002)	Maize seedlings treated by seed priming	(+) Root length (p = 0.0012)
	(+) Apex height (p = 0.0338)		(+) Apex height (p = 0.0042)
	(+) FAW (p = 0.0476)		(+) FAW (p = 0.0296)
	(+) DAW (p = 0.0192)		(+) DAW (p = 0.0471)
	(+) FRW (p = 0.0195)		(+) FRW (p = 0.0151)
	(+) DRW(p = 0.0334)		(+) Chlorophyll (p = 0.0001)
	(+) Chlorophyll (p = 0.0165)		
	(+) Total phenols (p = 0.0001)		
Melon seedlings treated with extract spraying	(+) Root length (p = 0.001)	Maize seedlings treated with extract spraying	(+) Root length (p = 0.0001)
	(+) Apex height (p = 0.049)		(+) Apex height (p = 0.0312)
	(+) FAW (p = 0.0094)		(+) FAW (p = 0.0001)
	(+) DAW (p = 0.0131)		(+) DAW (p = 0.0001)
	(+) FRW (p = 0.0316)		(+) FRW (p = 0.0046)
	(+) DRW(p = 0.0142)		(+) DRW (p = 0.0032)
	(+) Chlorophyll (p = 0.0001)		(+) Chlorophyll (p = 0.0001)
	(+) Total phenols (p = 0.0025)		(+) Total phenols (p = 0.002)

DW: Dry weight, FAW: Fresh apex weight, DAW: Dry apex weight, FRW: Fresh radicle weight, DRW: Dry radicle weight, AC: Absolute control, BE: Botanical extracts. Only contrasts with statistically significant results are shown. Signs (+/-) indicate the coding of the corresponding results and the direction of the effect

($p \leq 0.001$), apex height ($p = 0.0082$), and chlorophyll ($p = 0.0165$) in melon, and for root length ($p = 0.0003$), root dry weight ($p = 0.0046$), and chlorophyll ($p = 0.0001$) in maize, although phenols decreased in melon ($p = 0.0032$). Overall, botanical extracts improved growth and physiological traits compared to the absolute control (Table 12), increasing root length, biomass, chlorophyll, and phenols ($p \leq 0.049$), confirming their positive effect in both species.

DISCUSSION

Metabolite extraction depends on the interaction between plant material and solvent, as well as on processing conditions such as particle size, time, and solid:liquid ratio¹⁵. In this study, ethanolic extracts showed higher contents of total phenols, flavonoids, proteins, and glutathione, together with greater antioxidant capacity, indicating a more efficient recovery of bioactive compounds than methanolic extracts^{16,17}. This agrees with previous reports showing that ethanol water mixtures effectively extract phenolic compounds and thiols, while also offering practical advantages due to their lower toxicity and greater suitability for agrifood applications¹⁷.

The metabolite profile also depended on the crucifer species. Cabbage showed the highest contents of proteins, phenols, and glutathione, as well as the greatest antioxidant capacity, which is consistent with studies reporting higher flavonoid levels and antioxidant activity in cabbage than in broccoli or cauliflower¹⁸. These results highlight cabbage, particularly in ethanolic extracts, as a promising raw material for the development of botanical biostimulants.

Seed imbibition is a widely recognized technique for improving germination rate and uniformity by reactivating physiological and metabolic processes prior to emergence, such as enzymatic activation, respiratory metabolism, the synthesis of proteins, nucleic acids, and antioxidants, and reserve mobilization^{19,20}. In this study, priming with botanical extracts increased germination rate in melon and maize compared with the control, suggesting that the compounds present in the extracts promoted early growth activation. This result agrees with previous studies attributing these effects to the stimulation of reserve-mobilizing enzymes and the regulation of antioxidant and hormonal networks during the early stages of germination²¹.

The effect was more evident in early growth traits, particularly plumule and radicle length, whereas the response in biomass was less pronounced. This agrees with reports indicating that priming effects are usually expressed first in emergence and early growth, and that changes in biomass may require more time to become evident²². In addition, phenolic compounds and flavonoids have been described as modulators of hormonal and antioxidant processes linked to root development, which reinforces the practical relevance of these extracts as biostimulants to improve early crop establishment²³.

Priming acts as an early stimulus that induces physiological and molecular adjustments associated with faster seedling establishment²⁴. Physiologically, it promoted early activation of seed metabolism, accelerated reserve mobilization, and led to faster germination²⁵; it may also have stimulated hormonal and antioxidant networks, mainly favoring root elongation, greater biomass allocation to the

root system, and, in some treatments, improved chlorophyll accumulation during the transition to autotrophy. In this study, melon and maize showed a stronger response in root elongation than in shoot growth, which agrees with previous studies describing priming as a process that improves reserve use, accelerates early growth, and can generate a stress memory that enhances later responses^{24,26}. The effect was more evident in the intermediate evaluation, whereas differences became less pronounced at later stages, probably because seed reserves were depleted before full autotrophy was reached^{27,28}; this pattern is consistent with reports indicating that priming responses are usually stronger during early establishment than in more advanced stages²². Overall, methanolic extracts were the most effective in promoting germination and early vigor, whereas extracts rich in antioxidant metabolites also favored chlorophyll accumulation and phenolic status in seedlings²⁹. Taken together, these results agree with previous studies showing that Brassica extracts can increase phenol content, antioxidant capacity, and early seedling performance, supporting their practical value as biostimulants to improve crop establishment.

Unlike seed priming, foliar spraying produced a clearer response in root growth and chlorophyll content. Physiologically, this application method allowed the bioactive compounds to act directly on young leaf tissues, where they may have modulated antioxidant and hormonal status, protected the photosynthetic apparatus, and promoted chlorophyll accumulation; as a result, photoassimilate availability may also have increased, indirectly stimulating root development^{29,30}. In melon, the ethanolic cabbage extract equaled or exceeded the commercial control for root length, whereas in maize, only the methanolic cabbage extract showed values comparable to the commercial control. These results suggest that cabbage extracts were the most effective under foliar application, in agreement with their higher phenolic content and with previous studies linking Brassica-derived compounds to root growth stimulation and improved seedling performance²⁸⁻³¹.

Overall, ethanolic extracts performed better under foliar spraying, which agrees with reports indicating that foliar extracts rich in phenols, flavonoids, and other antioxidant metabolites can improve leaf activity and support growth³². In this study, cauliflower and cabbage extracts, which showed higher antioxidant capacity and metabolite content, also promoted higher total chlorophyll, consistent with studies showing that antioxidant-rich biostimulants help preserve photosynthetic pigments and leaf function²⁹. In addition, the

application method influenced total phenol accumulation, with spraying producing the greatest increase, in line with previous evidence that foliar application can directly stimulate phenolic metabolism and act as an elicitor in treated tissues^{33,34}. Taken together, these results indicate that foliar spraying with Brassica extracts, especially cabbage-based formulations, is a promising strategy to improve early root development, chlorophyll content, and the biochemical status of seedlings.

CONCLUSION

Hydroalcoholic extracts from crucifer residues showed clear biostimulant potential in melon and maize, although their effectiveness depended on the solvent, crucifer species, crop, and application method. Ethanolic extracts showed more efficient recovery of bioactive compounds, whereas cabbage stood out for its higher metabolite content and more consistent biological response. Seed priming mainly promoted germination and early root growth, with a better response to methanolic extracts, whereas foliar spraying produced clearer effects on root development, chlorophyll content, and phenols of seedlings, with cabbage extracts showing the best performance. Overall, these results support the potential use of Brassica extracts as an alternative to improve early crop establishment and the physiological quality of seedlings.

SIGNIFICANCE STATEMENT

This study highlights critical advancements in crop resilience by optimizing agricultural management strategies. By systematically evaluating plant development across distinct growth phases, the findings provide actionable insights to maximize yield efficiency and enhance research integrity in plant sciences. Ultimately, this work offers a valuable framework for researchers and practitioners aiming to bridge the gap between theoretical data and sustainable, field-level applications.

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