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Article

Evaluation of four natural biostimulants in the sprouting and rooting of sugarcane cuttings [*Saccharum officinarum L.*] and their resistance to transplantation

Evaluación de cuatro bioestimulantes naturales en la brotación y enraizamiento de esquejes de caña de azúcar [*Saccharum officinarum L.*] y su resistencia al trasplante

Hernández-Guzmán, Eros De Jesús [a], Sandoval-Rojas, Martha Elvira [b], Rodríguez-Rivera, Viviana [c] and Ortiz-Celiseo, Araceli *[d]

[a] Tecnológico Nacional de México - Instituto Tecnológico de Orizaba • • 0009-0006-0441-9724• : 2039966
[b] Tecnológico Nacional de México - Instituto Tecnológico de Orizaba • • 0009-0005-7566-5079, • 80904
[c] Tecnológico Nacional de México - Instituto Tecnológico de Orizaba • • 0000-0003-3139-7939• 344862
[d] Tecnológico Nacional de México - Instituto Tecnológico de Orizaba • 0000-0002-6726-8453 • : 350246

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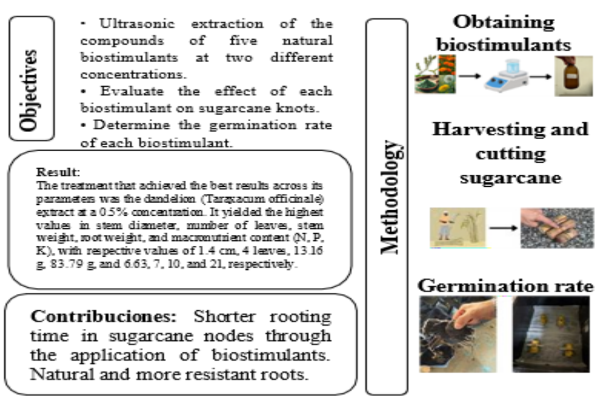
* [araceli.oc@orizaba.tecnm.mx]

Abstract

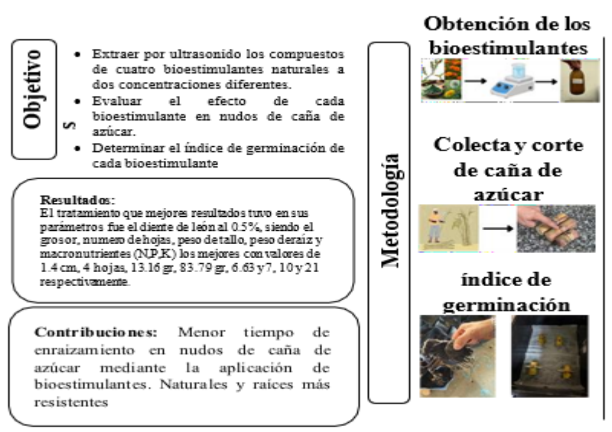
The sugarcane agroindustry holds significant importance in Mexico, particularly in the state of Veracruz, which is home to 17 of the country's 50 sugar mills. Although this sector provides substantial economic and productive benefits, it faces several challenges, including low productivity, water scarcity, and the limited effectiveness of biofertilizers against pests and diseases. As an alternative, this study evaluated four natural extracts used as biostimulants—*Elodea densa*, dandelion [*Taraxacum officinale*], marigold [*Calendula officinalis*], and spirulina [*Arthrospira platensis*]—to analyze their effects on sugarcane nodes through growth and germination indicators. The results indicated that the dandelion extract at a 0.5% concentration was the most effective, significantly improving the analyzed characteristics of sugarcane. *Elodea densa* ranked second, showing consistently favorable effects across all evaluated parameters.



Sugarcane, Natural Biostimulants, Sprouting and Rooting of nodes.

Resumen

La agroindustria de la caña de azúcar es de gran relevancia en México, especialmente en Veracruz, que alberga 17 de los 50 ingenios del país. Aunque esta actividad genera importantes beneficios económicos y productivos, enfrenta retos como la baja productividad, la escasez de agua y la resistencia de biofertilizantes ante plagas y enfermedades. Como alternativa, este estudio evaluó cuatro extractos naturales utilizados como bioestimulantes, [*Elodea densa*, diente de león, caléndula y espirulina], se analizó su efecto en nudos mediante indicadores de crecimiento y germinación. Los resultados mostraron que el extracto de diente de león con una concentración de 0.5% fue el más eficaz, logrando mejorar significativamente las características analizadas de la caña de azúcar, como segundo extracto con mejores beneficios fue la *Elodea densa*, ambos con efectos consistentemente favorables en todos los parámetros evaluados.



Caña de azúcar, Bioestimulantes naturales, Brotación y Enraizamiento de nudo

Area: Promotion of frontier research and basic science in all fields of knowledge

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Introduction

The sugarcane [Mex 69-290] agroindustry is one of the most significant economic activities in Mexico, generating multiple benefits such as employment, energy production, sugar, paper, biofuels, fertilizers, and animal feed, among others. In the state of Veracruz, 17 of the 46 sugar mills that participated in the 2024/2025 harvest season are located [CONADESUCA, 2025]. Moreover, more than 330,000 hectares are harvested annually out of the 850,000 cultivated nationwide, allowing the processing of approximately 22.5 million tons of sugarcane, with a field yield of less than 68 t/ha and an industrial yield exceeding 11% [Herrera et al., 2023].

Currently, the sugarcane agroindustry faces several challenges, including water scarcity and the limited effectiveness of biofertilizers against pests, which affect profitability and damage crops, thereby impacting producers and related employment.

A promising alternative to address these issues is the use of natural biostimulants. These products provide essential nutrients and minerals that stimulate physiological processes and root development, enhancing nutrient absorption, soil quality, and consequently, the productivity and quality of sugarcane.

Sugarcane

Saccharum officinarum L. is primarily used for sugar production, while its by-products or residues are utilized as fertilizer, animal feed, or in paper manufacturing. Sugarcane juice, another derivative, is used in the production of industrial alcohol and alcoholic beverages. Sugarcane is considered a cost-effective energy source and one of the crops with the greatest capacity to convert solar energy into biomass [SADR, 2020].

Sugarcane Growth Cycle

The growth cycle of the sugarcane crop, from planting to harvest, can last between 14 and 18 months [Figure 1]. During this period, sugarcane undergoes five distinct stages [SADR, 2019]:

Germination and emergence: This stage begins approximately 7 to 10 days after planting and lasts up to 35 days, during which the initial growth of the sugarcane occurs.

Emergence: This phase starts around 35 to 40 days after planting and is characterized by the development of secondary shoots from the basal nodes. It is a critical stage, as it determines the potential number of harvestable stalks.

Rapid growth: In this stage, the sugarcane exhibits rapid stem elongation and reaches its maximum leaf area. Its duration depends on the variety, temperature, and humidity.

Maturation: This phase occurs during the last 2 to 3 months of the cycle and involves the synthesis and accumulation of sucrose in the stalks, beginning from the base and progressing toward the upper sections.

Harvest: Carried out between 11 and 16 months after planting, mature stalks are identified by dry leaves and a brittle rind. Prior to harvesting, the field is typically burned to eliminate weeds and pests, and the sugarcane is then harvested either manually or mechanically.

Box 1

Figure 1
Stages of sugarcane growth
Source: SADR, 2020

Biostimulants

A plant biostimulant is any substance or microorganism applied to plants with the aim of improving nutritional efficiency, abiotic stress tolerance and/or crop quality characteristics, regardless of its nutrient content [du Jardín, 2015].

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Types of Biostimulants

Biostimulants originate from natural sources and can be effectively categorized into four main groups [Figure 2]:

Microbial: Microbial biostimulants, such as Plant Growth-Promoting *Rhizobacteria* [PGPR], arbuscular mycorrhizal fungi, and *Trichoderma* spp., enhance plant growth through biological nitrogen fixation, nutrient solubilization, and increased nutrient availability, even under adverse conditions.

They also produce volatile organic compounds and strengthen tolerance to abiotic stress. These can be applied to the rhizosphere or through seed treatments, using single strains or mixtures of microorganisms with synergistic effects [Hasanuzzaman et al., 2021].

Acids: Humic substances [humic acid, HA; fulvic acid, FA; humins], amino acids, fatty acids, and organic acids are considered members of this group of biostimulants [Hasanuzzaman et al., 2021]. Humic substances are primarily soil organic matter naturally produced not only through decomposition processes but also via microbial activity [Rouphael & Colla, 2018]. They improve plant growth and stress tolerance by enhancing water and nutrient uptake [Bulgari et al., 2019].

Other types: Beneficial elements such as Al, Si, Na, Se, and Co are not required by all plant species but are essential for the growth promotion of specific plants. These elements are found in plants and soils as inorganic salts such as chlorides, carbonates, silicates, phosphates, and phosphites [Hasanuzzaman et al., 2021].

Extracts: This is a vast group consisting of different types of products originating from different organisms, including seaweed, chitosan, bioactive substances derived from plants, polyphenols, and allelochemicals.

These extracts stimulate faster recovery of the plant from abiotic stress through increased membrane stability, elimination of reactive oxygen species [ROS] due to their role as cofactors in antioxidant activity and thus improve tolerance to oxidative stress [Van Oosten et al., 2017].

Box 2

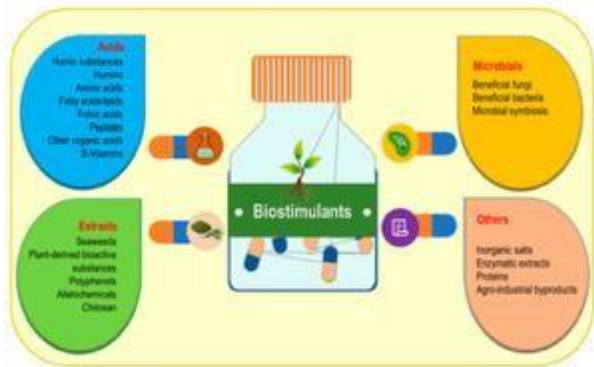

Figure 1

Main categories of biostimulants

Source: Hasanuzzaman et al., 2021

Advantages and Benefits of Biostimulants

The use of biostimulants helps stimulate root system development and plant growth throughout all reproductive stages, optimize nutrient availability and uptake, and improve the quality of fruits and vegetables in terms of firmness, diameter, sugar content, and postharvest shelf life.

Additionally, biostimulants enhance crop tolerance to both abiotic and biotic stress factors, promote soil fertility through the enrichment of microbiota and improvement of soil structure, and regulate water availability for plants, thereby reducing production losses.

Agricultural production must move toward more productive, efficient, and sustainable systems. In this context, the use of biostimulants contributes to a more efficient utilization of resources such as water and reduces the dependence on chemical products [MANVERT, 2023].

Extracts Used as Biostimulants

Marigold [*Calendula officinalis*], belonging to the Asteraceae family, has been shown to possess a wide range of pharmacological properties, including anti-inflammatory, anti-edematous, antioxidant, antibacterial, antifungal, wound-healing, and immunostimulant activities [Patil et al., 2022].

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Known for its biopesticide activity on various strawberry cultivars—particularly enhancing root system development—it has also demonstrated a positive impact on plant growth, especially on root traits associated with nutrient absorption [Furmanczyk et al., 2023].

Spirulina is a fast-growing microalga widely produced and valued in agroindustry as an agricultural biostimulant. It generates bioactive molecules [such as polysaccharides, amino acids, and phytohormones] that promote plant growth and increase tolerance to both biotic and abiotic stress [Arahou et al., 2022]. Studies have reported that treatment with spirulina increased the size of tomato and pepper plants by 20–30% compared to untreated plants, and also enhanced leaf size, measured as leaf area, by 57% and 100% in the same species, respectively [Farmaquimica Sur, 2020].

Elodea densa, also known as *Egeria densa*, is a freshwater aquatic plant that grows submerged and forms dense, homogeneous populations that restrict water flow, retain sediments and cause fluctuations in water quality [GISD, 2019]. It can be found in abundance in freshwater ecosystems such as ditches, lakes, ponds, rivers, and streams. These algae could be considered a pest due to its easy propagation and the damage it causes to infrastructure and ecosystems [North Carolina University, 2023]. Studies conducted by [Munhoz et al. 2025] showed that *Elodea densa* extract resulted in a 15% increase in height and dry biomass in *Cucumis saltivus* [cucumber] and *Urochloa decumbens* [grass] plants.

These findings highlight the potential of *Elodea densa* as a sustainable biostimulant and a solution to macrophyte overgrowth, supporting agricultural and environmental objectives.

Dandelion, also known as *Taraxacum officinale*, has been shown in recent studies to be effective as a plant biostimulant. Research by [Biesiada et al. 2019] revealed that dandelion-based extracts showed the highest biostimulant activity in cabbage seedlings compared to other plant extracts analysed [common mugwort, chamomile, basil, or purple coneflower].

Specifically, cabbage seedlings treated with a 2.5% concentration of dandelion flower extract showed 37% longer shoots, while those treated with 0.5% leaf extract had 76% longer roots compared to the control groups [Biesiada et al., 2019].

Methodology

Sugar cane harvesting

The sugar cane [variety Mex 69-290] was provided by farmers from the municipality of Santa Ana, Atzacan, Veracruz, located in the central mountainous region of the state, at coordinates 18°53'09"N 97°05'37 W [Figure 3], at an altitude of 1280 meters above sea level. Twenty stems in good condition and 18 months old were selected.

Box 3

Figure 3

Location of the site where the raw material is obtained

Source: Google Earth

Selection of sugarcane stalks

The best stalks were selected from which knots and seedlings in good condition and without defects were obtained for subsequent rooting. To do this, the leaves were removed to expose the knots and buds; The healthiest nodes, free of physical or mechanical abnormalities, were then selected and cut vertically, leaving a margin of 3 cm above and below the bud, for a total of 6 cm [Figure 4].

The nodes were classified according to their diameter and size, and two washes were carried out: 1] with water and multi-purpose disinfectant, adding 10 ml of disinfectant per 1 L of water, and 2] with citric acid dissolved in water at a concentration of 10 g of citric acid per 1 L of water.

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Obtaining biostimulants

Biostimulants were obtained from four different plant sources: microalgae [*Spirulina* and *Egeria densa*], dandelion flowers [*Taraxacum officinale*] and coyol bark [*Acrocomia aculeata*]. The samples were dried in a convection oven [VEVOR] at 40 °C for 12 hours. They were then ground in a blade mill [Estrella, model 11540] at 300 rpm and sieved with a #40 mesh.

Box 4

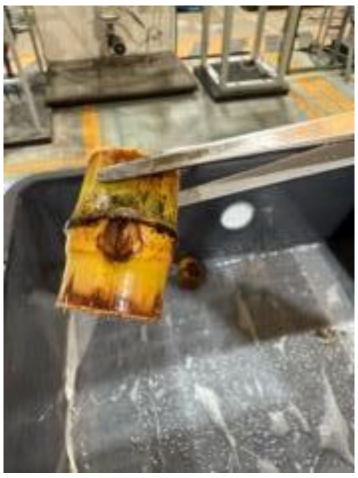

Figure 4
Cutting the sugarcane knot

For extraction, 200 g of each sample was weighed and placed in Erlenmeyer flasks, adding 500 ml of 70% ethanol. The samples were subjected to an ultrasonic bath [Cole-Parmer, 40 kHz] for 30 min and filtered with Whatman #1 paper. The filtrate obtained was concentrated to dryness in a rotary evaporator [Buchi] at 40 °C. Finally, the extracts were prepared at concentrations of 0.5% and 1% [w/v].

Physicochemical characterization of plant species

Physicochemical analysis was performed on plant species that served as natural biostimulants: calendula, spirulina, dandelion, and *Elodea densa*, in accordance with Mexican standards, as shown in Table 1.

Box 5

Table 1
Standards used for the physicochemical characterization of natural biostimulants

Characterization	Standard and/or method for such characterization
pH	NOM-F-317-S-1978
Acidity	NMX-F-102-NORMEX-2010
Humidity	NOM-008-SCFI-1993
Ash	NMX-F-607-NORMEX-2020
Fat	PROY-NMX-F-615-NORMEX-2018
Protein	NOM-F-68-S-1980
Color	CIELAB [Spectrophotometer CM-36d]

Application of Treatments in the Planting of Sugarcane Buds

The experiments were conducted using experimental units, with seven replicates per treatment and a control group [distilled water] also consisting of seven replicates. Cultivation bags were used for this procedure, each filled with 80 g of soil without compost and 20 g of gravel at the bottom.

Subsequently, 500 mL of solution was prepared for each of the biostimulants under study at two concentrations [0.5% and 1% w/v], and the sugarcane nodes were immersed in the corresponding solution for 15 minutes according to the treatment. After immersion, the cuttings were planted at a depth of 5 cm in each of the experimental units of this study [Figure 6] and arranged randomly according to the design 2^4.

Box 6

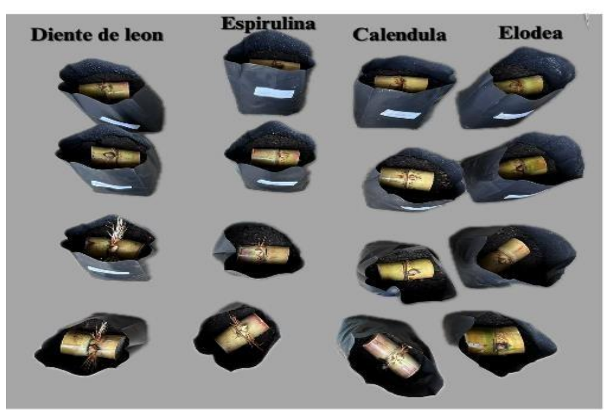

Figure 5
Planting sugarcane knots

Source: Own work

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Irrigation During Sprouting and Rooting of Sugarcane Nodes

The biostimulants at both concentrations [0.5% and 1% w/v] were applied to the sugarcane nodes every seven days after planting, for a total period of 60 days. Applications were performed by spraying until reaching a moisture level of 60%, and distilled water was added every three days to maintain this level of humidity.

Plant Growth Evaluation

Once the nodes germinated, growth data was collected every third day over a 60-day period. During this time, the germination index was monitored and determined by evaluating several parameters: longitudinal stem growth [measured with a tape measure], stem thickness or diameter [measured with a Mitutoyo Series 500 digital caliper], root area—assessed through horizontal, vertical, and diagonal measurements [using a tape measure]—and the number of leaves.

After 60 days of growth, the aerial part [leaves and stem] and the basal part [root] were separated and placed in kraft bags for subsequent analysis. They were then placed in a drying oven [Thermo Scientific Heratherm, Model IGS-60] for 72 hours at 65°C to remove moisture from the tissues. A digital scale [Uline Balanza, model H-9885] was used to determine the total dry matter [TDM]. Prior to drying, the length [cm] was determined.

Soil Characterization with Treatments

At the end of the 60-day period and following plant removal, a physicochemical characterization of the soil was conducted using the most effective biostimulant. The analysis included the evaluation of organic matter, pH, bulk density, gravimetric moisture, extractable phosphorus, inorganic nitrogen, and cation exchange capacity.

The characterization was performed in triplicate and in accordance with the NOM-021- RECNAT-2000 standard to assess the nutrients contributed by the biostimulant to the sugarcane cuttings. These parameters were compared with those obtained from the untreated control [Sample].

Statistical Analysis of Sprouting and Rooting Growth in Sugarcane Nodes

The study of sugarcane involves multiple variables which, when analyzed together, can be complex. To simplify interpretation and identify relationships among them, Principal Component Analysis [PCA] was employed a statistical technique that condenses information into a few components without losing the most relevant aspects.

Morphological characteristics [stem thickness, height, number of leaves], soil nutrients [nitrogen, phosphorus, potassium], chemical parameters [pH, electrical conductivity, moisture], and biometric weights [stem and root] were considered. The data were standardized to avoid bias caused by differences in measurement units and subsequently analyzed in R using the factoextra package.

The resulting biplot allowed for the visualization of how each variable contributed to the principal components and how samples clustered according to their experimental conditions, revealing common patterns and key trends in sugarcane development.

Results

Physicochemical Characterization of Plant Species

The results of the physicochemical characterization of the plant species used as natural biostimulants are presented in Table 2.

Box 7

Table 2

Results of characterization of natural biostimulant

Tratamiento	pH	Acidity [%]	Humidity [%]	Ash [%]	Fat [%]	Protein [gr]	Color
Elodea	7 ±0.5	0.0013 ±0.01	94.3 ±0.24	22.87 ±0.08	37.62 ±0.14	1.44 ±0.30	6.92 ±0.18
Dandelion	5.33 ±0.09	0.23 ±0.02	76.77 ±1.65	10.40 ±0.28	0.7 ±0.01	2.7 ±0.22	10.63 ±0.24
Marigold	5.33 ±0.09	0.0026 ±0.01	75.70 ±1.15	10.40 ±0.28	0	0.8 ±0.11	28.35 ±0.04
Spirulina	6.49 ±0.22	0.4537 ±0.05	90.1 ±0.3	9.04 ±0.01	7.5 ±0.01	58.8 ±0.14	3.182 ±0.01

Based on the results presented in Table 2, spirulina exhibits the most favorable characteristics for use as a biostimulant.

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This is primarily due to its high protein content, as some plants are capable of utilizing not only inorganic nitrogen but also intact amino acids and short peptides [proteins].

According to studies, the roots of various agricultural and wild plant species can exude proteases and use them to create an accessible nitrogen reserve [Adamczyk et al., 2010]. Additionally, its slightly acidic to neutral pH favors optimal nutrient availability and overall plant health [Yara, 2019]. Likewise, fat and acidity levels are considered non- representative values, whereas ash content indicates the amount of minerals present in the biostimulants.

Plant growth assessment

Sixty days after planting the cane nodes, they were sacrificed and the best biostimulant at the germination stage was determined.

Effect of biostimulant application on plant height.

The height of sugarcane plants with each natural biostimulant is shown in Table 3.

Box 8

Table 3

Sugarcane height data

Treatment	Height [cm]
Marigold 0.5%	13
Marigold 1%	11
Dandelion 0.5%	14
Dandelion 1%	14.5
Elodea 0.5%	13
Elodea 1%	10
Spirulina 0.5%	11
Spirulina 1%	15
Control	9

According to the results obtained, the most effective biostimulant for promoting plant height was spirulina at a 1% concentration, achieving an average height of 15 cm, followed by dandelion at 1% with 14.5 cm, and dandelion at 0.5% with 14 cm, with no significant difference observed between the latter two treatments. These results indicate a notable increase in height compared to those reported by Ghallab et al. [2024], who documented a maximum height of 253.63 cm for spirulina-treated plants over an 18-month period.

However, no studies have been found on the use of dandelion as a natural biostimulant for sugarcane growth. Conversely, the lowest growth in terms of height was recorded for the control treatment [distilled water], which reached only 9 cm.

Similarly, based on the findings of Medina [2025], the use of the bacterium *Azospirillum* resulted in a plant height of 59.33 cm in blueberry plants when applied at a dose of 20 mL with a single application.

However, when the Kelpak biostimulant derived from seaweed was applied at a 10 mL dose with five applications, a greater height of 60.25 cm was obtained. In contrast, the control treatment, which received neither dosage nor application, reached only 47.50 cm. This experiment was conducted over a period of 152 days.

Another study conducted by Vega et al. [2025], investigated the use of growth regulators on forage triticale over a six-month period. The Biozyme regulator proved to be the most effective, achieving a plant height of 1.06 m, differing significantly from the treatment without growth regulators, which was applied at a rate of 1 liter per 200 liters of water.

Effect of biostimulant application on plant diameter

The diameter values presented in Table 4 show no significant differences between the treatments with biostimulants and the control treated only with water.

Box 9

Table 4

Results of sugarcane diameter treatments

Treatment	diameter [cm]
Marigold 0.5%	1.3
Marigold 1%	1.2
Dandelion 0.5%	1.4
Dandelion 1%	1.1
Elodea 0.5%	1.2
Elodea 1%	1.2
Spirulina 0.5%	1.2
Spirulina 1%	1.2
Control	1.15

According to the results obtained, the most favorable treatment for the stem diameter parameter corresponded to dandelion at a 0.5% concentration. In second place was marigold at 0.5%, with a difference of only 0.1, which does not represent a statistically significant variation. In contrast, dandelion at 1% exhibited the lowest value recorded for this parameter. When comparing the value obtained for spirulina with the findings of Ghallab et al. [2024], a stem diameter of 2.97 cm was reported in sugarcane compared to 2.30 cm in the control treatment. These measurements were taken at harvest, corresponding to a growth period of 16–18 months.

Similarly, Medina [2025] reported that treatments with *Azospirillum* [20 mL, five applications] and Kelpak [10 mL, one and five applications] resulted in the same stem diameter of 0.86 cm, compared to 0.48 cm in the control after 152 days.

In another study, Lanche et al. [2024], focused on determining the effect of pseudostem colostrum on the vegetative growth of suckers, evaluating multiple doses. For the diameter parameter, no statistically significant differences were found among the treatments evaluated. However, it is worth noting that the T5 [control] treatment showed the highest average value of 15.10 cm, compared to the lowest value of 15.06 cm obtained with a 0.75 L/ha dose, both measured 45 days after treatment.

Effect of Biostimulant Application on the Number of Leaves

The number of leaves per plant was manually counted, considering only those that exhibited uniform growth. The results are presented in Table 5.

Box 10

Table 5

Sugar cane leaf data numbers

Treatment	Number of sheets
Marigold 0.5%	3
Marigold 1%	3
Dandelion 0.5%	4
Dandelion 1%	3
Elodea 0.5%	3
Elodea 1%	3
Spirulina 0.5%	2
Spirulina 1%	3
Control	2

The biostimulant that resulted in the greatest number of leaves was dandelion at a 0.5% concentration. Most of the other biostimulants produced between two and three leaves, indicating that there was no notable variation in this parameter.

Effect of Biostimulant Application on Stem and Root Weight

The results presented in Table 6 are particularly relevant, as they reflect one of the primary objectives of biostimulant application.

Box 11

Table 6

Data on the weight of the stem and root of sugar cane

Treatment	Stem Weight [gr]	Root Weight [gr]
Marigold 0.5%	5.31	59.33
Marigold 1%	4.30	57.89
Dandelion 0.5%	13.16	83.79
Dandelion 1%	7.83	81.88
Elodea 0.5%	8.61	77.76
Elodea 1%	4.61	62.69
Spirulina 0.5%	1.77	47.10
Spirulina 1%	3.66	55.59
Control	1.75	55.07

The best-performing treatment was dandelion at 0.5%, which produced the highest stem and root weight compared to the other treatments.

The least effective treatment was spirulina at 1%; however, Ghallab et al. [2024] reported a stem weight of 1.47 kg, although under longer observation periods.

Effect of Biostimulant Application on pH

The optimum soil pH is around 6.5, which maximizes nutrient availability. However, crops can tolerate a considerable level of acidity and alkalinity in the soil. [Yara, 2019].

In this context, Table 7 presents the data obtained.

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Box 12

Table 7

Soil pH data with biostimulants

Treatment	pH
Marigold 0.5%	7
Marigold 1%	6.67
Dandelion 0.5%	6.63
Dandelion 1%	6.74
Elodea 0.5%	6.66
Elodea 1%	6.84
Spirulina 0.5%	6.65
Spirulina 1%	6.83
Control	6.27

Among the treatments evaluated in Table 7, calendula at 0.5% exhibited a pH of 7, the value closest to neutrality, making it the most favorable treatment compared to the others, whose pH values ranged between 6.27 and 6.84. Nevertheless, all treatments remained within or near the optimal range for sugarcane cultivation.

Effect of Biostimulant Application on Moisture

Moisture content was determined following the AS-05 method, adhering to all specified procedural steps. The results are shown in Table 8. For optimal sugarcane development, particularly during the maximum growth stage, a relative humidity [RH] between 80% and 85% is required to promote stalk elongation.

Box 13

Table 8

Soil moisture data with biostimulants

Treatment	Humidity
Marigold 0.5%	45.36
Marigold 1%	35.36
Dandelion 0.5%	44.36
Dandelion 1%	51.62
Elodea 0.5%	46.91
Elodea 1%	46.26
Spirulina 0.5%	46.15
Spirulina 1%	39.87
Control	47.20

Relative humidity [RH] above 40%, combined with a warm climate, promotes the vegetative growth of sugarcane, while an RH between 45% and 65%, along with limited water supply, is favorable during the maturation phase [Vandana et al., 2020].

Based on this information and considering that the samples were collected during the maturation stage, it can be concluded that moisture levels were within the optimal range for most biostimulant treatments, except for Spirulina at 1%, which showed a value below the optimal range.

Soil Characterization with the Best Biostimulant

After completing the germination analysis, a physicochemical characterization of the soil treated with the most effective natural biostimulants was conducted following a 60-day period.

Effect of Biostimulant Application on Electrical Conductivity

The soil's electrical conductivity [E.C.] serves as an indirect indicator of salt concentration. Naturally, soil contains dissolved salts; therefore, its electrical conductivity can be very low but never null [Cremona et al., 2020].

In this study, a device capable of directly measuring electrical conductivity was employed, and the resulting data are presented in Table 9**.**

Box 14

Table 9

Soil electrical conductivity [EC] data with biostimulants

Treatment	E.C. [us/cm]
Marigold 0.5%	31
Marigold 1%	26
Dandelion 0.5%	109
Dandelion 1%	49
Elodea 0.5%	55
Elodea 1%	40
Spirulina 0.5%	101
Spirulina 1%	66
Control	37

A lower electrical conductivity in plant extract solutions indicates a reduced concentration of dissolved salts, which translates into a lower risk of osmotic stress and phytotoxicity within biological systems. In this regard, the treatment with Calendula at 1% proved to be the most favorable, as it maintained conductivity within a low and safe range.

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This finding is consistent with the results reported by Moncada et al. [2020], who noted that high electrical conductivity levels in culture solutions can negatively affect nutrient absorption and seedling growth, whereas moderate or low values promote better physiological development and overall system stability.

Effect of Biostimulant Application on Organic Matter

The analysis of soil organic matter is essential for understanding soil quality and its influence on physical, chemical, and biological properties. This information enables farmers to determine which amendments [such as compost or organic fertilizers] and what quantities are required to enhance soil fertility, thereby optimizing crop productivity.

Box 15

Table 10

Data on organic matter in soil with biostimulants

Treatment	Organic matter
Marigold 0.5%	6.87
Dandelion 0.5%	6.66
Elodea 0.5%	7.66
Spirulina 1%	6.0
Control	5.91

A higher organic matter content in the soil is associated with improvements in structure, aeration, water retention, and nutrient availability, thereby creating a favorable environment for plant growth. In this context, the treatment with *Elodea* at 0.5% proved to be the most advantageous, as it contributed to the greatest increase in organic matter.

These results are consistent with the findings of Lal [2020], who emphasized that organic matter is a key indicator of soil health and that higher levels are directly linked to greater agricultural productivity and sustainability.

Effect of Biostimulant Application on Soil N, K, and P

The data on nitrogen [N], phosphorus [P], and potassium [K] indicate the concentration of dissolved nutrients in the soil before and after the experimental period, as presented in Table 11.

Box 15

Table 10

N, K and P data in soil with biostimulants

Treatment	N [mg/kg]	P [mg/kg]	K [mg/kg]
Marigold 0.5%	3	2	5
Marigold 1%	2	3	6
Dandelion 0.5%	3	4	9
Dandelion 1%	7	10	21
Elodea 0.5%	2	5	8
Elodea 1%	3	5	11
Spirulina 0.5%	4	6	13
Spirulina 1%	7	10	20
Control	2	3	7

Nitrogen participates in protein synthesis and is therefore vital for all metabolic activities in plants [Aguilar et al., 2019]. Phosphorus is an essential nutrient for plant growth and a key factor influencing vegetation development and long-term ecosystem sustainability [Hongtao et al., 2017]. Potassium, due to its high mobility, acts as a neutralizing agent for organic acids produced during metabolism, thus maintaining the hydrogen balance within plant cell sap [Menese et al., 2017].

Based on the results shown in the table and in comparison, with Aguilar et al. [2019], who evaluated nitrogen, phosphorus, and potassium to determine the agronomic performance of maize canguil the treatment consisting of 150 kg/ha of nitrogen, 27 kg/ha of phosphorus, and 102 kg/ha of potassium produced improvements in plant height, stem diameter, and grain weight. Therefore, considering these findings, the dandelion extract, which presented the highest content of these nutrients, demonstrated superior results in parameters such as stem thickness, leaf number, stem weight, and root weight.

Results of the Statistical Analysis of the Experimental Design

The Principal Component Analysis [PCA] was performed to reduce the dimensionality of the dataset and to examine the relationships among the morphological, chemical, and biometric variables of the samples grouped into five experimental treatments: Calendula [C], Dandelion [D], *Elodea* [E], Spirulina [S], and Control [T]. The first two principal components [Dim1 and Dim2] together explained 69.7% of the total variance [41.7% and 28.0%, respectively], indicating that they adequately represent the variability of the dataset.

Hernández-Guzmán, Eros De Jesús, Sandoval-Rojas, Martha Elvira, Rodríguez-Rivera, Viviana and Ortiz-Celiseo, Araceli. [2025]. Evaluation of four natural biostimulants in the sprouting and rooting of sugarcane cuttings [Saccharum officinarum L.] and their resistance to transplantation. Journal of Engineering Applications. 12[33]1-13: e71233113.
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The biplot derived from the multivariate analysis revealed a clear separation among the experimental groups. Samples from groups C and S were primarily located in the positive region of the first component, associated with higher nutrient content in the soil [N, P, K] and greater stem weight, whereas groups D, E, and T were positioned in the negative region, highlighting distinct morphological and growth- related differences.

Box 16

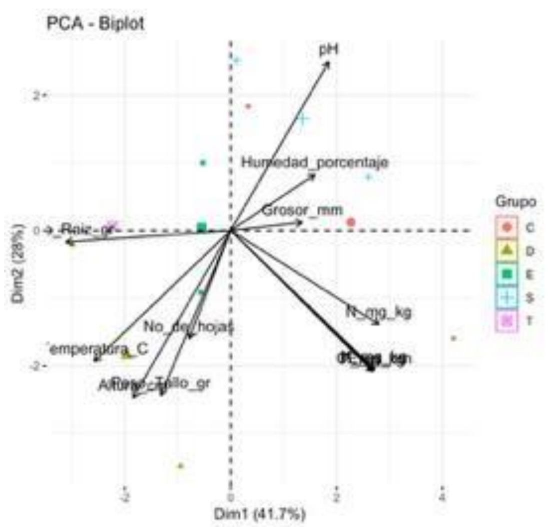

Figure 6
Principal Component Analysis [PCA]

The biplot from the multivariate analysis revealed a clear separation among the experimental groups. Samples from groups C and S were primarily located in the positive region of the first component, associated with higher soil nutrient content [N, P, K] and greater stem weight, whereas groups D, E, and T were positioned in the negative region, highlighting morphological and growth-related differences.

Additionally, variables such as pH, moisture, and stem diameter influenced differentiation along the second component, while temperature, plant height, and number of leaves were more closely related to groups D and T. Finally, root weight showed an opposite trend to nutrient content and stem weight, suggesting a possible negative relationship between these traits. These findings indicate that both soil chemical properties and plant biometric attributes vary depending on the treatment, contributing significantly to the observed differentiation in sugarcane growth.

Conclusion

The results of the Principal Component Analysis [PCA] revealed that variables related to nutrient concentration [N, P, K] are strongly associated with stem weight, suggesting that the availability of these nutrients in the soil promotes the biometric development of plants.

This finding aligns with previous studies emphasizing the critical role of nitrogen and potassium in plant growth and biomass accumulation [Ma et al., 2022].

The results demonstrated that the application of biostimulants is beneficial for both root development and various morphological and physiological characteristics of sugarcane.

Among the treatments evaluated, the 0.5% dandelion-based biostimulant showed the most outstanding performance across most analyzed parameters, recording the highest values in stem diameter, leaf number, stem and root weight, and electrical conductivity [EC]. In second place, the 1% dandelion treatment also exhibited positive effects, particularly on plant height and moisture content.

Conversely, the spirulina biostimulant, at both concentrations [0.5% and 1%], showed the lowest performance in most of the evaluated indicators, including leaf number, stem and root weight, electrical conductivity, and moisture— ranking only slightly above the control treatment.

Overall, these results confirm that the application of natural biostimulants produces significant benefits in sugarcane development, supporting their use as an effective agronomic strategy to enhance crop yield and quality.

Declarations

Conflict of interest

The authors declare no interest conflict. They have no known competing financial interests or personal relationships that could have appeared to influence the article reported in this article.

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Author contribution

The contribution of each researcher in each of the points developed in this research was defined based on:

Hernández-Guzmán, Eros De Jesús: Contributed to the conceptualization of the study, as well as the research method and technique. Participated in the analysis and interpretation of the results.

Rodriguez-Rivera Viviana: Contributed to the experimental design and the analysis of results.

Sandoval-Rojas Martha Elvira: Contributed to the physicochemical characterization of the plant species and the soil analysis.

Ortiz-Celiseo, Araceli: Contributed to the project conception, methodological design, research approach, and overall supervision of the study.

Availability of data and materials

The yields generated by biostimulants were obtained at the Orizaba Institute of Technology.

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Abbreviations

PGPR	Rizobacterias Promotoras del Crecimiento Vegetal
C.E	Conductividad Electrica
MST	Materia Seca Total

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