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Biostimulants Use to Improve Quality and Stress Resistance of *Echinocereus rigidissimus* var. *rubrispinus* and *Bulbine abyssinica*

Francesco Attanasio [1] and Domenico Prisa [2,*]

[1] *Attanasio Farm, Località San Martino 17, Torraca, Italy.*

[2] *Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria (CREA), Centro di Ricerca Orticoltura e Florovivaismo, Via dei Fiori 8, 51012 Pescia, PT, Italy.*

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Abstract

The use of biostimulants is gaining increasing importance as a sustainable strategy to improve plant growth, quality, and tolerance to abiotic stress in ornamental horticulture. This study evaluated the effects of different biostimulant approaches on *Echinocereus rigidissimus* var. *rubrispinus* and *Bulbine abyssinica* grown in pots under greenhouse conditions during the winter–spring season at the Pescia, CREA Research Centre for Vegetable and Ornamental Crops. All treatments were applied in combination with a uniform fertilization program. Six treatments were compared: fertilized control, *Inula viscosa* extract, electroculture, *Inula viscosa* combined with electroculture, a commercial seaweed-based product derived from *Ascophyllum nodosum*, and a microbial inoculum containing *Glomus mosseae* and *Rhizophagus irregularis*. Growth parameters, root development, chlorophyll content (SPAD index), ornamental quality, and stress tolerance under controlled drought conditions were assessed. Results showed that all biostimulant treatments significantly improved plant performance compared to the control. The combined application of *Inula viscosa* and electroculture produced the highest increases in vegetative growth, biomass accumulation, and chlorophyll content in both species, indicating a synergistic effect. The mycorrhizal treatment markedly enhanced root system development and improved plant survival under water deficit conditions. The seaweed-based product provided consistent improvements in plant vigor and ornamental traits. Overall, the integration of plant extracts, beneficial microorganisms, and physical stimulation techniques demonstrated a strong potential to enhance both growth and stress resilience in succulent and xerophytic species. These findings support the adoption of combined biostimulant strategies as an effective tool for sustainable ornamental plant production under greenhouse conditions.

Keywords: Abiotic stress tolerance; Greenhouse cultivation; Arbuscular mycorrhizal fungi; Seaweed extract; Sustainable horticulture; Root architecture

1. Introduction

The increasing emphasis on environmentally responsible horticulture has encouraged the development of cultivation strategies that enhance plant performance while minimizing external inputs. In this context, biostimulants have emerged as valuable tools capable of promoting plant growth, improving nutrient efficiency, and strengthening tolerance to environmental constraints without functioning as traditional fertilizers [1]. These products include a diverse group of substances and organisms such as botanical extracts, marine-derived compounds, beneficial soil microbes, and innovative physical techniques, each contributing to plant development through different mechanisms [2]. Their relevance is particularly evident in ornamental plant production, where visual quality and adaptability to controlled environments are key commercial traits [3]. Succulent and xerophytic species have gained significant popularity in recent years due to their distinctive morphology and reduced water requirements [4]. Among these, *Echinocereus rigidissimus* var. *rubrispinus* is highly valued for its decorative spination and compact form, while *Bulbine*

* Corresponding author: Domenico Prisa

abyssinica is appreciated for its ornamental and ethnobotanical significance. Despite their natural adaptation to arid conditions, these species may experience suboptimal growth when cultivated in containers under greenhouse conditions, where root restriction, substrate composition, and irrigation regimes can influence physiological responses [5]. Environmental stresses such as water imbalance and temperature fluctuations may lead to reduced biomass production and diminished aesthetic quality [6]. Consequently, the application of biostimulants represents a promising strategy to improve cultivation outcomes in these plants [7]. Plant-derived extracts are increasingly investigated as natural biostimulants due to their richness in biologically active molecules. *Inula viscosa*, a Mediterranean species, contains a wide array of secondary metabolites including phenolic compounds and terpenes that have been associated with antimicrobial activity and plant growth stimulation [8]. These compounds may enhance plant defense systems and modulate metabolic processes, contributing to improved resilience under stress conditions [9]. Similarly, seaweed extracts derived from *Ascophyllum nodosum* are widely used in horticulture because of their content of polysaccharides, trace elements, and hormone-like substances that can positively influence plant growth and stress tolerance [10]. Their application has been linked to increased chlorophyll content, improved nutrient uptake, and enhanced overall vigor [11]. Microbial biostimulants, particularly arbuscular mycorrhizal fungi (AMF), play a fundamental role in sustainable plant production systems. Species such as *Glomus mosseae* and *Rhizophagus irregularis* establish symbiotic associations with plant roots, facilitating the uptake of water and essential nutrients, especially phosphorus [12]. In addition, AMF can improve plant tolerance to drought and other abiotic stresses by enhancing root system development and physiological efficiency [13]. Their use has been successfully demonstrated in various horticultural crops, including ornamental and drought-adapted species [14]. Alongside biological inputs, physical stimulation methods are gaining attention as complementary approaches to plant enhancement. Electroculture, which involves the exposure of plants to low-intensity electrical fields or atmospheric energy, has been proposed as a technique capable of influencing plant metabolism and growth dynamics [15]. Although the underlying mechanisms remain partially understood, it is suggested that electrical stimuli may affect ion mobility, membrane permeability, and enzymatic activity, thereby promoting physiological processes [16]. Preliminary findings indicate potential benefits in terms of plant growth and stress adaptation across different species [17]. The integration of multiple biostimulant approaches may result in additive or synergistic effects, offering greater improvements than single treatments alone [18]. However, there is still limited research on the combined application of plant extracts, microbial inoculants, and electroculture, particularly in succulent species cultivated under protected conditions. Therefore, the objective of this study was to assess the impact of *Inula viscosa*, electroculture, their combined application, and two commercial biostimulants based on *Ascophyllum nodosum* and arbuscular mycorrhizal fungi on the growth, ornamental quality, and stress tolerance of *Echinocereus rigidissimus* var. *rubrispinus* and *Bulbine abyssinica*. The experiment was conducted under greenhouse conditions at the Pescia CREA Research Centre for Vegetable and Ornamental Crops, with the aim of identifying sustainable practices for improving the cultivation of these species.

2. Materials and methods

2.1. Experimental Site and Growth Conditions

The experiment was conducted in a greenhouse at the Pescia CREA Research Centre for Vegetable and Ornamental Crops during the winter–spring growing period. Environmental conditions inside the greenhouse were monitored but not artificially forced beyond standard management practices. Air temperature ranged from 12 to 26 °C, reflecting seasonal variation, while relative humidity fluctuated between 60% and 80%. Natural daylight was used without supplemental lighting, and shading systems were applied only during periods of increased solar radiation toward late spring. Ventilation was automatically regulated to maintain stable internal conditions and prevent excessive humidity.

2.2. Plant Material and Substrate

Two species were included in the study: *Echinocereus rigidissimus* var. *rubrispinus* and *Bulbine abyssinica*. Uniform plants, free from visible defects and with comparable size and developmental stage, were selected at the beginning of the trial. Plants were transplanted into 12 cm diameter plastic pots filled with a substrate composed of peat and pumice (1:1, v/v). This mixture ensured optimal drainage and aeration, essential for succulent and xerophytic species. No base fertilization was added to the substrate to allow a clearer evaluation of treatment effects.

2.3. Experimental Design

A randomized complete block design was adopted. Six treatments were tested with three replicates per treatment for each species. Each replicate consisted of ten plants, resulting in 180 plants per species. Pots were arranged on greenhouse benches with uniform spacing to minimize microenvironmental variability.

2.4. Treatments

Six treatments were evaluated:

- Control (fertilization only)
- *Inula viscosa* extract + fertilization
- Electroculture + fertilization
- *Inula viscosa* extract + electroculture + fertilization
- *Ascophyllum nodosum* + fertilization
- *Glomus mosseae* + *Rhizophagus irregularis* + fertilization

All treatments received the same basal fertilization program in order to exclude nutrient deficiency as a limiting factor and to specifically assess the biostimulant effects. A balanced water-soluble fertilizer (N–P–K 20–20–20) was applied through irrigation at a concentration of 1 g L^{-1} once per week throughout the experimental period.

The *Inula viscosa* extract was prepared from dried aerial parts using aqueous extraction and applied as a foliar spray at 10-day intervals. The commercial seaweed-based product derived from *Ascophyllum nodosum* was applied via irrigation according to the manufacturer's recommended dosage. The mycorrhizal inoculum containing *Glomus mosseae* and *Rhizophagus irregularis* was incorporated into the substrate at transplanting by placing it in direct contact with the root system.

Electroculture treatment consisted of the installation of low-intensity electrical field devices above the plants. These systems operated continuously throughout the trial. The combined treatment included both electroculture exposure and *Inula viscosa* foliar applications.

2.5. Irrigation and Crop Management

Irrigation was applied uniformly across all treatments using a drip system. Watering frequency varied from once per week during winter to two or three times per week in spring, depending on substrate moisture levels. Care was taken to avoid waterlogging conditions.

Standard cultural practices were followed throughout the experiment. No chemical pesticides were used, and any pest occurrence was managed manually.

2.6. Data Collection

Evaluations were carried out 120 days after transplanting. The following parameters were measured:

- Vegetative growth: plant height, number of shoots or offsets, fresh and dry biomass
- Root development: root length and root dry weight
- Physiological parameters: chlorophyll content measured using a SPAD meter
- Ornamental quality: visual scoring based on uniformity, coloration, and plant compactness
- Stress tolerance: plants were subjected to a controlled drought period (10 days without irrigation), followed by rewatering. Wilting symptoms, recovery time, and survival rate were recorded

2.7. Statistical Analysis

The experiment was designed as a randomized complete block design. The data collected were analyzed for significant differences ($P \leq 0.05$, 0.01 and 0.001) among treatments using a one-way ANOVA technique using the GLM univariate procedure. Once the analysis proportions were made, means were separated using the LSD multiple range test ($P = 0.05$). All the statistics and graphics were generated in Costat (version 6.451) and Excel (Office 2010).

3. Results and Discussion

The application of different biostimulant strategies, in combination with a uniform fertilization regime, resulted in significant improvements in growth, physiological activity, ornamental quality, and stress tolerance in both *Echinocereus rigidissimus* var. *rubrispinus* and *Bulbine abyssinica*. The response patterns observed confirm that biostimulants can enhance plant performance beyond the baseline provided by mineral nutrition alone [19]. However, the magnitude and nature of the response varied between the two species, reflecting differences in growth habit, physiology, and adaptation to environmental conditions.

In *Echinocereus rigidissimus*, vegetative growth was moderately responsive to treatments, consistent with the inherently slow growth rate of this cactus species. Nevertheless, statistically significant increases in plant height and biomass were observed in all treated plants compared to the fertilized control (Table 1). The combined application of *Inula viscosa* extract and electroculture produced the highest values for these parameters, indicating a synergistic interaction between chemical and physical stimulation (Figure 1) [20]. This combined treatment likely enhanced metabolic efficiency by simultaneously activating biochemical pathways and improving ion transport processes. The seaweed-based biostimulant derived from *Ascophyllum nodosum* also promoted growth, supporting previous findings that attribute such effects to the presence of phytohormones, amino acids, and polysaccharides [21,22].

Root development in *Echinocereus rigidissimus* showed a distinct response pattern. The application of arbuscular mycorrhizal fungi (*Glomus mosseae* and *Rhizophagus irregularis*) resulted in the greatest increases in root length and root biomass (Table 1). This outcome is consistent with the well-documented role of AMF in improving root system architecture and enhancing nutrient and water uptake [23,24]. The development of a more extensive root network likely contributed to improved plant stability and resource acquisition under both optimal and stress conditions. The combined *Inula viscosa* and electroculture treatment also stimulated root growth, although to a lesser extent than AMF, suggesting that while biochemical and physical stimuli can promote root activity, symbiotic interactions remain the most effective mechanism for root system enhancement [25].

In *Bulbine abyssinica*, vegetative growth responses were more pronounced, reflecting the faster growth dynamics of this species. All treatments significantly increased plant height, biomass accumulation, and shoot development compared to the control (Table 2). As observed in *Echinocereus*, the combined *Inula viscosa* and electroculture treatment produced the highest values, confirming the effectiveness of this integrated approach across different plant types. The seaweed-based treatment also showed strong performance, particularly in promoting leaf expansion and overall plant vigor. These effects are likely associated with the presence of cytokinin-like compounds and micronutrients that stimulate cell division and elongation [26-28].

Root development in *Bulbine abyssinica* was again most strongly influenced by mycorrhizal inoculation. Plants treated with AMF exhibited the highest root length and dry weight values (Table 2), indicating improved below-ground growth and nutrient acquisition capacity (Figure 2). This enhanced root system likely contributed to the superior performance of these plants under drought conditions. The combined treatment and seaweed extract also improved root parameters, although their effects were less pronounced than those of AMF, highlighting the importance of microbial symbiosis in root development [29,30].

Physiological parameters, particularly chlorophyll content, were significantly affected by all treatments in both species. The highest SPAD values were recorded in plants treated with the combined *Inula viscosa* and electroculture approach, as well as in those receiving the seaweed-based biostimulant (Tables 1 and 2). Increased chlorophyll content is indicative of enhanced photosynthetic capacity and improved nitrogen assimilation [31]. Seaweed extracts are known to contain compounds such as betaines and trace elements that protect chloroplast integrity and support pigment synthesis [32]. Electroculture may further enhance these processes by influencing membrane permeability and enzyme activity, thereby facilitating nutrient uptake and metabolic efficiency [33].

Ornamental quality, a key parameter for both species, was significantly improved by biostimulant application. In *Echinocereus rigidissimus*, treated plants exhibited more intense spine coloration, improved symmetry, and greater compactness compared to the control (Table 1). In *Bulbine abyssinica*, improvements were observed in leaf turgidity, uniformity, and overall visual appeal (Table 2). The highest ornamental scores were consistently associated with the combined treatment and the seaweed-based product, suggesting that these approaches effectively enhance both structural and aesthetic traits. These improvements are closely linked to better physiological balance and more efficient resource utilization.

The evaluation of stress tolerance under controlled drought conditions revealed clear differences among treatments. In both species, plants treated with AMF and those receiving the combined *Inula viscosa* and electroculture treatment exhibited the highest survival rates and the fastest recovery following rehydration (Tables 1 and 2). These results indicate that biostimulants can significantly enhance plant resilience to water deficit. Mycorrhizal fungi likely improved drought tolerance by increasing root hydraulic conductivity and facilitating access to soil moisture [34]. Meanwhile, the bioactive compounds in *Inula viscosa* may have contributed to enhanced antioxidant activity, reducing oxidative damage during stress [35].

Electroculture alone produced moderate improvements in most parameters but consistently enhanced the effects of other treatments when applied in combination [36]. This suggests that electrical stimulation may act as a complementary factor, amplifying plant responses to biochemical inputs. Although the exact mechanisms remain unclear, it is plausible that electroculture influences ion movement, cellular signaling, and enzymatic activity, thereby improving overall plant performance [37].

Overall, the results demonstrate that integrating multiple biostimulant strategies can produce additive and, in some cases, synergistic effects on plant growth and stress tolerance [38]. The combined application of *Inula viscosa* and electroculture emerged as the most effective approach for enhancing above-ground growth and physiological performance, while mycorrhizal inoculation was particularly beneficial for root development and drought resistance [39]. These findings highlight the potential of combining natural extracts, beneficial microorganisms, and innovative physical techniques to develop sustainable cultivation protocols for ornamental and xerophytic species grown under greenhouse conditions [40].

Table 1 Effect of biostimulant treatments on *Echinocereus rigidissimus* var. *rubrispinus*

Treatment	Height (cm)	Biomass (g DW)	Root Length (cm)	Root DW (g)	SPAD	Ornamental Score	Survival (%)
Control (fertilized)	7.5 ± 0.3 d	3.8 ± 0.2 d	8.5 ± 0.4 d	1.6 ± 0.1 d	30.2 ± 1.0 d	2.7 ± 0.2 d	60 ± 3 d
Inula viscosa	8.8 ± 0.4 c	4.6 ± 0.2 c	9.8 ± 0.5 c	1.9 ± 0.1 c	34.5 ± 1.1 c	3.4 ± 0.2 c	70 ± 2 c
Electroculture	8.4 ± 0.3 c	4.3 ± 0.2 c	9.5 ± 0.4 c	1.8 ± 0.1 c	33.2 ± 1.2 c	3.2 ± 0.2 c	68 ± 3 c
Inula viscosa + Electroculture	10.2 ± 0.5 a	5.5 ± 0.3 a	10.9 ± 0.6 b	2.2 ± 0.2 b	38.8 ± 1.3 a	4.5 ± 0.1 a	88 ± 2 a
Ascophyllum nodosum	9.6 ± 0.4 b	5.0 ± 0.2 b	10.2 ± 0.5 bc	2.0 ± 0.1 bc	37.2 ± 1.1 ab	4.1 ± 0.2 ab	82 ± 3 ab
AMF (*Glomus mosseae* + *Rhizophagus irregularis*)	9.3 ± 0.3 b	4.8 ± 0.3 b	11.8 ± 0.7 a	2.5 ± 0.2 a	36.5 ± 1.0 b	3.9 ± 0.2 b	85 ± 2 a
ANOVA	***	***	***	***	***	***	***

Mean values (± standard error) of vegetative growth (plant height, dry biomass), root development (root length and root dry weight), physiological status (SPAD index), ornamental quality (visual score), and stress tolerance (survival percentage after drought stress) recorded 120 days after transplanting under greenhouse conditions. All treatments were applied in combination with a uniform fertilization program. Different letters within each column indicate statistically significant differences among treatments according to Tukey's HSD test ($p \leq 0.05$). Analysis of variance (ANOVA) revealed highly significant effects of treatments for all measured parameters ($p < 0.001$).

Table 2 Effect of biostimulant treatments on *Bulbine abyssinica*

Treatment	Height (cm)	Biomass (g DW)	Root Length (cm)	Root DW (g)	SPAD	Ornamental Score	Survival (%)
Control (fertilized)	9.0 ± 0.4 d	5.2 ± 0.3 d	10.2 ± 0.5 d	2.0 ± 0.1 d	34.6 ± 1.2 d	3.0 ± 0.2 d	70 ± 3 d
Inula viscosa	10.5 ± 0.5 c	6.3 ± 0.3 c	11.6 ± 0.6 c	2.4 ± 0.1 c	38.2 ± 1.1 c	3.8 ± 0.2 c	80 ± 2 c
Electroculture	10.0 ± 0.4 c	5.9 ± 0.2 c	11.0 ± 0.5 c	2.3 ± 0.1 c	37.1 ± 1.3 c	3.6 ± 0.2 c	78 ± 3 c
Inula viscosa + Electroculture	12.3 ± 0.6 a	7.5 ± 0.4 a	12.8 ± 0.7 b	2.9 ± 0.2 b	42.0 ± 1.4 a	4.7 ± 0.1 a	95 ± 2 a
Ascophyllum nodosum	11.5 ± 0.5 b	6.9 ± 0.3 b	12.0 ± 0.6 bc	2.6 ± 0.1 bc	40.8 ± 1.2 ab	4.4 ± 0.2 ab	90 ± 3 ab
AMF (*Glomus mosseae* + *Rhizophagus irregularis*)	11.0 ± 0.4 b	6.6 ± 0.3 b	13.5 ± 0.8 a	3.2 ± 0.2 a	39.9 ± 1.1 b	4.2 ± 0.2 b	92 ± 2 a
ANOVA	***	***	***	***	***	***	***

Mean values (± standard error) of vegetative growth (plant height, dry biomass), root development (root length and root dry weight), physiological parameters (chlorophyll content expressed as SPAD index), ornamental quality (visual rating), and stress tolerance (survival percentage following controlled drought conditions) measured at the end of the experimental period (120 days). All treatments were conducted under the same fertilization regime in greenhouse conditions. Different letters within columns denote significant differences among treatments based on Tukey's HSD test ($p \leq 0.05$). ANOVA results indicated statistically significant treatment effects for all variables ($p < 0.001$).

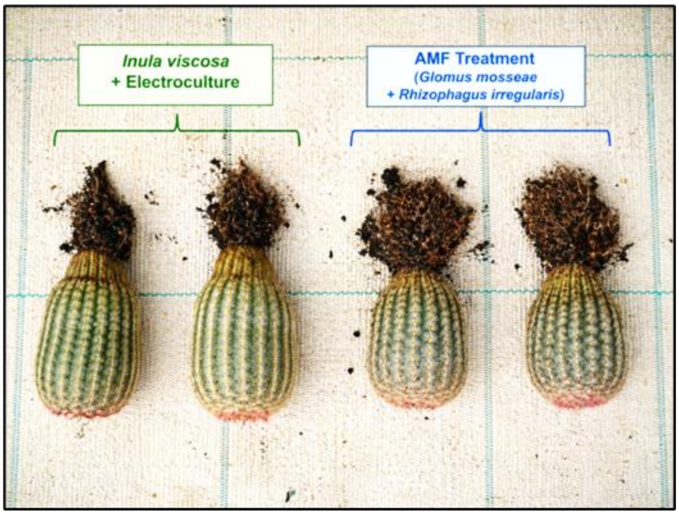

Figure 1 Visual comparison of root and shoot development in *Echinocereus rigidissimus* var. *rubrispinus* under different biostimulant treatments. Plants on the left were treated with *Inula viscosa* extract combined with electroculture, while plants on the right received arbuscular mycorrhizal fungi (AMF) inoculation (*Glomus mosseae* + *Rhizophagus irregularis*). The combined treatment promoted greater shoot size and overall biomass, whereas AMF treatment resulted in a more developed and highly branched root system. This image highlights the contrasting allocation patterns between above-ground and below-ground growth induced by the different treatments

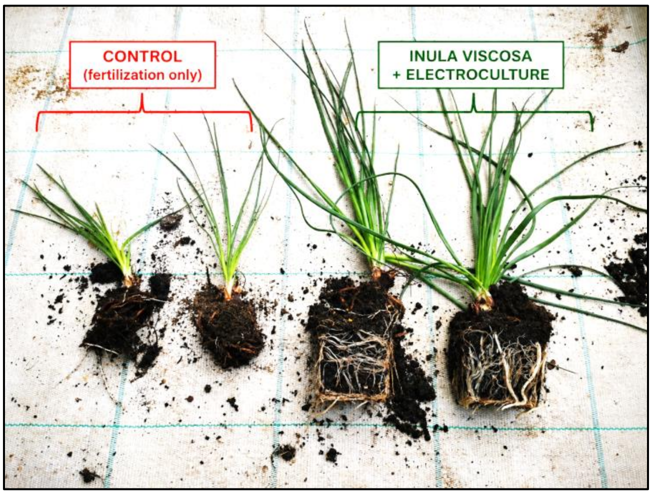

Figure 2 Comparison of root and shoot development in *Bulbine abyssinica* under different treatments. The two plants on the left represent the control treatment (fertilization only), while the two plants on the right were treated with *Inula viscosa* extract combined with electroculture. Plants subjected to the combined treatment show enhanced vegetative growth, with greater leaf length and biomass, as well as a more developed and structured root system compared to the control. The image highlights the positive effect of the combined biostimulant and electroculture approach on both above-ground vigor and root architecture.

4. Conclusion

The present study demonstrates that the application of biostimulants, in combination with a consistent fertilization program, can substantially improve growth performance, physiological efficiency, ornamental quality, and stress tolerance in both *Echinocereus rigidissimus* var. *rubrispinus* and *Bulbine abyssinica*. Although both species benefited from all tested treatments, distinct response patterns were observed, highlighting the importance of selecting appropriate biostimulant strategies according to plant type and desired outcomes. Among the treatments evaluated, the combined application of *Inula viscosa* extract and electroculture proved to be the most effective in enhancing above-ground growth and overall plant vigor. This approach consistently resulted in higher biomass accumulation, improved chlorophyll content, and superior ornamental characteristics. The results suggest that the integration of natural plant-derived compounds with physical stimulation techniques can create synergistic effects, leading to improved metabolic activity and resource utilization. In contrast, the application of arbuscular mycorrhizal fungi (*Glomus mosseae* and *Rhizophagus irregularis*) was particularly effective in promoting root system development and increasing tolerance to water deficit. Plants treated with AMF exhibited more extensive and structured root systems, which translated into improved resilience under drought conditions. This confirms the critical role of symbiotic microorganisms in enhancing below-ground functionality and stress adaptation. The seaweed-based biostimulant derived from *Ascophyllum nodosum* also produced positive and consistent effects across most parameters, supporting its widespread use in horticultural systems as a reliable growth enhancer. Electroculture applied alone showed moderate benefits but demonstrated greater effectiveness when combined with *Inula viscosa*, reinforcing its potential as a complementary technique rather than a standalone solution. Overall, the findings of this study highlight the value of integrating multiple biostimulant approaches to optimize plant performance in greenhouse cultivation systems. The combined use of botanical extracts, beneficial microorganisms, and innovative physical methods represents a promising strategy for improving the sustainability and efficiency of ornamental plant production. Future research should focus on understanding the underlying mechanisms of interaction among these treatments and on optimizing application protocols for different species and growing conditions.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declares no conflict of interest.

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