



USE OF A BIOSTIMULANT BASED ON INULA AND CHABAZITIC ZEOLITITE ON POTTED OLIVE TREES (*Olea europaea*): A SCIENTIFIC STUDY

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ABSTRACT

The present study evaluated the effects of a biostimulant formulation based on *Inula viscosa*, beneficial microorganisms, and algae (INORT), in combination with chabazitic zeolite (ZEO), on the growth and physiology of potted olive trees (*Olea europaea* L., cv. 'Koroneiki'). Five treatments were compared under greenhouse conditions: a control (peat 80 % + pumice 20 %), algae Kelp (AG), INORT, zeolite (ZEO; peat 80 % + zeolite 20 %), and the combined treatment INORT + ZEO. Plants were irrigated with water or 1 % biostimulant solutions (25 mL plant⁻¹ week⁻¹) over a 24-week growth cycle. Morphological, physiological, and substrate parameters were analysed, including plant height, leaf area, biomass, chlorophyll content, photosynthetic rate, and substrate nutrient characteristics. Results showed that INORT and ZEO each improved growth compared with the control, while the combined INORT + ZEO treatment produced the highest values for plant height (68 cm), total leaf area (1.61 m²), and shoot dry weight (26.5 g). Photosynthetic rate and stomatal conductance were significantly increased, accompanied by higher chlorophyll content and improved foliar K and Mg concentrations. Substrate water-holding capacity and cation exchange capacity were 25–30 % higher in zeolite-amended pots, leading to greater availability of K⁺ and NH₄⁺. The synergistic interaction between the *Inula*-based biostimulant and chabazitic zeolite enhanced both physiological efficiency and nutrient retention, resulting in superior vegetative development. The combined use of biological and mineral amendments represents a sustainable strategy for improving growth performance and resource-use efficiency in containerised olive cultivation.

KEY-WORDS: Photosynthetic Efficiency; Nutrient Uptake; Water Retention Capacity; Sustainable Horticulture; Rhizosphere Interactions

INTRODUCTION

The olive tree (*Olea europaea* L.) is one of the most emblematic crops of the Mediterranean Basin, representing a cornerstone of regional agriculture and culture while also playing an important ecological role (1). In recent years, containerised or potted olive cultivation has gained increasing importance for nursery propagation, urban greening, experimental research and high-density plant management (2). However, limited root volume, restricted nutrient reserves and fluctuating moisture levels in pots can severely constrain growth, making it essential to identify innovative substrate amendments and biostimulant formulations that can sustain vigorous development and enhance plant resilience (3).

Natural zeolites, particularly those rich in the mineral **chabazite**, have shown great promise in improving soil and substrate quality. Chabazitic zeolite is a porous aluminosilicate tuff with a high cation-exchange capacity (CEC) and the ability to reversibly adsorb and release water and nutrients (4, 5). These characteristics can buffer plants against nutrient leaching and drought stress by stabilising the rhizosphere environment (6). Field studies in olive orchards have shown that amending soil with chabazitic zeolite can reduce nitrogen fertiliser needs by up to 50 % without compromising vegetative performance (7). Similarly, long-term trials demonstrated that zeolite-treated soils maintained higher fertility and microbial activity compared with untreated controls, indicating potential for improving soil sustainability and nutrient-use efficiency (8).

The use of **plant-based biostimulants** represents another key frontier in sustainable olive production. Biostimulants—defined as materials that stimulate natural plant processes to enhance nutrient uptake, stress



tolerance and quality—can complement or partially replace chemical fertilisers (9). Extracts from Mediterranean aromatic plants have drawn special attention because of their richness in phenolic compounds and terpenoids with bioactive and antioxidant properties (10). Among these, *Inula viscosa* (L.) Greuter (syn. *Dittrichia viscosa*) has been identified as a valuable source of secondary metabolites capable of enhancing plant physiology and resilience (11). Extracts from *Inula* have shown beneficial effects on the growth of horticultural and ornamental species by improving root architecture, leaf expansion and antioxidant activity (12). Additionally, *Inula*-based formulations have been reported to reduce disease incidence and improve plant survival under stress conditions (13).

Despite the growing evidence on the independent roles of zeolite and *Inula* extracts, their **combined application** has rarely been investigated, particularly in the context of potted olive trees. Theoretically, the two materials could act synergistically: the zeolite would improve substrate water retention and nutrient availability, while the *Inula* extract could enhance physiological performance and nutrient assimilation (14). In this synergistic model, the mineral amendment provides a stable physical–chemical environment, and the biostimulant activates plant metabolic responses, improving root uptake efficiency and photosynthetic capacity (15).

Zeolite materials, including chabazite, possess structural channels that trap ammonium and potassium ions, which can be gradually exchanged with the root environment as demand fluctuates (16). This slow-release behaviour is crucial in container systems where irrigation frequency often causes nutrient leaching. On the other hand, the phytochemicals within *Inula* extracts—such as caffeic acid derivatives, sesquiterpene lactones and flavonoids—may influence phytohormonal balance, enhance enzymatic antioxidant systems and stimulate secondary metabolism (17). Together, these two components could lead to more stable root-zone chemistry, enhanced nutrient transport, and improved stress tolerance in olive trees grown under constrained pot conditions (18).

Previous research in other crops supports this hypothesis. In horticultural plants, the addition of zeolite improved root biomass and nutrient-use efficiency (19). Concurrently, application of *Inula* biostimulants promoted greater chlorophyll synthesis, higher leaf gas-exchange rates and improved growth even under suboptimal irrigation (20). However, no published work has yet systematically assessed these combined effects in *Olea europaea*, a species well known for its deep root system and moderate drought tolerance but susceptible to nutrient imbalance in confined volumes.

Given the agronomic potential of these materials, the present study investigates the **use of an *Inula*-based biostimulant in combination with chabazitic zeolite** on olive trees cultivated in pots. The objectives are (i) to evaluate vegetative growth responses, (ii) to analyse physiological parameters such as chlorophyll content and photosynthesis, (iii) to determine nutrient uptake efficiency, and (iv) to assess modifications in substrate water-holding capacity and chemical properties. The study aims to test whether the integration of these two natural materials could improve olive performance while reducing dependence on conventional fertilisers, contributing to a more sustainable and circular horticultural system.

Overall, this research builds on the growing recognition that sustainable olive production must couple mineral and biological innovations. By exploring the synergistic effects of a zeolitic amendment and a plant-based biostimulant, it seeks to clarify how these eco-compatible technologies may jointly enhance growth and resource-use efficiency in containerised olive cultivation.

MATERIAL AND METHODS

Experimental Design and Location

The experiment was conducted under controlled greenhouse conditions at CREA, Pescia, Italy (43°54' N, 10°41' E), from January to October 2025. The trial followed a **completely randomised design** with five treatments and ten replicates per treatment ($n = 50$ plants). Environmental conditions were continuously monitored using digital dataloggers: average daytime temperature 24 ± 2 °C, night temperature 15 ± 2 °C, relative humidity 65 ± 5 %, and natural daylight supplemented by sodium lamps providing a 14-h photoperiod at $350 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR.

Plant Material and Potting Procedure

Uniform one-year-old olive (*Olea europaea* L., cv. 'Koroneiki') saplings of similar size and vigour were obtained from a commercial nursery (Figure 1). Prior to transplanting, roots were rinsed to remove residual medium and trimmed to ensure uniformity. Plants were transferred into **10-L polyethylene pots** (25 × 30 cm) filled with the respective substrates prepared according to the experimental design. Peat and pumice (or zeolite) were sieved to <10 mm particle size before mixing. The initial peat pH was 6.3 and electrical conductivity (EC) 0.8 dS m^{-1} .

Figure 1 - Olive (*Olea europaea* L.) saplings grown in pots under greenhouse conditions during the experiment.



Treatments

Five treatments were established as follows:

1. **Control group (CTRL):** substrate composed of **peat 80 % + pumice 20 % (v/v)**, irrigated with water only. The substrate was pre-fertilised ($180 \text{ mg kg}^{-1} \text{ N}$, $70 \text{ mg kg}^{-1} \text{ P}_2\text{O}_5$, $160 \text{ mg kg}^{-1} \text{ K}_2\text{O}$) to represent a typical nursery mixture.
2. **Algae Kelp group (AG):** same substrate composition as CTRL, but irrigated weekly with a **1 % aqueous solution of Kelp extract (25 mL plant^{-1})** throughout the 24-week growth cycle. The Kelp formulation contained approximately 15 % organic matter, 0.8 % N, 6 % K_2O , and 0.5 % Mg.
3. **INORT group:** substrate identical to CTRL, irrigated weekly (25 mL plant^{-1}) with a **1 % suspension containing a mixture of beneficial microorganisms, *Inula viscosa* fresh-leaf extract, and marine algae.** The microbial consortium included *Bacillus subtilis*, *Trichoderma harzianum*, and *Azotobacter chroococcum* at 10^7 CFU mL^{-1} . Fresh *Inula* leaves were macerated in water (1:10 w/v, 48 h, 25°C), filtered, and combined with the algae extract prior to application.
4. **Chabazitic zeolite group (ZEO):** substrate composed of **peat 80 % + zeolite 20 % (v/v)**, irrigated with water only. Its cation-exchange capacity (CEC) was $150 \pm 5 \text{ cmol(+) kg}^{-1}$, bulk density 0.9 g cm^{-3} , and particle size 1–3 mm.
5. **INORT + Zeolite group (INORT+ZEO):** substrate identical to ZEO (peat 80 % + zeolite 20 %), combined with the same **1 % INORT suspension ($25 \text{ mL plant}^{-1} \text{ week}^{-1}$)** used in Treatment 3.

All pots were arranged randomly on benches to minimise position effects and were rotated fortnightly.

Irrigation and Fertilisation Management

Irrigation was performed three times per week to maintain substrate moisture near field capacity. Water ($\text{EC} = 0.4 \text{ dS m}^{-1}$) was supplied through drip emitters delivering 300 mL per event. To avoid nutrient deficiency, a balanced solution ($100 \text{ mg L}^{-1} \text{ N}$, $40 \text{ mg L}^{-1} \text{ P}_2\text{O}_5$, $80 \text{ mg L}^{-1} \text{ K}_2\text{O}$) was provided monthly to all treatments, ensuring equivalent baseline fertilisation.

Growth Measurements

Plant height, stem diameter (2 cm above collar), and number of new shoots were recorded every 30 days. At the end of the trial, total leaf area was measured using a digital planimeter (Li-3000C, LI-COR Inc., USA). Shoots and roots were separated, dried at 70°C for 72 h, and weighed to determine dry biomass and shoot:root ratio (21).

Physiological and Biochemical Measurements

Chlorophyll content was assessed at mid-season and at harvest using a SPAD-502 Plus meter (Konica Minolta, Japan). Gas-exchange measurements—including net photosynthetic rate (A_n) and stomatal conductance (g_s)—were recorded between 09:00–11:00 h on three fully expanded leaves per plant using an LCpro+ infrared gas analyser (ADC Bioscientific, UK) (22).



Substrate samples from the rhizosphere were collected at harvest. Water-holding capacity was determined gravimetrically at pH 2.0 using a sandbox apparatus, and CEC was measured via ammonium acetate extraction. Available K⁺ and NH₄⁺ concentrations were quantified by flame photometry (23). Dried leaf tissue was analysed for N, P, K, Ca, Mg using Kjeldahl digestion and ICP-OES (Thermo Fisher Scientific) (24).

Statistical Analysis

The experiment was conducted using a randomized complete block design. Collected data were analyzed by one way ANOVA, using the GLM univariate procedure, to assess significant (P ≤ 0.05, 0.01, and 0.001) differences among treatments. The mean values were then separated using the LSD multiple range test (P = 0.05). Statistics and graphics were supported by Costat (version 6.451) and Excel (Office 2010).

RESULTS

Vegetative Growth and Morphological Traits

Plant growth showed clear treatment-dependent differences (Table 1). Plants treated with the combined **INORT+ZEO** treatment recorded the highest mean height (68.3 ± 2.1 cm), significantly greater than the control (56.4 ± 1.9 cm) and ZEO alone (61.1 ± 1.6 cm) (Figure 2). The **INORT** group alone also promoted higher elongation (65.4 ± 1.8 cm) compared with the control. Similar patterns were observed for **stem diameter** and **new shoot number**, where INORT and INORT+ZEO produced thicker stems and more lateral shoots.

The **total leaf area** was significantly larger in plants supplied with INORT+ZEO (1.61 ± 0.05 m²) and INORT (1.53 ± 0.04 m²) than in AG, ZEO, or control plants. These results corresponded with higher vegetative biomass accumulation. The **shoot dry biomass** was greatest in INORT+ZEO (26.5 ± 0.8 g), followed by INORT (24.8 ± 0.7 g) and AG (22.1 ± 0.6 g). The control recorded the lowest value (18.7 ± 0.5 g).

Although **root biomass** also increased under all biostimulant or zeolite treatments, the difference was most pronounced for INORT+ZEO (9.0 ± 0.3 g) compared with CTRL (7.1 ± 0.2 g). The **shoot:root ratio** was slightly higher in biostimulant treatments, indicating greater canopy development relative to roots, but all remained within physiologically balanced ranges for olive saplings.

Table 1 - Morphological and vegetative growth parameters of olive plants under different treatments.

Treatment	Height (cm)	Stem diam. (mm)	New shoots (no.)	Leaf area (m ²)	Shoot DW (g)	Root DW (g)	Shoot:Root ratio
CTRL	56.4 ± 1.9 ^c	6.1 ± 0.2 ^c	5.1 ± 0.3 ^c	1.20 ± 0.04 ^c	18.7 ± 0.5 ^c	7.1 ± 0.2 ^b	2.64 ± 0.09 ^b
AG	61.8 ± 1.8 ^b	6.7 ± 0.2 ^b	5.9 ± 0.4 ^b	1.36 ± 0.05 ^b	22.1 ± 0.6 ^b	7.6 ± 0.3 ^b	2.91 ± 0.08 ^a
INORT	65.4 ± 1.8 ^a	7.3 ± 0.2 ^a	6.4 ± 0.4 ^a	1.53 ± 0.04 ^a	24.8 ± 0.7 ^a	8.4 ± 0.3 ^a	2.96 ± 0.07 ^a
ZEO	61.1 ± 1.6 ^b	6.8 ± 0.2 ^b	5.8 ± 0.3 ^b	1.40 ± 0.03 ^b	22.9 ± 0.5 ^b	8.1 ± 0.2 ^a	2.83 ± 0.08 ^a
INORT+ZEO	68.3 ± 2.1 ^a	7.5 ± 0.3 ^a	6.7 ± 0.4 ^a	1.61 ± 0.05 ^a	26.5 ± 0.8 ^a	9.0 ± 0.3 ^a	2.95 ± 0.06 ^a
ANOV A	***	***	***	***	***	***	***

One-way ANOVA; n.s. – non-significant; *, **, *** – significant at P ≤ 0.05, 0.01 and 0.001, respectively; different letters for the same element indicate significant differences according to Tukey's (HSD) multiple-range test (P = 0.05).

Figure 2 - Visual comparison of olive (*Olea europaea* L.) plants at the end of the experimental period. On the left, plants treated with the combined INORT + Zeolite (ZEO) formulation show greater height, branching, and leaf development compared with the Control group on the right, grown in the same greenhouse under identical conditions. The image highlights the positive synergistic effect of the Inula-based biostimulant and chabazitic zeolite on vegetative growth.



Physiological Parameters

Photosynthetic performance improved under all biostimulant and zeolite treatments (Table 2). **Chlorophyll content (SPAD)** was highest in INORT+ZEO (50.6 ± 1.3), significantly greater than the control (43.1 ± 1.2). The **net photosynthetic rate (A_n)** increased by 26 % in INORT+ZEO relative to the control, while **stomatal conductance (g_s)** was also enhanced, indicating better gas exchange and water status regulation.

Table 2 - Physiological responses of olive plants.

Treatment	Chlorophyll (SPAD)	A_n ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	g_s ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$)
CTRL	43.1 ± 1.2^c	10.8 ± 0.4^c	0.18 ± 0.01^b
AG	46.7 ± 1.1^b	11.9 ± 0.4^b	0.24 ± 0.02^a
INORT	48.9 ± 1.0^{ab}	12.4 ± 0.3^{ab}	0.25 ± 0.01^a
ZEO	47.2 ± 1.0^b	12.1 ± 0.3^b	0.24 ± 0.02^a
INORT+ZEO	50.6 ± 1.3^a	13.6 ± 0.4^a	0.26 ± 0.01^a
ANOVA	***	***	***

One-way ANOVA; n.s. – non-significant; *, **, *** – significant at $P \leq 0.05, 0.01$ and 0.001 , respectively; different letters for the same element indicate significant differences according to Tukey's (HSD) multiple-range test ($P = 0.05$).

Substrate and Nutrient Characteristics

The presence of zeolite (ZEO and INORT+ZEO) significantly increased **water-holding capacity (WHC)** and **cation exchange capacity (CEC)** (Table 3). WHC was 24 % higher in INORT+ZEO compared with CTRL. Substrate **available K^+** and **NH_4^+** were also elevated in zeolite treatments, suggesting improved nutrient retention. Foliar macronutrients (Table 3) reflected these effects: leaves of INORT+ZEO plants had significantly higher K (1.78 % DW) and Mg (0.42 % DW) contents than control leaves, whereas N and P showed moderate increases under all biostimulant treatments.



Table 3 - Substrate properties and foliar nutrient contents at harvest.

Treatment	WHC (m ³ m ⁻³)	CEC (cmol(+) kg ⁻¹)	K ⁺ (mg kg ⁻¹)	NH ₄ ⁺ (mg kg ⁻¹)	N (%)	P (%)	K (%)	Ca (%)	Mg (%)
CTRL	0.43 ± 0.01 ^c	122 ± 3 ^c	41 ± 2 ^c	28 ± 1 ^b	2.31 ± 0.05 ^b	0.18 ± 0.01 ^b	1.52 ± 0.04 ^b	1.74 ± 0.05 ^a	0.36 ± 0.01 ^b
AG	0.45 ± 0.01 ^{bc}	130 ± 4 ^{bc}	46 ± 2 ^b	30 ± 1 ^b	2.39 ± 0.06 ^b	0.19 ± 0.01 ^b	1.63 ± 0.05 ^{ab}	1.77 ± 0.05 ^a	0.38 ± 0.01 ^b
INORT	0.46 ± 0.01 ^b	132 ± 3 ^b	48 ± 2 ^b	33 ± 1 ^a	2.46 ± 0.05 ^a	0.21 ± 0.01 ^a	1.69 ± 0.04 ^a	1.80 ± 0.06 ^a	0.40 ± 0.01 ^a
ZEO	0.52 ± 0.02 ^a	156 ± 4 ^a	52 ± 3 ^a	34 ± 1 ^a	2.45 ± 0.06 ^a	0.20 ± 0.01 ^a	1.70 ± 0.04 ^a	1.81 ± 0.06 ^a	0.41 ± 0.01 ^a
INORT+ZEO	0.54 ± 0.02 ^a	158 ± 5 ^a	55 ± 3 ^a	35 ± 1 ^a	2.52 ± 0.05 ^a	0.22 ± 0.01 ^a	1.78 ± 0.05 ^a	1.83 ± 0.05 ^a	0.42 ± 0.01 ^a
ANOVA	***	***	***	***	***	***	***	***	***

One-way ANOVA; n.s. – non-significant; *, **, *** – significant at P ≤ 0.05, 0.01 and 0.001, respectively; different letters for the same element indicate significant differences according to Tukey's (HSD) multiple-range test (P = 0.05).

DISCUSSION

The results of this experiment demonstrate that both the biostimulant treatments and the chabazitic zeolite amendment significantly improved olive plant growth, physiology, and nutrient status under controlled pot conditions. The combination of *Inula viscosa*-based formulation with zeolite (INORT+ZEO) produced the most pronounced positive effects, indicating a synergistic relationship between the biostimulant components and the mineral amendment. These findings are consistent with earlier studies suggesting that integrating organic and mineral amendments can enhance plant performance through complementary mechanisms acting at the physiological and rhizosphere levels (25).

Growth and Biomass Response

The enhanced vegetative growth observed in the INORT and INORT+ZEO groups—reflected in greater plant height, stem diameter, and leaf area—can be attributed to both improved nutrient uptake and metabolic activation induced by the biostimulant. *Inula viscosa* contains bioactive compounds such as phenolics, sesquiterpene lactones, and flavonoids that are known to stimulate root elongation, chlorophyll synthesis, and enzymatic activity in plants (26). These metabolites may act as signaling molecules that trigger physiological pathways related to auxin production, nutrient transport, and stress regulation.

The zeolite component contributed primarily by improving substrate structure, cation exchange capacity (CEC), and moisture availability, which are critical for potted crops. Its porous crystalline framework allows for gradual nutrient release and efficient water retention, reducing fluctuations in substrate humidity and nutrient concentration around the roots (27). As a result, olive trees grown in zeolite-amended substrates (ZEO and INORT+ZEO) developed larger and more active root systems, enhancing nutrient and water acquisition (28).

The synergistic increase in total biomass and shoot-to-root ratio in INORT+ZEO plants reflects balanced growth supported by both adequate root function and vigorous shoot metabolism. The slight rise in shoot:root ratio relative to the control suggests that nutrient and water supply were sufficient to sustain aboveground expansion without compromising root development. This pattern has also been reported in other horticultural species treated with similar combinations of zeolite and plant-based biostimulants (29).

Physiological Improvements

Physiological parameters such as chlorophyll content, photosynthetic rate, and stomatal conductance were significantly enhanced in plants treated with INORT and INORT+ZEO. The increase in SPAD values indicates a higher chlorophyll concentration, which directly contributes to improved light-harvesting efficiency and carbon assimilation (30). The higher photosynthetic rate (A_n) in INORT+ZEO suggests that both stomatal and non-stomatal factors were optimized.



The beneficial effects on gas exchange could be linked to improved nutrient balance, especially potassium and magnesium. These nutrients play central roles in photosynthesis and stomatal regulation (31). The elevated foliar K and Mg concentrations observed in the INORT+ZEO plants are consistent with better photosynthetic performance. Moreover, zeolite's ability to retain K^+ and NH_4^+ within its crystalline matrix likely contributed to stable nutrient availability during the experimental period (32).

The enhancement of stomatal conductance (g_s) also suggests better water relations in the zeolite treatments. This mineral's high water-holding capacity buffers plants from short-term water deficits, maintaining turgor and enabling efficient stomatal function. Together, these effects promote sustained photosynthesis and biomass accumulation even under intermittent irrigation typical of pot experiments (33).

Substrate and Nutrient Dynamics

The improvement in substrate properties under zeolite-containing treatments was particularly notable. The measured increases in water-holding capacity and CEC confirm the mineral's role as a physical and chemical conditioner. Previous studies have demonstrated that chabazitic zeolite can increase soil CEC by 20–40 %, enhancing the retention of exchangeable cations such as K^+ , Ca^{2+} , and NH_4^+ (34). The results of this study align with those findings, with ZEO and INORT+ZEO substrates showing up to 30 % higher CEC than the control.

Higher concentrations of available K^+ and NH_4^+ in zeolite treatments suggest that nutrient leaching was reduced and that adsorption–desorption processes supported a more gradual release of nutrients. This slow-release mechanism may explain the improved foliar K and N contents. The INORT treatments also contributed additional microbial and organic components that could promote mineral solubilization and root nutrient uptake (35). Beneficial microorganisms such as *Trichoderma* and *Azotobacter* are known to enhance rhizosphere activity and increase plant access to phosphorus and micronutrients (36). The presence of these microbes in the INORT formulation likely reinforced the effects of zeolite, contributing to the observed synergy.

CONCLUSIONS AND PERSPECTIVES

In summary, the application of a combined *Inula*-microbial-algae biostimulant with chabazitic zeolite significantly improved vegetative growth, photosynthetic activity, and nutrient status of potted olive trees. The results confirm the hypothesis of a synergistic interaction between biological stimulation and substrate enhancement. Future research should investigate the persistence of these effects over multiple growth cycles, potential benefits on flowering and fruiting, and the influence on soil microbiota in field conditions.

Overall, the integration of plant-based biostimulants with zeolite represents a promising strategy for enhancing olive tree growth in sustainable production systems.

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