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Biostimulant Application, under Reduced Nutrient Supply, Enhances Quality and Sustainability of Ornamental Containerized Transplants

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Abstract: Ornamental containerized transplant production needs high doses of controlled release fertilizers (CFR), but it is known that there is an environmental risk caused by inadequate fertilization management. To the best of our knowledge, amino acid-(AaB) and seaweed extract-(SeB) based biostimulant application, in ornamental transplant production, is still poorly studied. Therefore, the aim of this work was to assess the hypothesis that, under reduced nutrient supply, SeB and AaB applications, via foliar spray, can promote quality and sustainability in the production of high-quality ornamental seedlings with a 90-day growing cycle. The CRF incorporated into the peat-growing medium was Osmocote Exact Mini in formulation N:P:K = 15 + 9 + 11 (3 months). Six treatments were compared in two economically important potted (0.3 L in volume) ornamentals: *Abelia* × *grandiflora* and *Lantana camara*: T1 = conventional full CRF dose: 4 gL^{-1} per pot; T2 = limited CRF dose: 50% of T1; T3 = T2 + MC-Extra® [SeB 0.5 gL^{-1}]; T4 = T2 + MC-Extra® [SeB 1.0 gL^{-1}]; T5 = T2 + Megafol® [AaB 1.5 mL L^{-1}]; T6 = T2 + Megafol® [AaB 2.5 mL L^{-1}]. The research results showed that the application of 50% CRF plus biostimulant application resulted in plant performance greater than or equal to those raised under the conventional CRF full dose. In particular, S1 (*Abelia* × *grandiflora* 'Edward Goucher') and S2 (*Lantana camara* 'Little Lucky') behaved differently concerning the Megafol® dose under 50% CRF; compared to T1, in *A.* × *grandiflora* young transplants, T5 increased root morphological characteristics, as well as number of leaves, leaf area, and dry biomass accumulation; in *L. camara*, T6 achieved higher performance. The application of biostimulants under 50% CRF also improved, in both *A.* × *grandiflora* and *L. camara*, the physiological and agronomical Nitrogen Use Efficiency, compared to a full CRF dose. This study can support decision-making in terms of agronomic technique choices in line with the sustainable development of high-quality ornamental transplant production.

Keywords: amino acid; controlled release fertilizer; nutrient stress; seaweed extract

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1. Introduction

Nutrition is the main vehicle that maximizes crop production [1] and mineral fertilizers are fundamental for crop nutrition [2,3]. In conventional agriculture, chemical fertilizers are always applied to exceed the recommended dose, so they have become a potential source of environmental pollution [4–7]. Fertilizer overuse has, therefore, introduced key problems such as soil infertility [8], climate change [9,10], environmental degradation [11], and N and P runoff [12,13]. In our opinion, environmental sustainability has become an essential requirement [14], leading to an increased focus on a balanced fertilization strategy [15] that also involves minimizing the use of mineral fertilizers to enhance both crop production and quality and nutrient uptake under low input conditions [16].

Ornamental container-plant production is intensive, reliant on significant contributions of labor, water, and fertilizers [17]. Chen and Wei [18] and Vejan et al. [19] recommended controlled-release fertilizers (CRF). Lawrencia et al. [20] define CRF: "products containing sources of water-soluble nutrients, the release of which in the soil is controlled by a coating applied to the fertilizer", as a best management practice to reduce nutrient leaching and runoff from container-plant production. Mello et al. 2017 [21] showed that polymer-coated urea reduced N leaching by 64.5% compared to conventional urea in container production of *Lantana camara* L. Pitton et al. [22] identified significant leaching of nutrients in the production cycle. For this reason, mineral fertilizers should be reduced and replaced by organic fertilizers [23,24], beneficial microbial inoculants [25,26], and non-microbial biostimulants [27]. A 'plant biostimulant' is any substance or microorganism stimulating plant nutrition efficiency, independently of the product's nutrient content, biotic and abiotic stress tolerance and crop quality traits [28–30]. Biostimulants include, for example, seaweed extracts (SeB), amino acids (AaB), humic substances, chitin, chitosan, minerals, poly-vitamins, and oligosaccharides [31–34], beneficial fungi, and bacteria [34,35]. It is important to consider that the complex and variable nature of raw materials used for their production and the heterogeneous mixture of components of the final product can make it difficult to attribute a specific mode of action to each biostimulant [36–39].

SeB are used in sustainable agriculture to increase growth, quality, and shelf life [40–43]; many studies have demonstrated the positive effects of these extracts on a wide range of crops, including cereals [44], ornamental and flowering plants [45], vegetables [46], and field crops [47]. Several studies of SeB [48], made on *Ascophyllum nodosum* (L.) Jolie [33,49], have shown a positive influence on cell signaling, due to molecules such as polyphenols, peptides, carotenoids [50], polysaccharides [51–54], and betaines. It has been observed that in seaweed extracts there are some essential phytohormones (e.g., auxins, gibberellins, and cytokinins), which have a positive influence on metabolism and development [55–58], promoting growth, quality, and yield [59–62].

Amino acid biostimulants (AaB) have several beneficial effects on hormone biosynthesis and plant growth and development [63–67], including vegetable crops [68–70]. Trovato et al. and Kolukisaoglu [71,72] reported that AaB indicate growth regulator activity and activate plant metabolic processes.

Understanding the biostimulant-plant interactions at molecular, cellular, and physiological levels is also an important prerequisite [73–75]. Biostimulants can be applied as a foliar spray or soil drenching depending on dose and time of application, environmental factors and plant species and cultivar [76,77]. Youssef et al., [78] showed that a foliar spray treatment quickly provides plants with maximal nutrient absorption and utilization compared to soil drenching.

Some research studies also propose the use of biostimulants as a partial replacement for chemical fertilizers in terms of plant growth performance and quality improvement [79,80]. Quille et al. [81] reported that the co-application of mineral N fertilizer and a plant biostimulant is suitable for implementation in current agronomic practice and is aligned with best agricultural practices. Therefore, it is vital for plant nutrition based on sustainable agronomical strategies to reduce the consumption and adverse effects of chemical fertilizers [80,82,83]. In the example illustrated above, of partial replacement of chemical fertilizers, SeB and AaB, applied via foliar spray, can improve young plants' development since these substances act as signals of various beneficial physiological processes [84,85].

Ornamental growers require vigorous containerized transplants in order to improve plant performance (or tolerance) in a post-field establishment [86]. The ornamental plant industry is characterized by great diversity and a continuous need for new genetic materials [87].

L. camara L. (family *Verbenaceae*), commonly known as wild or red sage, is the most widespread species of this genus, native to tropical regions of the Americas and Africa [88] and was introduced twenty years ago to Mediterranean ornamental nurseries. It is adapted to all types of climates from temperate to tropical regions [89]. In the natural pharmacopeia, *L. camara* extract is used to treat bronchitis, asthma, pulmonary ailments, fever, and rheumatism [90–92]. *Abelia* × *grandiflora* (family *Caprifoliaceae*) is a semi-evergreen shrub, derived from interspecific hybridization between *A. chinensis* R. Brown and *A. uniflora* R.

Brown [93]; it is frequently cultivated all over the world [94], since it is generally used for hedge, borders, and screens. It grows well in rich, moist but well-drained soil in full sun or lightly shaded locations and has good drought tolerance [95].

In Mediterranean nursery farms, glossy abelia and wild sage are propagated with vegetative multiplication by cutting, which is rooted and subsequently transplanted into a low-volume pot (0.3 L), and grown in a greenhouse for three months. To the best of our knowledge, AaB and SeB application in ornamental transplant production is still poorly studied. Therefore, the aim of this work was to assess the hypothesis that, under reduced nutrient supply, SeB and AaB foliar spray applications can promote quality and sustainability in the production of high-quality ornamental seedlings with a 90-day growing cycle.

2. Materials and Methods

2.1. Greenhouse Environment

The experiment was carried out from 10 February to 11 May 2021 (90 days), in a heated greenhouse, covered with ethyl vinyl acetate (EVA) plastic film, located in a commercial ornamental nursery (Monopoli, Bari, Italy, 40°54′19.1′′ N, 17°18′21.4′′ E; 66 m a.s.l.). Natural photoperiod, mean air temperature of 22/10 °C day/night, and 60% relative humidity inside the greenhouse were maintained throughout the growth stage.

2.2. Plant Material

Two commercial species, representing high-value ornamental crops, were selected: *Abelia* × *grandiflora* cv 'Edward Goucher' (S1) and *Lantana camara* cv 'Little Lucky' (S2) [96]. Rooted stem cuttings were three months old, with average leaf numbers of 12 and 5 and 10 and 9 roots, respectively, in *Abelia* and *Lantana*. The selection of these species was justified by the fact that, although not native to the Mediterranean area, they are both widely used for ornamental purposes in this environment, which also underlines their adaptability to the climatic conditions in the Mediterranean basin. Rooted cuttings were randomly selected as plant material and grown in a Styroblock® container growing system (model 112/95). Each rooted cutting was chosen to ensure uniformity, vigor, absence of disease, and trueness to cultivar.

2.3. Transplant and Growing Medium Analysis

On 10 February 2021, plastic pots (7 × 7 × 8 cm in size, 0.3 L in volume with four outlets to collect leachate water) were used for transplanting rooted cuttings (one for each pot).

Pots were filled with a sterile growing medium (marked as GM) made of 75% sphagnum peat moss, 15% pumice (3–6 mm in diameter), 4% pumice (7–12 mm in diameter), and 6% lapillus (1–3 mm in diameter)

Physicochemical features of the GM (Tables 1 and 2) were determined before the addition of the CRF. Physical parameters were determined according to De Boodt et al. [97]. In addition, total N and C contents were assessed on a dry matter basis [98,99], while other chemical parameters were analyzed in the 1:5 (V:V) solid:water extract, i.e., pH [100], EC [101], P_2O_5, K_2O, Ca, Mg, and Na [102].

Table 1. Physical characterization of the tested growing medium. BD, Bulk density; TP, Total Porosity; WHC, Water holding capacity.

Parameters	U.M.	GM
BD	g cm^{-3}	0.33 ± 0.09
TP	%	74.2 ± 4.8
WHC	%	66.3 ± 4.0

Values for chemical characteristics are the average of three measurements ± standard error.

Table 2. Chemical characterization of the tested growing medium.

Parameters	U.M.	GM
pH		4.6 ± 0.2
EC	dS m^{-1}	0.14 ± 0.01
Organic N	g 100 g^{-1}	0.65 ± 0.02
Organic C	g 100 g^{-1}	41.5 ± 0.9
C/N ratio		63.8 ± 12.1
P-P_2O_5	g 100 g^{-1}	0.09 ± 0.01
Total Ca	g 100 g^{-1}	1.2 ± 0.6
K-K_2O	g 100 g^{-1}	0.32 ± 0.02
Total Mg	g 100 g^{-1}	0.08 ± 0.01
Total Na	g 100 g^{-1}	0.5 ± 0.03

EC was obtained in aqueous extract w/v 1:10 at 25 °C; pH in H_2O p/v 3:50. Values for chemical characteristics are the average of three measurements ± standard error.

2.4. Nutrient Mineral Supply

The CRF used in this study was Osmocote Exact Mini (granule contains NPK coated with organic resin) in the formulation N:P:K = 15 + 9 + 11 + 2.5 MgO + microelements (3 months). The conventional fertilization dose: 4 g L^{-1} GM was set according to the fertilizer manufacturer's recommendations.

2.5. Biostimulant Supplies

In this study, MC-Extra® (marked as B1) and Megafol® (marked as B2) (Valagro, Atessa, Chieti, Italy) were used. MC-Extra® is derived from the SeB *A. nodosum* [103]; according to the manufacturer's specification, it was composed of organic C (20%), organic N (1%), K_2O (20%), mannitol (4%), betaines (0.2%), and cytokinins.

The MC-Extra® doses used were: D1: 0.5 gL^{-1}; D2: 1.0 gL^{-1}. Megafol® (marked as B2) is a plant AaB, a mixture of proline and tryptophan, sugars (glycosides and polysaccharides), vitamins, and betaines that have been recognized as stress signaling and response molecules [104–106]. It contains total N: 3%, organic N: 1%, ureic N: 2%, K_2O: 8%, and organic C (by fluid vinasse): 9% [107–109].

The Megafol® doses used were: D1: 1.5 mL L^{-1}; D2: 2.5 mL L^{-1}.

2.6. Treatments and Experimental Design

Six treatments were compared:

- T1 = conventional full CRF dose: 4 gL^{-1} GM;
- T2 = limited CRF dose: 50% of T1: 2 gL^{-1} GM;
- T3 = T2 + B1D1 (0.5 gL^{-1});
- T4 = T2 + B1D2 (1.0 gL^{-1});
- T5 = T2 + B2D1 (1.5 mL L^{-1});
- T6 = T2 + B2D2 (2.5 mL L^{-1}).

The biostimulant treatment, started on 10 February, was applied four times as a foliar spray on the leaves of plants at the dose of 30 mL/plant, using a hand sprayer. Care was taken to ensure no dripping occurred on the growing medium. The same volume of distilled water was applied to T1 and T2 plants. Apart from nutrition, standard agricultural practices were adopted for pest and disease management.

Plants were watered using sprinkler irrigation, and they were monitored continuously in terms of general status.

The treatments were arranged in a randomized complete-block design with three replicates (each one consisted of 14 plants) with 18 experimental units per species. A total of 540 pots were placed on the mulched ground, at a density of 100 plants m^{-2}.

2.7. Root Morphology and Growth Measurements

At the end of the cultivation period (90 days after transplant), nine plants per replicate were harvested, the growing medium was gently washed from the roots, and plants were divided into roots, leaves, and shoots. Fresh root systems were sampled, carefully washed with tap water after harvest, spread out on a transparent tray, and scanned at 400 dpi with a scanner (Epson v700 Perfection, Japan). The captured images were then processed using image analysis software (WinRHIZO v. 2005b ©, Regent Instruments Inc., Québec, QC, Canada) to determine total root length, surface area, and volume. For each replicate and treatment, the roots of nine plants were scanned. Above-ground and ground plant traits were measured, those being the number of leaves and total leaf area per plant (by Delta-T, Decagon Devices, Pullman, WA, USA leaf area meter). A hand-help SPAD-502 (Minolta Camera Co., Osaka, Japan) was used to estimate the chlorophyll SPAD index.

The above-ground (shoot + leaves) and ground fresh weight were also determined. Subsequently, these were oven dried at 70 °C ± 1 °C until they reached a constant dryness, and the above-ground, ground, whole dry weights (d.w.), and the root to shoot ratio (i.e., the ratio between root dry mass and shoot dry mass) were calculated.

2.8. Above-Ground Macronutrients Analysis

Above-ground samplings were performed for both *A.* × *grandiflora* and *L. camara* species to determine macronutrients (N, P, K, Ca, and Mg). The concentrations of nutrients were determined based on the percentage of dry matter. The nitrogen Kjeldahl was determined using the Kjeldahl method after 96% H_2SO_4 hot digestion; the phosphorus (P) was determined using the colorimetric method with a spectrophotometer. Potassium (K), calcium (Ca), and magnesium (Mg) concentrations were analyzed by obtaining a digest that was diluted with 25 mL of ultrapure water before nutrient determination by simultaneous inductively coupled plasma-atomic emission spectrometry.

2.9. Nitrogen Use Efficiency (NUE)

Nitrogen Use Efficiency (NUE) indices were calculated as follows [110]:

i. Physiological Use Efficiency as the ratio between above-ground dry biomass and total amount of N in above-ground tissues;
ii. Agronomic Use Efficiency as the ratio between above-ground dry biomass and total N supplied with CRF.

Above-ground dry biomass was calculated as the difference between transplant above-ground d.w. at the end of experiment and rooted cutting above-ground d.w. at transplanting.

2.10. Statistical Analysis

One-way ANOVA was performed within each species. All the above data analyses were performed using SAS version 9.3 statistical software (SAS, 1999); treatment means were separated by the S.N.K. (Student Newman–Keuls) test ($p \leq 0.05$).

3. Results

The most striking results in *A.* × *grandiflora* young transplants, for the root morphological traits (Table 3), were an increase in length (+15.5%), surface area (+55.2%), and volume (+236.7%) with the application of the limited CRF supply plus B2 (AaB = Megafol®) at 1.5 mL L^{-1} dose (T5) compared to the conventional CRF supply (T1). Root length, surface area, and volume in *A.* × *grandiflora* under 50% CRF plus B2 at 2.5 mL L^{-1} (T6) were significantly different to T5, with decreases, respectively of 17.1, 39.5, and 107%. Both B1 (SeB = MC-Extra®) doses, under the limited CRF supply, exhibited similar effects and were slightly lower than T5 for root length. On the other hand, young plants showed surface area and volume decreases under the limited CRF supply plus 0.5 g L^{-1} (T3) and 1.0 g L^{-1} (T4) compared to those under T5.

Table 3. Transplant root morphological traits: root length (mm), root surface area (mm^2), and volume (mm^3) in *A.* × *grandiflora* (S1) and *L. camara* (S2), are influenced by different nutrient supplies.

TMTS	Root					
	Length (mm)		Surface Area (mm^2)		Volume (mm^3)	
	A. × *grandiflora*	*L. camara*	*A.* × *grandiflora*	*L. camara*	*A.* × *grandiflora*	*L. camara*
T1	5527 ± 75 b	6144 ± 59 d	1138 ± 27 d	1891 ± 55 b	1299 ± 52 f	5482 ± 194 b
T2	6050 ± 136 b	7599 ± 223 c	1513 ± 18 b	2129 ± 137 b	2824 ± 76 d	5333 ± 144 b
T3	6019 ± 204 b	8252 ± 282 b	1572 ± 78 b	2408 ± 157 a	3196 ± 44 c	5757 ± 118 b
T4	5650 ± 253 b	8356 ± 224 b	1593 ± 17 b	2421 ± 55 a	4154 ± 20 b	5650 ± 216 b
T5	6382 ± 78 a	6617 ± 55 d	1766 ± 19 a	2197 ± 147 b	4375 ± 59 a	5302 ± 388 b
T6	5448 ± 269 b	9478 ± 100 a	1266 ± 48 c	2421 ± 79 a	2113 ± 85 e	6217 ± 59 a

In columns, numbers followed by different letters are statistically different within parameters (S.N.K. test, $p \leq 0.05$; mean ± SD, n = 3). T1 = conventional dose of CRF [4 gL^{-1} GM]; T2 = 50% T1; T3 = T2 + MC-Extra® [0.5 gL^{-1}]; T4 = T2 + MC-Extra® [1.0 gL^{-1}]; T5 = T2 + Megafol® [1.5 mL L^{-1}]; T6 = T2 + Megafol® [2.5 mL L^{-1}].

In *L. camara* (Table 3), the limited CRF dose plus B2 (AaB) at 2.5 mL L^{-1} (T6) significantly affected both root surface area and volume, compared to all other treatments; in particular, T6 had consistent increases in length (+54.3%) and volume (+13.4%) compared to the conventional full CRF dose (T1).

As revealed in the statistical analysis, in *A.* × *grandiflora* (Table 4) the limited CRF supply plus B2 (AaB) (Megafol®) at the dose of 1.5 mL L^{-1} (T5) affected the number of leaves (+13.3%), leaf area (+4.2%), and leaf fresh weight (+8.5%) compared to the full CRF supply (T1). The worst result was achieved by the young plants grown under limited CRF alone (T2): 42 leaves, 136 cm^2 in leaf area, and 3.60 g in fresh leaf weight. By applying B2 (AaB), at the dose of 2.5 mL L^{-1} (T6), compared to T5, *A.* × *grandiflora* young plants showed decreases of 11.7% (leaves), 4.7% (leaf area), and 11.1% in fresh leaf weight. On the contrary, both T3 and T6 exhibited differences for the SPAD index, respectively, 445 (+9%) and 442 (+8%) compared to T1 (full CRF supply). In *L. camara* (Table 4), the highest number of leaves was obtained with T6 (60), with an increase of 15% compared to T1 (52); the lowest leaf number values were found in T2 (46) and T4 (48). The seedlings with the highest leaf area, were those under both T1 (full CRF dose) and T6 (half CRF plus B2D2), with 143 and 139 cm^2, respectively, with an average increase of 33% compared to T2 (106 cm^2). The fresh leaf biomass (Table 4) was found to be 10% higher in T6-treated plants (6.91 g) compared to those in T1 (6.26 g). The worst result was achieved by the plants grown under the 50% CRF dose (T2): 4.77 g. Both T3 and T4 fresh leaf weight values (respectively 5.76 and 5.36 g) were lower than T5 and T6 (respectively 6.46 and 6.91 g); SPAD was found to be unaffected.

Table 4. Transplant above-ground traits: leaves per transplant (no.), leaf area per transplant (cm^2), leaf fresh weight (g) and chlorophyll index (SPAD) in *A.* × *grandiflora* (S1) and *L. camara* (S2), influenced by different nutrient supplies.

TMTS	Plant Above-Ground Traits							
	Leaves (n)		Leaf Area (cm^2)		Leaf Fresh Weight (g)		Chlorophyll Index (SPAD)	
	A. × *grandiflora*	*L. camara*	*A.* × *grandiflora*	*L. camara*	*A.* × *grandiflora*	*L. camara*	*A.* × *grandiflora*	*L. camara*
T1	45 ± 1.2 bc	52 ± 1.5 ab	143 ± 2.9 b	143 ± 1.0 a	3.90 ± 0.10 bc	6.26 ± 0.14 b	409 ± 5.2 b	366 ± 8.1 a
T2	42 ± 1.0 c	46 ± 2.5 b	136 ± 0.6 c	106 ± 3.7 c	3.60 ± 0.05 c	4.77 ± 0.12 e	432 ± 1.3 ab	344 ± 3.3 a
T3	49 ± 0.9 ab	52 ± 3.2 ab	141 ± 2.9 b	118 ± 4.8 bc	4.03 ± 0.06 ab	5.76 ± 0.12 c	445 ± 3.5 a	354 ± 3.0 a
T4	44 ± 0.8 bc	48 ± 0.5 b	138 ± 3.7 bc	120 ± 3.9 bc	3.66 ± 0.06 bc	5.36 ± 0.14 d	426 ± 1.4 ab	345 ± 5.4 a
T5	51 ± 0.9 a	54 ± 2.4 ab	149 ± 2.6 a	130 ± 6.0 ab	4.23 ± 0.14 a	6.46 ± 0.08 b	431 ± 5.7 ab	351 ± 4.4 a
T6	45 ± 1.3 bc	60 ± 3.1 a	142 ± 2.3 b	139 ± 5.5 a	3.76 ± 0.07 bc	6.91 ± 0.11 a	442 ± 1.6 a	365 ± 5.8 a

In columns, numbers followed by different letters are statistically different within parameters (S.N.K. test, $p \leq 0.05$; mean ± SD, n = 3). T1 = conventional dose of CRF [4 gL^{-1} GM]; T2 = 50% T1; T3 = T2 + MC-Extra® [0.5 gL^{-1}]; T4 = T2 + MC-Extra® [1.0 gL^{-1}]; T5 = T2 + Megafol® [1.5 mL L^{-1}]; T6 = T2 + Megafol® [2.5 mL L^{-1}].

In general, the nutrient supply type had a significant effect on ground, above-ground and root-shoot dry weights (Table 5). In *A.* × *grandiflora*, ground and above-ground dry weights increased with the 50% CRF supply plus Megafol® at a dose of 1.5 mL L^{-1} (T5), compared to the full CRF dose (T1) by +26.9 and +15.7%, respectively. Young transplants, under T5, had decreases of 27 and 11% for ground and above-ground dry weights. T2, T3 and T4 showed lower performance than T5 plants. Root to shoot ratio was not significant. Table 5 showed that, in *L. camara*, ground and above-ground dry weights increased with the 50% CRF supply plus Megafol® at a dose of 2.5 mL L^{-1} (T6), compared to T1: respectively +21 and +18% in ground and above-ground dry weights. Furthermore, the limited CFR supply (T2) showed the highest root to shoot ratio (0.70), compared to other treatments.

Table 5. Transplant ground, above-ground dry weight (g) and root to shoot dry weight ratio in *A.* × *grandiflora* (S1) and *L. camara* (S2), influenced by different nutrient supplies.

TMTS	Dry Weight per Plant (g)					
	Ground		Above-Ground		Root: Shoot	
	A. × *grandiflora*	*L. camara*	*A.* × *grandiflora*	*L. camara*	*A.* × *grandiflora*	*L. camara*
T1	0.26 ± 0.05 cd	1.05 ± 0.06 b	1.53 ± 0.03 b	1.86 ± 0.03 b	0.17 ± 0.02 a	0.56 ± 0.02 b
T2	0.24 ± 0.03 d	0.98 ± 0.03 b	1.40 ± 0.05 c	1.40 ± 0.05 d	0.17 ± 0.02 a	0.70 ± 0.04 a
T3	0.29 ± 0.05 b	1.00 ± 0.05 b	1.60 ± 0.05 b	1.63 ± 0.03 c	0.18 ± 0.01 a	0.61 ± 0.05 b
T4	0.27 ± 0.03 c	1.06 ± 0.03 b	1.36 ± 0.06 c	1.73 ± 0.03 c	0.20 ± 0.02 a	0.61 ± 0.02 b
T5	0.33 ± 0.05 a	1.13 ± 0.03 ab	1.77 ± 0.06 a	1.90 ± 0.05 b	0.19 ± 0.01 a	0.59 ± 0.05 b
T6	0.24 ± 0.06 d	1.27 ± 0.03 a	1.57 ± 0.03 b	2.20 ± 0.03 a	0.15 ± 0.02 a	0.58 ± 0.03 b

In columns, numbers followed by different letters are statistically different within parameters (S.N.K. test, $p \leq 0.05$; mean ± SD, n = 3). T1 = conventional dose of CRF [4 gL^{-1} GM]; T2 = 50% T1; T3 = T2 + MC-Extra® [0.5 gL^{-1}]; T4 = T2 + MC-Extra® [1.0 gL^{-1}]; T5 = T2 + Megafol® [1.5 mL L^{-1}]; T6 = T2 + Megafol® [2.5 mL L^{-1}].

Table 6 shows that the nutrient supply type statistically influenced the N, P, and K content in epigeal organs (stem plus leaves) in both species. In *A.* × *grandiflora*, the highest N accumulation was observed in the conventional CRF supply (T1 = 21.5 mg g^{-1} d.w.) and in T5 and T6 (on average 20.9 mg g^{-1} d.w.). Lower contents were observed in T2, T3 and T4 (on average 19.3 mg g^{-1} d.w.). The P content was higher and without significant differences between the seedlings treated with T1, T2, T3, T5 and T6 (on average 1.4 mg g^{-1} d.w.). The lowest value was shown in T4. As regards the K content, the highest values were obtained in T1 and T6 with 27.0 and 26.2 mg g^{-1} d.w., respectively, while the lowest value in the plants treated with T4 (on average 15.3 mg g^{-1} d.w.). In *L. camara*, the highest N and P content was observed in T1, compared to all other treatments, with 33.4 and 2.8 mg g^{-1} d.w., while K was influenced by T6 and T1 with higher values (respectively 28.4 and 26.0 mg g^{-1} d.w.).

Table 6. Transplant above-ground Nitrogen (N), Phosphorous (P) and Potassium (K) content (mg g^{-1} dry weight) in *A.* × *grandiflora* (S1) and *L. camara* (S2), influenced by different nutrient supplies.

TMTS	Nutrient Content (mg g^{-1} d.w.)					
	N		P		K	
	A. × *grandiflora*	*L. camara*	*A.* × *grandiflora*	*L. camara*	*A.* × *grandiflora*	*L. camara*
T1	21.5 ± 0.01 a	33.4 ± 0.16 a	1.3 ± 0.01 a	2.8 ± 0.01 a	27.0 ± 0.03 a	26.0 ± 0.01 ab
T2	19.2 ± 0.01 b	22.8 ± 0.08 b	1.4 ± 0.01 a	1.6 ± 0.01 b	18.7 ± 0.01 c	24.4 ± 0.01 b
T3	19.3 ± 0.01 b	21.7 ± 0.03 bc	1.3 ± 0.01 a	2.1 ± 0.01 b	18.0 ± 0.01 c	23.8 ± 0.01 b
T4	19.3 ± 0.02 b	19.1 ± 0.02 c	1.0 ± 0.01 b	1.8 ± 0.01 b	15.3 ± 0.02 d	24.8 ± 0.01 b
T5	20.8 ± 0.02 a	19.6 ± 0.05 c	1.5 ± 0.01 a	1.9 ± 0.03 b	21.7 ± 0.04 b	24.5 ± 0.08 b
T6	20.9 ± 0.01 a	23.0 ± 0.04 b	1.6 ± 0.01 a	2.0 ± 0.02 b	26.2 ± 0.02 a	28.4 ± 0.02 a

In columns, numbers followed by different letters are statistically different within parameters (S.N.K. test, $p \leq 0.05$; mean ± SD, n = 3). T1 = conventional dose of CRF [4 gL^{-1} GM]; T2 = 50% T1; T3 = T2 + MC-Extra® [0.5 gL^{-1}]; T4 = T2 + MC-Extra® [1.0 gL^{-1}]; T5 = T2 + Megafol® [1.5 mL L^{-1}]; T6 = T2 + Megafol® [2.5 mL L^{-1}].

In *A. × grandiflora* (Table 7) the highest value of the Ca content resulted from T6 (36.3 mg g^{-1} d.w.) with an increase of 46.4% compared to the lowest value obtained in T2 (24.8 mg g^{-1} d.w.). In *L. camara* as well, the highest Ca content value was obtained by applying T6 (41.7 mg g^{-1} d.w.) with an increase of 53% compared to T4 (27.2 mg g^{-1} d.w.). As regards the Mg content (Table 7), the species behaved differently; in *A. × grandiflora*, the highest value occurred in T1 (5.6 mg g^{-1} d.w.), while the lowest values were found in T2 and T4 (mean 3.3 mg g^{-1} d.w.). In *L. camara*, on the other hand, the highest Mg content (6.7 mg g^{-1} d.w.) was obtained in T6, with an increase of +31% compared to T1 and of +63% compared to T4. However, no corresponding deficiency symptoms were observed at any nutrient supply treatment.

Table 7. Transplant above-ground Calcium (Ca) and Magnesium content (Mg) (mg g^{-1} dry weight) in *A. × grandiflora* (S1) and *L. camara* (S2), influenced by different nutrient supplies.

TMTS	Nutrient Content (mg g^{-1} d.w.)			
	Ca		Mg	
	A. × grandiflora	*L. camara*	*A. × grandiflora*	*L. camara*
T1	29.6 ± 0.04 b	31.7 ± 0.04 ab	5.6 ± 0.01 a	5.1 ± 0.01 b
T2	24.8 ± 0.03 c	38.7 ± 0.07 ab	3.3 ± 0.01 c	5.4 ± 0.01 b
T3	26.6 ± 0.02 c	39.8 ± 0.01 ab	3.6 ± 0.02 bc	4.8 ± 0.01 bc
T4	29.6 ± 0.04 b	27.2 ± 0.07 b	3.3 ± 0.01 c	4.1 ± 0.01 c
T5	26.4 ± 0.02 c	35.3 ± 0.08 ab	3.8 ± 0.01 b	5.4 ± 0.01 b
T6	36.3 ± 0.016 a	41.7 ± 0.09 a	4.8 ± 0.01 ab	6.7 ± 0.01 a

In columns, numbers followed by different letters are statistically different within parameters (S.N.K. test, $p \leq 0.05$; mean ± SD, n = 3). T1 = conventional dose of CRF [4 gL^{-1} GM]; T2 = 50% T1; T3 = T2 + MC-Extra® [0.5 gL^{-1}]; T4 = T2 + MC-Extra® [1.0 gL^{-1}]; T5 = T2 + Megafol® [1.5 mL L^{-1}]; T6 = T2 + Megafol® [2.5 mL L^{-1}].

The type of nutrient supply had a significant effect on the Nitrogen Use Efficiency (NUE) (Table 8): in *A. × grandiflora*, the physiological NUE value increased with MC-Extra® at both doses (on average 51 gg^{-1}); in *L. camara*, the highest values occurred in T4 (52.4 gg^{-1}) and T5 (51.0 gg^{-1}). As regards the agronomic NUE, in *A. × grandiflora*, the highest values were recorded in T3 (+113.3%), T5 (+141.1%), and T6 (+110%) compared to T1. In *L. camara*, the treatment with the best performance was T6 (+125.7%) compared to T1. The remaining treatments, however, all had a NUE above T1.

Table 8. Transplant Physiological (Ph.) and Agronomical (Agr.) Nitrogen Use Efficiency (NUE) in *A. × grandiflora* (S1) and *L. camara* (S2), influenced by different nutrient supplies.

TMTS	Nitrogen Use Efficiency (NUE)			
	Physiological NUE (gg^{-1})		Agronomical NUE (gg^{-1})	
	A. × grandiflora	*L. camara*	*A. × grandiflora*	*L. camara*
T1	48.8 ± 0.14 b	29.9 ± 1.10 c	90 ± 6.3 b	163 ± 7.3 c
T2	48.1 ± 0.33 b	43.9 ± 1.49 c	162 ± 18.8 ab	251 ± 18.3 b
T3	51.3 ± 0.40 a	46.1 ± 0.70 b	192 ± 16.5 a	279 ± 16.7 b
T4	51.2 ± 0.75 a	52.4 ± 0.72 a	162 ± 13.2 ab	294 ± 9.0 ab
T5	48.2 ± 0.46 b	51.0 ± 1.08 a	217 ± 11.7 a	323 ± 12.9 ab
T6	47.8 ± 0.20 b	43.5 ± 0.75 b	182 ± 7.4 a	368 ± 15.2 a

In columns, numbers followed by different letters are statistically different within parameters (S.N.K. test, $p \leq 0.05$; mean ± SD, n = 3). T1 = conventional dose of CRF [4 gL^{-1} GM]; T2 = 50% T1; T3 = T2 + MC-Extra® [0.5 gL^{-1}]; T4 = T2 + MC-Extra® [1.0 gL^{-1}]; T5 = T2 + Megafol® [1.5 mL L^{-1}]; T6 = T2 + Megafol® [2.5 mL L^{-1}].

4. Discussion

In vegetable crops, several pieces of research have highlighted that plant biostimulants, applied in the growing cycle, can reduce the level of inorganic fertilizer required [53,111–113] to optimize growth, quality, and yield [37,114,115]. On the other hand, in ornamental production, which is characterized by high agrochemical use and energy consumption [114], there has been little work [40], as far as we know, on reducing the conventional mineral CRF dose with the supplementation of biostimulants, while maintaining a high agronomic quality of ornamental plants for transplantation.

The results of this study highlighted that with both *A.* × *grandiflora* and *L. camara*, young plants had a well-developed root system, which is needed for nutrient uptake, especially when they were grown under a limited CRF supply plus B2 (AaB = Megafol®) (Table 3). In *A.* × *grandiflora*, our results show that an improvement in the root architecture is due to the role of Megafol® and its amino acid content, in line with Walch-Liu et al. [116] and Repke et al. [117]. Amino acids are considered to be precursors and constituents of proteins, which are well-known for stimulating cell growth [118]. Tryptophan is the precursor for the synthesis of the auxin IAA-indole acetic acid [119]. Among the exogenous rooting factors, the auxin IBA (indole-3-butyric acid) is widely used to stimulate rooting processes in cuttings, because of its strong ability to promote root initiation [120]. Izadi et al. [121] reported that this hormone, at the base of the cuttings, results in a localized IAA production. In *L. camara*, a positive effect of the application of both biostimulants was noted for the surface area and volume traits (Table 3). Our results, in *L. camara*, are in agreement with those of Crouch and van Staden [122] and Ertani et al. [41], which pointed out that, in maize, *A. nodosum* extract significantly promotes root morphology because of higher levels of indole-3-acetic acid (IAA). The presence of biostimulant molecules that have auxin-like activities has been highlighted by several pieces of research [41,65]. Therefore, the specific species response of products with biostimulant activity is confirmed. AaB could reduce the N fertilization as their application could benefit root development and establishment, as Merzt et al. [123] reported on creeping bentgrass.

Plant growth is defined as the increase in plant mass, measured on a weight basis or by other features such as leaf area [124]; in this study, under a limited CRF supply, the Megafol® biostimulant at 1.5 mL L^{-1} (in *A.* × *grandiflora*) and at 2.5 mL L^{-1} (in *L. camara*) intensified vegetative growth (Table 4). Results are in agreement with those of Shehata et al. [76] that showed that AaB application increased biomass production. In *A.* × *grandiflora*, biostimulants should be used with caution as an overdose may have adverse effects [125].

Niu et al. [126] applied Megafol® to the leaf surfaces of tomato seedlings at the four-leaf stage, after transplantation (500 times dilution) at 10 °C, 25 °C and 35 °C. They observed that 25 °C Megafol® increased aboveground f.w. and d.w. by 49.5% and 50.4% and ground f.w. and d.w. by 85.1% and 116.3% compared to untreated seedlings. Our results underlined that, in *A.* × *grandiflora*, biostimulants should be used with caution as an overdose may have adverse effects [125]. The results obtained in Table 4 diverge from those of other researchers who applied seaweed extracts, achieving positive results in ornamental pepper on a number of leaves [127], in *Chrysanthemum* spp., on leaf area [128], in lettuce on shoot growth and chlorophyll content, resistance to thermal stress [129,130], and root dry weight [131] (Table 5).

In this work, McExtra® and Megafol® biostimulant supplies, in *A.* × *grandiflora*, improved the SPAD index (Table 4). These results are consistent with those of Cristofano et al. [132] and Ottaiano et al. [133].

Nitrogen (N) is a vital nutrient that influences plant growth, as well as the quality and quantity of the produce [134–136]. The taxa *A.* × *grandiflora* and *L. camara* showed different behavior regarding the nitrogen content in the above-ground plant. In *A.* × *grandiflora*, the application of amino acids (Megafol®) had also shown positive effects on N content; this is supported by [137] (Table 6). Moreover, results of the research carried out by Bettoni et al. [138] indicate that aa-based-biostimulant application leads to the improved absorption of nitrogen from roots to shoots in plants.

In ornamental plant nurseries, N fertilization is often excessive, due to the diversity of genera, species and cultivars, plant ages, and production systems. As shown by Chen et al. [139], N rates for some container-grown crops ranged from 1067 to 2354 kg per hectare per year, which is 10–15 times higher than those recommended for many agronomic field crops.

Results regarding P, K, and Mg content (Tables 6 and 7) were partially in agreement with those of Radkowski et al. [140] that recorded increased amounts of chemical elements in lawn turf tissues under foliar application of an amino acid-based biostimulant. Adopting appropriate N management strategies is crucial for improving NUE and efficient crop production [141]. The use of environmentally friendly inputs of natural and organic origin resources can stimulate plant growth by improving the efficient use of synthetic fertilizers [142]. Both biostimulants (McExtra® and Megafol®) in a low fertilization input, improved the NUE of young seedlings, compared with treatments without biostimulants (T1 and T2). Of the two species, *L. camara* presented, in all treatments, the highest NUE compared to *A.* × *grandiflora* (Table 8).

5. Conclusions

The global market for mineral fertilizers is projected to be worth $143.34 billion by 2028. The amount of mineral fertilizers-nitrogen and phosphorus-used in agricultural production in the EU reached 11.2 million tons in 2020. Mineral fertilizer prices have increased by almost 30% since the start of 2022, after increases of 80% last year. The price increase is driven by a confluence of factors, including rising input costs, supply disruptions caused by sanctions (Belarus and Russia), and export restrictions (China). Nitrogen fertilizers are produced with the energy contribution of natural gas, the price of which is strongly linked to that of oil; phosphates are mined outside the EU, resulting in high production and transport costs, also linked to oil prices. It should be noted that the EU nitrogen fertilizer industry is heavily dependent on gas of Russian origin and that Russia and Belarus are key players in the world production of phosphate and especially potash fertilizers. The war in Ukraine, the application of sanctions on Russia and export restrictions from China will lead to a sharp increase in fertilizer prices in the years to come, which is likely to have a profound impact on conventional agriculture in the EU.

Ornamental containerized transplant production needs high doses of mineral fertilizers, but there is an environmental risk caused by inadequate fertilization management. Therefore, it is essential to apply sustainable techniques to promote plant growth and improve quality features.

The results reported herein highlighted that, under limited mineral nutrient supply (50% CRF), Megafol® (AaB) was more efficient than Mc Extra® (SwB), increasing the quality performance of Abelia and Lantana transplants compared to those produced without biostimulants. Both biostimulants, McExtra® and Megafol®, in a low fertilization input, improved the NUE of young cuttings, compared to the full and half CRF supplies. Nevertheless, additional studies are needed to validate these preliminary findings and to investigate the mechanism/mode of action.

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